

Research Note

Vector and reservoir host of a case of human *Brugia pahangi* infection in Selangor, peninsular Malaysia

Muslim, A.^{1*}, Fong, M.Y.², Mahmud, R.² and Sivanandam, S.²

¹Faculty of Medicine, Universiti Teknologi MARA (UiTM), 47000, Sungai Buloh, Selangor, Malaysia

²Department of Parasitology and TIDREC, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding author email: azdayanti@salam.uitm.edu.my

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Abstract. A case of human eye infection caused by *Brugia pahangi* was reported in 2010 in a semi rural village in Selangor, peninsular Malaysia. Our report here reveals results of investigation on the vector and animal host for the transmission of the infection. We conducted entomological survey and cat blood examination in the vicinity of the patient's home. The mosquito species *Armigeres subalbatus* was incriminated as the vector, whereas cat served as the reservoir host.

Brugia pahangi is a lymphatic filarial worm that infects cats, dogs and monkeys (Denham & McGreevy, 1977). Mosquitoes of species *Mansonia* spp. have been reported to be the natural vector of *B. pahangi* (Edeson *et al.*, 1960a). This worm has been experimentally transmitted to human and the infection produced signs and symptoms of lymphatic filariasis, but hardly produced microfilaraemia (Edeson *et al.*, 1960b). Although there was a report on the presence of *B. pahangi* microfilariae (mf) in human blood in South Kalimantan, the finding was not conclusive as the method used to identify the mf relied on measuring phosphatase distribution in the mf (Palmieri *et al.*, 1985). The first confirmed cases of human lymphatic filariasis caused by *B. pahangi* in Malaysia were reported in 2010 (Tan *et al.*, 2011). Shortly after that, an isolated case of human eye infection caused by *B. pahangi* was reported in Sungai Buloh, a semi-rural town in the state of Selangor in Peninsular Malaysia (Muslim, 2010). The present report here describes the vector and animal reservoir

host of the *B. pahangi* infection in Sungai Buloh.

The patient, a 24 year old Malay woman, presented with complaints of persistent right eye redness associated with mild pain and mild grade fever for one month. She was given topical antibiotic eye drops but there was no improvement. During further examination using slit-lamp, entangled tiny transparent thread-like mobile worms were detected at the subconjunctival area. The worms were extracted and confirmed as *B. pahangi* by PCR (Muslim, 2010). On further questioning the patient said that she had four pet cats at home. She had no history of travelling and lived with her family in a village in Sungai Buloh.

The village (3° 12' 51" North, 101° 30' 57" East) is highly populated and surrounded by orchard farms and an oil palm estate. Houses are built far apart from each other. Most of the people living in this area are Malays of Banjar and Mendailing ethnic groups. Similar to other typical villages elsewhere, this village also has improper drainage system

and some of the houses have unsatisfactory sanitary facilities.

Blood samples were collected only from three of the patient's pet cats. Thick blood smear (TBS) on microscope slides were prepared and stained with Giemsa solution. Microscopy examination revealed one of cats was positive for mf with a density of 20 mf/60 μ l blood. The mf were sheathed with obvious Innenkorper body and terminal nuclei (Figure 1). The average length of the mf and Innenkorper body were 245.8 ± 6.3 μ m and 49.5 ± 4.5 μ m, respectively. These morphometric measurements were within the range for *B. pahangi* mf, as reported previously by Buckley & Edeson (1956) and Sivanandam & Fredericks (1966).

Mosquito collection was conducted in the surrounding area from 0600-0900 and 1800-2030 hours using human landing catch (HLC) method. The mosquitoes were identified to species level and then dissected for the presence of filarial larvae. Of the 381 mosquitoes collected, 363 were dissected. Three species, *Amigeres subalbatus* (87.7%), *Aedes albopictus* (9.9%) and *Aedes aegypti* (2.4%) were found in the area. Only *Ar. subalbatus* was found to be positive (5%) for filarial larvae. Of these infected *Ar. subalbatus*, 2.5% had stage 3 larvae (L_3). The highest number of larvae recovered from a

single mosquito was 21. Overall, the total larvae recovered were 121 L_3 , 56 L_2 and 9 L_1 . Summary of the entomological findings is shown in Table 1.

Larvae from the mosquitoes and mf from the infected cat's blood were subjected to PCR and sequencing of the PCR fragments. The PCR amplified a region in the *B. pahangi* cytochrome oxidase subunit I (COXI) gene. Nucleotide sequence of the PCR fragments matched a *B. pahangi* COXI sequence (Accession Number AJ271611) in the GenBank database. In addition, multiple alignment analysis (Figure 2) revealed >99% similarity in the sequences the larvae, mf and *B. pahangi* COXI AJ271611. These findings further confirmed the identity of the larvae and mf as *B. pahangi*.

Ocular infection by filarial worms has previously been reported in Malaysia, in which *Dirofilaria* spp. were implicated as the aetiologic agents (Dissanaike *et al.*, 1977; Mak & Thanalingam, 1984; Rohela *et al.*, 2009). Our report here is the first record of human eye infection caused by *B. pahangi*. In this infection, human serves as accidental or dead end host. Hence, *B. pahangi* larva does not reach adulthood or sexual maturity in the human body. The worm is present as occult infection as no mf is present in the blood.

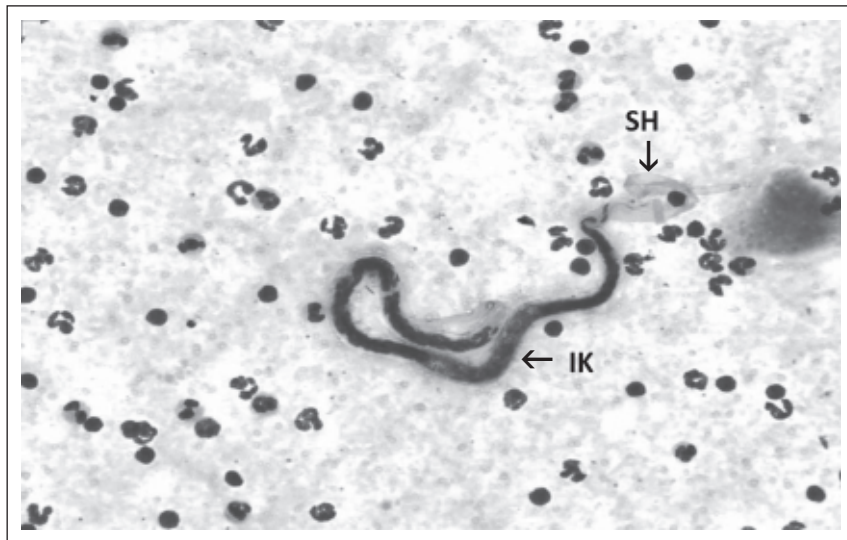


Figure 1. *Brugia pahangi* microfilaria in the blood of infected cat (40X). The sheath (SH) and Innenkorper body (IK) are arrowed

Table 1. Result of entomological findings in the Kg. Merbau Sempak

Mosquito species	Total	Dissection results
POSITIV		
<i>Armigeres subalbatus</i>		Total mosquitoes infected: 18 (5.0%)
<i>Caught</i>	334	with L ₃ : 9 (2.5 %)
<i>Dissected</i>	334	with L ₂ : 7 with L ₁ : 2
NEGATIVE		
<i>Aedes aegypti</i>		
<i>Caught</i>	9	
<i>Dissected</i>	9	
NEGATIVE		
<i>Aedes albopictus</i>		
<i>Caught</i>	38	
<i>Dissected</i>	20	
NEGATIVE		
Grand Total		
<i>Caught</i>	381	
<i>Dissected</i>	363	

Filarial larvae Microfilariae <i>B. pahangi</i> COXI	GCCTTGCTTTTTGATTTACTTTTGTAGCTTTGTTGATGGTTTATCAATCTTTTTTATT GCCTTGCTTTTTGATTTACTTTTGTAGCTTTGTTGATGGTTTATCAATCTTTTTTATT GCCTTGCTTTTTGATTTACTTTTGTAGCTTTGTTGATGGTTTATCAATCTTTTTTATT *****	60
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	GGTGGTGGGCCCGGTAGTAGTTGAACCTTTTATCCTCCTTTGAGAGTTGAGGGTCAGCCG GGTGGTGGGCCCGGTAGTAGTTGAACCTTTTATCCTCCTTTGATAGTTGAGGGTCATCCG GGTGGTGGGCCCGGTAGTAGTTGAACCTTTTATCCTCCTTTGAGAGTTGAGGGTCAGCCG *****	120
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	GAAGTTCTTTGGATGTTATAATTTGGGGTTGCACACTGTTGGAATTGGATCTTTGCTT GAAGTTCTTTGGATGTTATAATTTGGGGTTGCACACTGTTGGAATTGGATCTTTGCTT GAAGTTCTTTGGATGTTATAATTTGGGGTTGCACACTGTTGGAATTGGATCTTTGCTT *****	180
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	GGTGTATTAATTTTATAGTTACTACTCAAATATGCGTTCGTGCTGTTACTTTAGAT GGTGTATTAATTTTATAGTTACTACTCAAATATGCGTTCGTGCTGTTACTTTAGAT GGTGTATTAATTTTATAGTTACTACTCAAATATGCGTTCGTGCTGTTACTTTAGAT *****	240
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	CAGGCTAGTATGTTTGTGGACTTCTTATTTAACTTCTTTTTTGTAGTGTGCTGTT CAGGCTAGTATGTTTGTGGACTTCTTATTTAACTTCTTTTTTGTAGTGTGCTGTT CAGGCTAGTATGTTTGTGGACTTCTTATTTAACTTCTTTTTTGTAGTGTGCTGTT *****	300
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	CCTGTGTTAGCTGGTCTTTGTTGTTTTATTATTTGATCGTAATTTAACTTCTTTT CCTGTGTTAGCTGGTCTTTGTTGTTTTATTATTTGATCGTAATTTAACTTCTTTT CCTGTGTTAGCTGGTCTTTGTTGTTTTATTATTTGATCGTAATTTAACTTCTTTT *****	360
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	TATGATACTAAAAATGGAGGGAATCCGTTGCTGTATCAACATTTGATTTGGTTTTTGGT TATGATACTAAAAATGGAGGGAATCCGTTGCTGTATCAACATTTGATTTGGTTTTTGGT TATGATACTAAAAATGGAGGGAATCCGTTGCTGTATCAACATTTGATTTGGTTTTTGGT *****	420
Filarial larvae Microfilariae <i>B. pahangi</i> COXI	CACCTGAAGTTTATGTTATTATTTACCTGTTTTCCGGTATTATTAGT CACCTGAAGTTTATGTTATTATTTACCTGTTTTCCGGTATTATTAGT CATCCTGAAGTTTATGTTATTATTTACCTGTTTTCCGGTATTATTAGT ** *****	468 468 468

Figure 2. Multiple sequence alignment of fragments from PCR on filarial larvae and mf in infected cat blood, and *B. pahangi* COXI (GenBank Accession Number AJ271611). Asterisks* indicate nucleotide identity among the sequences

Tan *et al.* (2011) reported several cases of zoonotic *B. pahangi* lymphatic filariasis in a suburbia of Kuala Lumpur City, Malaysia. Microfilaria-positive domestic cats were the source of the zoonotic infections in the suburbia. The vector implicated was *Ar. subalbatus* as only adult females of this mosquito species were infected with *B. pahangi* larvae. Our findings in the present study further underline the role of *Ar. subalbatus* and cat in the transmission of zoonotic *B. pahangi* in Malaysia.

Armigeres subalbatus is widely distributed in Malaysia. Previously, this species has never been considered a medically important mosquito in Malaysia as compared to other mosquitoes such as *Culex*, *Aedes*, *Mansonia* and *Anopheles* spp. *Armigeres subalbatus* has been observed in the laboratory to be an efficient vector for *B. pahangi*. It can rapidly produce high number of *B. pahangi* L₃ larvae after a bloodmeal on infected cat (Edeson *et al.*, 1960a). Thus, with a capacity to naturally harbour high number of *B. pahangi* larvae, and being a human biter which thrives in areas of human habitations, *Ar. subalbatus* should now be considered a medically important mosquito species in Malaysia.

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