Case Report

Zoonotic Brugia pahangi filariasis in a suburbia of Kuala Lumpur City, Malaysia

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A B S T R A C T

Five local Malaysian patients with clinical manifestations consistent with lymphatic filariasis were referred to our medical centre between 2003 and 2006. Although no microfilariae (mf) were detected in their nocturnal blood samples, all were diagnosed to have lymphatic filariasis on the basis of clinical findings and positive serology results. PCR on their blood samples revealed that two of the patients were infected with Brugia pahangi, an animal filarial worm hitherto not known to cause human disease in the natural environment. All the patients were successfully treated with anti-filarial drugs: four patients were treated with a combination of diethylcarbamazine (DEC) and albendazole, and one with doxycycline. Four of them were residents of Petaling Jaya, a residential suburbia located 10 km southwest of Kuala Lumpur city, Malaysia. The fifth patient was a frequent visitor of the suburbia. This suburbia has no history or record of B. malayi infection. The most likely vector of the worm was Armigeres subalbatus as extensive entomological surveys within the suburbia revealed only adult females of this mosquito species were infected with B. pahangi larvae. Wild monkeys caught in the suburbia were free from B. pahangi mf, but domestic cats were mf positive. This suggests that infected cats might be the source of the zoonotic infection in the suburbia.

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1. Introduction

Lymphatic filariasis is a disease caused by the filarial worms Wuchereria bancrofti, Brugia malayi and B. timori. It is estimated that more than 120 million people worldwide are infected with one of these worms. More than 90% of the infections are due to W. bancrofti, the remainders are mostly due to B. malayi. An estimated 40 million infected individuals are seriously incapacitated and disfigured by the disease [1].

Brugia pahangi, a closely related species of B. malayi, is a lymphatic filarial worm of mammals, particularly of cats, dogs and wild carnivores [2]. This parasite is not known to cause human disease in the natural environment although there was a report on the presence of its mf in human blood samples [3]. Experimental B. pahangi infection on human volunteers produced signs and symptoms of lymphatic filariasis, but only one volunteer was found to have mf in the blood [4].

Between 2003 and 2006, five local Malaysian patients with clinical manifestations consistent with lymphatic filariasis were referred to our medical centre. All were diagnosed to have filariasis based on clinical findings and serology results. Further investigation using PCR revealed that two of the patients were infected with B. pahangi. In this communication, we present the history, diagnosis and treatment of the cases. We also present brief results of entomological and animal surveys which were conducted in the vicinity where the cases originated.

2. Case studies

The five patients (P1–P5) were referred to us between October 2003 and September 2006 (Table 1). All patients except P4 lived near a hill in Petaling Jaya (3°5.82′N, 101°39.52′E), a residential suburbia located 10 km southwest of Kuala Lumpur, the capital city of Malaysia. The hill and its surrounding are a popular place for recreational activities. However, there is no history or record of Brugia malayi infection in this suburbia. Although P4 did not reside in this residential area, he frequently visited the area for regular jogs. P1 and P2 had together climbed Mount Kinabalu in Sabah (on Borneo Island) years before the illness. P3 had history of travel to India as well as other Southeast Asian countries due to job requirement. P4 and P5 did not report any history of travel in the past.

All five patients were referred to us by various private physicians after having been treated for unusual and unresolved lower limb cellulitis. The first four patients presented with descending lymphangitis and cellulitis of the lower limb, whereas P5 presented with recurrent cellulitis of his right foot without a history of descending lymphangitis. Apart from P3 and P5, who presented relatively early
following the acute onset of their first episode of descending lymphangitis and cellulitis respectively, the other patients were referred to us after recurrent episodes of symptoms. Fever was a transient symptom associated with the acute presentation of lymphangitis or cellulitis in all the patients (Table 1).

The first four patients were treated with diethylcarbamazine (DEC, single dose of 50 mg on day 1, 50 mg ×3/day on day 2, 100 mg ×3/day on day 3, 150 mg ×3/daily on days 4–14) and albendazole (single dose of 400 mg) with complete resolution of lymphangitis and cellulitis. P5 was treated with an 8-week course of doxycycline (100 mg ×2/daily for 8 weeks). His symptoms also resolved completely. No recurrence of symptoms was recorded.

All the patients were seropositive in Brugia Rapid test [5] at the time of diagnosis, but became seronegative following treatment. All of them were negative for mf on nocturnal peripheral blood smear. None of them had eosinophilia on full blood picture. Family members of the patients (including their housemaids, all of whom were migrant workers) also had their blood screened for mf and Brugia Rapid test at the time of diagnosis. None of them were positive for any of these tests.

PCR was only available in our Parasitology Diagnostic Laboratory in 2006, hence the first four patients did not have PCR test done on their fresh blood samples at the time of diagnosis. For each blood sample, 100 μl was used for DNA extraction (QIAamp DNA Blood Mini Kit, QIAGEN). Primer pairs specific for the cytochrome oxidase I (COXI) gene of B. pahangi (forward primer 5′ TATGTCCTGTATGC 3′; reverse primer 5′ TGATATGTC GTAC 3′; expected size of PCR product, 633 bp) and B. malayi (forward primer 5′ GGACCAGGAATGT 3′; reverse primer 5′ TATACATGTGCACTC 3′; expected size of PCR product, 517 bp) were used in the PCR. The reaction was carried out in a 25 μl mixture containing 10 mM Tris–HCl (pH 8.3), 2 mM MgCl₂, 50 mM KCl, 0.01% gelatin, 200 mM of each deoxyribo)nucleoside triphosphate, 20 pmol of each primer, and 1 U of Taq polymerase (Fermentas Life Sciences, Canada). The PCR mixture was pre-heated at 95 °C for 10 min for initial denaturation before 30 cycles of amplification, which consisted of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, and elongation at 72 °C for 2 min. Final extension of the reaction was carried out at 72 °C for 10 min.

Patient P5 was the first found to be PCR positive for B. pahangi COXI when the test was performed on his fresh nocturnal blood sample. Following this, the previously stored blood samples were retrieved for PCR testing, but only patient P4 was found to be positive for B. pahangi COXI (Fig. 1). The nucleotide sequence of the PCR product (sequence data deposited into GenBank, accession no. EF406112) showed 99% similarity with that of a B. pahangi COXI sequence (GenBank accession no. AJ271611) reported previously by Casiraghi et al. [6].

A repeat screening using nocturnal blood mf examination, Brugia Rapid test and PCR was carried out in October 2006 on the first three patients and their family members including their maids, but all were found to be negative. Repeat screening tests were not carried out for P4 and his family members as they were not living in the suburb.

Our survey of mosquitoes within a 2 km radius of the suburb revealed that mosquitoes of the genera Armigeres, Aedes and Culex were present. Mosquitoes of Mansonia spp., the principal vectors of B. malayi in Peninsular Malaysia, were not found. Only adult female mosquitoes of the species Armigeres subalbatus were infected with filarial larvae. Of the 801 adult females A. subalbatus collected and dissected, 54 (7%) harbored larvae. PCR on the larvae DNA (Fig. 1), and subsequent DNA sequencing of the PCR product showed that the COXI nucleotide sequence of the B. pahangi larvae ([GenBank accession no. EF534735] was identical to that of the COXI of B. pahangi in patient P4, thus, suggesting that Ar. subalbatus was most likely the vector of the parasite.

In order to determine the reservoir animal host of the B. pahangi, blood samples were taken from wild monkeys and domestic cats in the suburb for microscopy examination and PCR. None of the 15 monkeys caught were positive for B. pahangi mf or COXI. Of the 12 domestic cat blood samples, 5 were positive for B. pahangi mf based on their morphology and length [7]. These mf-positive blood samples were also positive for B. pahangi COXI but negative for B. malayi COXI. The sequence of the PCR product was found to be identical to that of patient 4 and of the larvae in the Ar. subalbatus mosquitoes. These findings thus suggest that infected domestic cats might be the source of the zoonotic infection in the suburb.

### 3. Discussion

B. pahangi has not been known to cause human disease in natural environment. To the best of our knowledge, our report is the first clinical description of lymphatic filariasis caused by natural infection with B. pahangi. However, with the limited number of positive cases over a span of three years, it was suspected that the transmission rate was probably low. This is not surprising as a history of prolonged stay or exposure to an endemic area is usually required for the acquisition of infection. This is true in all the five patients in our report. Four of them were residents of the suburb of more than 10 years and one of them, although a non-resident, did have a long history of frequent visits to that area for recreational activity.

The clinical presentations of our patients were no different from classical brugian filariasis. All except one presented with typical

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**Table 1**

Epidemiological background and clinical presentations of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Onset</th>
<th>Clinical presentations</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>54</td>
<td>F</td>
<td>Indian</td>
<td>Jul 2003</td>
<td>Recurrent descending lymphangitis and cellulitis of lower right limb</td>
</tr>
<tr>
<td>P2</td>
<td>46</td>
<td>F</td>
<td>Indian</td>
<td>Jan 2003</td>
<td>Recurrent descending lymphangitis and cellulitis of lower right limb</td>
</tr>
<tr>
<td>P3</td>
<td>43</td>
<td>M</td>
<td>Indian</td>
<td>Sep 2003</td>
<td>Acute descending lymphangitis and cellulitis of lower left limb</td>
</tr>
<tr>
<td>P4</td>
<td>53</td>
<td>M</td>
<td>Chinese</td>
<td>Nov 2004</td>
<td>Recurrent descending lymphangitis and cellulitis of lower left limb</td>
</tr>
</tbody>
</table>

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![Fig. 1. Agarose gel electrophoresis of PCR products for Brugia pahangi and B. malayi cytochrome oxidase I (COXI) gene. Lane 1, DNA molecular mass standards (Fermentas, Lithuania); lane 3, amplicon (633 bp) from PCR on blood of patient P4 using primer pair specific for B. pahangi COXI; lane 5, amplicon (633 bp) from PCR on filarial larva recovered from an adult female Armigeres subalbatus mosquito using primer pair specific for B. pahangi COXI; no amplicon was detected from PCR on blood of patient P4 (lane 2) and filarial larva (lane 4) using primer pair specific for B. malayi COXI.](image-url)
descending lymphangitis involving the lower limb. However, painful or swollen inguinal nodes were not seen in our patients. The cellulitis found in all the patients, is a common sequelae of lymphatic filariasis [8]. The mild lymphoedema in our patients suggests that lymphatic destruction might be minimal, and the acquisition of the parasite occurred probably not too long ago.

Treatment response seemed to be satisfactory with the conventional approach for the first four patients. All of them tolerated relatively well with the conventional DEC followed by a dose of albendazole [9]. Patient P5 was treated with the novel approach using doxycycline, a drug known to deplete Wolbachia, the endosymbiont of adult filarial worms [10,11].

In the experimental infection study of Edeson et al. [4], the two human volunteers who were inoculated with infective B. pahangi larvae experienced signs and symptoms of clinical lymphatic filariasis. However, only one of them developed mf and the density was very low. It was postulated that the absence or low count of mf might be due to the inability of most of the larvae to develop fully into sexually-mature worms. Similarly, this might be the reason for the amicrolaemia in our patients. Laboratory diagnosis, therefore, should not rely solely on microscopic examination for mf in the blood sample. The diagnosis of our patients was aided by the use of the serology kit Brugia Rapid. However, Brugia Rapid is only a test for brugian filariasis, it is not able to distinguish the different species of Brugia. PCR was useful in helping us to establish the final diagnosis.

In conclusion, our study has provided evidence of two confirmed (PCR positive) and three probable cases of human B. pahangi infection. These findings thus raise concern of a potential zoonotic B. pahangi filariasis in urban and suburban areas of Malaysia. Further studies need to be carried out to determine the extent of this disease in Malaysia.

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References