**INTRODUCTION**

Human filariasis is caused by members of the Filaridae family, including species of *Dirofilaria, Brugia, Wuchereria, Onchocerca, Dipetalonema, Loa*, and *Meningonema*; it is transmitted to humans by various kinds of insect vectors [1]. Dirofilaria is typically a disease of animals, which can also be easily transmitted to humans by mosquitoes of the genera *Anopheles, Culex*, and *Aedes* [2]. All of these mosquitoes are found in Vietnam. Of the 30 different species of *Dirofilaria*, *D. repens* and *D. immitis* are the 2 most common species that frequently infect humans [3]. Other *Dirofilaria* species have also been reported to infect Vietnamese carnivore species [4].

There have been over 1,000 cases of dirofilariasis, reported throughout the world, including 300 cases involving the lungs or viscera and over 800 cases involving the subcutaneous tissues or eyes [1]. Most of these were caused by *D. immitis* or *D. repens*. *D. immitis* is a parasite of dogs and cats and it can occasionally become a causative agent of lung and subcutaneous dirofilariasis in humans. *D. repens* can also infect humans and is associated with diseases of various organs, including the conjunctiva, lungs, soft tissues (including the breast), brain, liver, intestine, lymphatic glands, and muscles [5,6].

In some cases, identification of *Dirofilaria* spp. based only on the morphology is difficult. Therefor, the use of molecular methods, such as PCR, is necessary for effective species identification [7]. Nuclear and mitochondrial genes are useful for the identification of helminth species, and especially the latter genes have been frequently used for identification of *Dirofilaria* spp. [8-10].

Given that there has been an increasing number of patients suffering from *D. repens* infection, further research is required on this newly emerging zoonotic disease as a public health threat in Vietnam. Accurate diagnosis, proper identification, and control measures are therefore needed to control human dirofilariasis in Vietnam.

**CASE RECORD**

During 2006 to 2010, a total of 9 patients with a swelling...
mass under their conjunctiva admitted to the National Eye Hospital (NEH), and a patient with a swelling in the subcutaneous tissue admitted to the Military Hospital 108. By surgery, live parasites were collected from these patients and species identification was tried. The total 10 patients, 27-77 years old, were from 4 provinces in the North Vietnam, including Hanoi City (4 patients), Ninh Binh province (2 patients), Ha Nam province (2 patients), and Hung Yen province (1 patient) (Table 1). Nine of them had similar symptoms, such as a painful, itchy, swollen, and tangible nodule in the eye; 6 cases involved the right eye and 3 involved the left. Another patient, 36-year-old, had a tumor (3 × 4 cm) in the left subcutaneous tissue, which appeared as a red nodule and itchy. Surgical biopsies were performed on all patients and living parasites were recovered from each patient.

Parasites measured 4.0-12.5 cm in length and 0.5–0.6 mm in width. The worms were identified by the morphology, and pictures were taken (Fig. 1). Among the worms, 3 were chosen (2 from the conjunctiva and 1 from the subcutaneous tissue), marked as GCA-VN1, GCH-VN2, and GCD-VN3, respectively, and analyzed by molecular methods.

**Table 1. Information of worms collected from patients**

| Serial no. cases | Sex  | Age (years) | Province | Parasitic place | Worm length (cm) |
|------------------|------|-------------|----------|-----------------|-----------------|
| 1                | Female | 50         | Hanoi   | Right conjunctiva | 4.0             |
| 2                | Male   | 47         | Hanoi   | Left conjunctiva  | 8.0             |
| 3                | Female | 27         | Hanoi   | Left conjunctiva  | 10.0            |
| 4                | Male   | 49         | Hanoi   | Right conjunctiva | 5.0             |
| 5                | Male   | 77         | Ninh Binh | Right conjunctiva | 11.0            |
| 6                | Female | 60         | Ninh Binh | Right conjunctiva | 15.0            |
| 7                | Female | 55         | Ninh Binh | Right conjunctiva | 11.0            |
| 8                | Female | 50         | Hung Yen | Right conjunctiva | 10.0            |
| 9                | Male   | 50         | Ha Nam  | Left conjunctiva  | 12.5            |
| 10               | Male   | 36         | Ha Nam  | Left subcutaneous side | 12.0 |

**Fig. 1.** *Dirofilaria repens* collected from the conjunctiva (A, B) and subcutaneous tissue (C, D) of humans in Vietnam. (A) A female worm from the conjunctiva of a patient. (B) Another specimen from another patient. (C) Anterior end of a worm showing the mouth and esophagus (×100). (D) Posterior part of a female worm extracted from the subcutaneous tissue of a patient (×40).

**Table 2. Sequencing of the portion cox1 of different filarial species from GenBank compared with *Dirofilaria repens* in Vietnam**

| Notation | Origin | Host | Length | Species | GenBank | Author |
|----------|--------|------|--------|---------|---------|--------|
| GCA-VN1  | Vietnam | Human | 461 bp | *Dirofilaria repens* | - | De, Le, and Chai* |
| GCH-VN2  | Vietnam | Human | 461 bp | *Dirofilaria repens* | - | De, Le, and Chai* |
| GCD-VN3  | Vietnam | Human | 461 bp | *Dirofilaria repens* | - | De, Le, and Chai* |
| Drep (ITA1) | Italy | - | 461 bp | *Dirofilaria repens* | AJ271614 | [8] |
| Drep (ITA2) | Italy | - | 461 bp | *Dirofilaria repens* | DG358814 | Serini et al. (GenBank) |
| Dimm     | Australia | Dog | 461 bp | *Dirofilaria immitis* | AJ537512 | [14] |
| Bmal     | GenBank | - | 461 bp | *Brugia malayi* | AF538716 | [15] |
| Ovol     | GenBank | - | 461 bp | *Onchocerca volvulus* | AF015193 | [16] |

*aResults of the present study.*
Fig. 2. Comparison of 461 nucleotide (A) and 153 amino acids (B) of portion cox1 mitochondrial genome between Vietnamese Dirofilaria and other species of the family Filaridae, including the Italian Dirofilaria repens (Drep [ITA1] and Drep [ITA2]), Australian D. immitis (Dimm), Brugia malayi (Bmal), and Onchocerca volvulus (Ovol). Note differences between the Vietnamese Dirofilaria (GCA-VN1; GCH-VN2; GCD-VN3) and other species showed by sign nucleotide (or amino acid) of them; mark (.) is similar each other in nucleotide (or amino acid).

5’GGTCTTGGTGAACGTCTATTCTTATC3’ and UCO1r2: 5’CC-
AACCATAAACATATGATGAGCCCA3’.

PCR products purified using a QIAquick Purification Kit (Qiagen) were subjected to direct sequencing using the BigDye Terminator Cycle Sequencing technology on an automated sequencer, ABI 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Sequences were then edited using SeqEdv1.03, aligned using Assembly LIGNv1.9c, and analyzed using the MacVector 8.2 package (Accelrys Inc., San Diego, California, USA). Sequences were searched against the GenBank database, using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), and approximately 500
Table 3. Percentage identity of nucleotide (above diagonal) and amino acid homology (below diagonal) of cox1 sequences of Vietnamese Dirofilaria repens and other Filaridae in GenBank.

|          | GCA-VN1 | GCH-VN2 | GCD-VN3 | Drep (ITA1) | Drep (ITA2) | Dimm | Bmal | Ovol |
|----------|---------|---------|---------|-------------|-------------|------|------|------|
| GCA-VN1  | 100     | 100     | 95      | 95          | 89          | 87   | 91   |      |
| GCH-VN2  | 100     | 100     | 95      | 95          | 89          | 87   | 91   |      |
| GCD-VN3  | 100     | 100     | 95      | 95          | 89          | 87   | 91   |      |
| Drep (ITA1) | 100 | 100  | 100    | 99          | 90          | 87   | 92   |      |
| Drep (ITA2) | 99  | 99   | 99     | 99          | 90          | 87   | 92   |      |
| Dimm     | 96      | 96      | 96      | 96          | 89          |      |      |      |
| Bmal     | 94      | 94      | 94      | 94          | 92          |      |      | 86   |
| Ovol     | 98      | 98      | 98      | 98          | 97          | 96   | 95   |      |

The phylogenetic analysis results are shown in Fig. 3. The phylogenetic tree based on a portion of cox1 sequence of Dirofilaria isolates and nematode strains, including 3 collected in Vietnam. Topology was constructed by MEGA 4.1 using the neighbor-joining method [17]. Dirofilaria repens from this study and 2 from Italy are shown by the vertical bar. The length of the cox1 sequence is indicated in brackets. Bootstrap values (%) are indicated in numerals from 1,000 replicates. GCA-VN1, GCH-VN2, and GCD-VN3 = Vietnamese Dirofilaria; Drep (ITA1) and Drep (ITA2) = Italian Dirofilaria repens (GenBank no. AJ271614 and DQ358814); Dimm: Dirofilaria immitis (GenBank no. AJ537512); Bmal: Brugia malayi (GenBank no. AF538716); Ovol: Onchocerca volvulus (GenBank no. AF015193).

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DISCUSSION

In this study, 10 Dirofilaria worm specimens from humans, including 9 from the conjunctiva and 1 from the subcutaneous tissue, were identified by the morphology and molecular methods as D. repens. In Vietnam, the filarial worm of this species was first reported from a human conjunctiva in 2008 [12], and another was reported from the human subcutaneous tissue in 2010 [13]. This is the 3rd report of human D. repens infection in Vietnam which involved the conjunctiva or subcutaneous tissue.

This species is parasitic in dogs, cats, and wild animals [1], and together with D. immitis it can cause complicated epidemiology in zoonotic diseases. Dirofilaria is transmitted to humans by mosquitoes, including Anopheles, Culex, and Aedes [2], and these mosquitoes are common in Vietnam. Feeding dogs and cats are very common in the whole country. Thus, a high risk for human dirofilariosis is existing everywhere in Vietnam.
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