Case Report: An Eyelid Nodule Caused by Candidatus Dirofilaria hongkongensis Diagnosed by Mitochondrial 12S rRNA Sequence

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Abstract. A 59-year-old female living in Rayong Province, eastern Thailand, presented with painless, right upper eyelid nodule for 3 months. Upon removal of the eyelid mass, a well-circumscribed, firm globular mass with diameter about 1 cm was found. Histopathological examination revealed an immature female dirofiilar worm reminiscent of Dirofilaria repens, characterized by prominent sharp longitudinal ridges at external surface of the cuticle. Analysis of the mitochondrial 12S rRNA sequence showed that the worm belongs to Candidatus Dirofilaria hongkongensis. It is likely that some infections previously reported as D. repens based on histological examination may have actually been due to Candidatus D. hongkongensis.

INTRODUCTION

Filarialis is an important vector-borne nematode infection in both humans and several domestic and wild mammals. Zoonotic filariasis is caused by animal filarial nematodes with wide geographic distribution, especially in areas where reservoir hosts and their vectors are abundant.1 Of these, Dirofilaria immitis is an important agent of human pulmonary dirofilariasis while it is responsible for cardiopulmonary infection in canine hosts. Meanwhile, subcutaneous dirofilariasis in humans are mainly caused by Dirofilaria repens while other species within the genus Dirofilaria have been sporadically reported such as Dirofilaria ursi, Dirofilaria striata, and Dirofilaria tenuis whose natural hosts are bears, felines, and raccoons, respectively.2 In most circumstances, diagnosis of zoonotic filariasis has been relied on characteristic features of these worms in tissue samples. However, species identification can be complicated by their stages of development, the presence of some overlapping characteristics and intraspecific variations across the length of the worms such as their longitudinal cuticular ridges.1,3 Meanwhile, molecular diagnosis of zoonotic dirofilariasis has led to the identification of cryptic species; one of which is Candidatus Dirofilaria hongkongensis that has been incriminated in human infections in Hong Kong.4–5 This novel species has been subsequently identified in travelers returning to Austria and Germany from India including indigenous cases in India and China.6–10 Although sporadic cases of pulmonary and subcutaneous dirofilariasis in humans have been reported in Thailand, it is likely that the number of cases may be underrepresented due to some undiagnosed and unreported cases.11–14 Herein, we described a Thai patient presenting with an eyelid mass in which histopathological examination was consistent with D. repens infection whereas the mitochondrial 12S rRNA sequence has confirmed it to be Candidatus D. hongkongensis.

CASE REPORT

A 59-year-old Thai female presented with a slowly progressive, painless right upper eyelid mass for 3 months. She noticed that after feeling like an insect bite at the right eyelid, it started swelling a few days later without any pain or redness. She sought medical diagnosis and treatment in a hospital near her home in Rayong Province, 180 km southeast of Bangkok. Orbital computerized tomography scan was performed, which revealed an enlarged right lacrimal gland (Figure 1A). With a suspicion of lacrimal gland infection, she received intravenous broad-spectrum antibiotics for 7 days. After treatment, a slight reduction in eyelid swelling was observed but an eyelid mass became palpable. Because of the persistence of eyelid mass, she was then referred to our hospital. The patient had no associated symptoms and no underlying illness. She had never been traveling abroad prior to her ailment. Her family kept a dog as a pet for almost a decade without apparent serious illness.

Ocular examination showed an eyelid mass, neither movable nor tender, at the lateral aspect of right upper eyelid (Figure 1B). The anterior eye segment was quiet with normal fundus and full ocular motility. No lymph node enlargement was found. Complete blood count showed hemoglobin 14.1 g/dL, neutrophils 44.6%, lymphocytes 44.5%, monocytes 8.7%, eosinophils 1.9%, and basophils 0.3%. Provisional diagnosis included lymphoproliferative disorders because there was no sign of inflammation. An incisional biopsy was attempted, but it was discovered during the operative procedure that the lesion was a well-defined, firm nodule with diameter about 1 × 1 cm that was fixed to the underlying tarsus. Therefore, the entire mass was removed (Figure 1C).

The hematoxylin-eosin–stained eyelid tissue revealed granulomatous formations in which each granula contains characteristic transverse or tangential feature of a nematode surrounded by lymphocytes, histiocytes, plasma cells, neutrophils, eosinophils, and multinucleated giant cells (Figure 2). The nematode had a relatively thick multilayered cuticle with lateral internal cuticular ridges. The lateral cords were inconspicuous. The worm exhibited variation of dimensions with the largest diameter of approximately 330–340 μm at the level containing well-defined reproductive organ,
suggesting that the worm could have tapered ends. The hypodermis was laid with multilayer, well-developed coelomary muscle cells. The external surface of cuticle possessed prominent sharp longitudinal ridges. The distance between the ridges was slightly wider than the width of the ridges. The total number of the ridges was around 100. The intestine was found at the central region of the body cavity along with paired uteri filled with unsegmented eggs. With this histopathological study, an initial diagnosis of subcutaneous dirofilariasis caused by *D. repens* was made based on characteristic external cuticular ridges and the high prevalence of this worm species known to be incriminated in subcutaneous infections in humans.1–3

To specifically determine the species of *Dirofilaria* in our patient, a portion of the tissue sample was subject to DNA extraction, followed by amplification of the mitochondrial 12S rRNA gene by polymerase chain reaction (PCR) using primers *Diro12SF0* (5'-TATAATATTAGTTTGATTTTGGTTTT-3') and *Diro12SR0* (5'-ATTGACGGATGGTTTGTACCAC-3'), which were derived from interspecies conserved regions of the gene (GenBank accession nos. AJ537512, KX265049, and KX265050). The thermal cycling profile included the preamplification denaturation at 94°C for 1 minute followed by 35 cycles of 96°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

Direct sequencing of the purified PCR products spanning 575 bp, excluding PCR primers, has shown a completely identical sequence with the previously reported sequence of Candidatus *D. hongkongensis* from the eyelid of a German patient returning from India (GenBank accession nos. KX265050) while it shared 99.3% sequence identity with isolates from dogs in Thailand (KX265092 and KX265093).6 The neighbor-joining phylogenetic tree inferred from the sequence obtained from the worm in our patient in comparison with other species of *Dirofilaria* has supported that the eyelid mass was caused by the newly proposed Candidatus *D. hongkongensis* (Figure 3). Meanwhile, no microfilaremia was found in this patient based on examination of a venous blood sample by using the Knott’s technique. Likewise, a negative result was obtained from the PCR analysis of DNA extracted from the patient’s blood sample using the dirofilarial 12S rRNA gene as a target. The nucleotide sequence from the patient reported herein has been available in the GenBank database with the accession no. MZ810545.

**DISCUSSION**

The genus *Dirofilaria* comprises two subgenera including *Dirofilaria* and *Nochtiella*. Subgenus *Dirofilaria* contains filarial worms devoid of cuticular ornamentation and the adult.
worms are defined as large filariae and confined to cardio-pulmonary system of the definitive hosts with *Dirofilaria immitis* Leidy, 1856 as type. On the other hand, subgenus *Nochtiella*, with *D. Nochtiella repens* Railleit and Henry, 1911 as type, includes relatively small filarial worms with longitudinal ridges on the outer surface of the cuticle and primarily infects subcutaneous and other tissues of the mammalian hosts. Canine populations serve as the main and reservoir host of both *D. immitis* and *D. repens* while various species of culicine mosquitoes are vectors. A survey of canine population in Hong Kong where the first human cases caused by *Candidatus D. hongkongensis* have been reported has

![Figure 2](image-url)  
(A) Section through a subcutaneous nodule removed from the right upper eyelid showing multiple transverse sections and a tangential section of a worm. The diameter of the nodule measures approximately 1 cm consisting of numerous inflammatory cells. Scale represents 100 μm. (B) Transverse section through an immature female worm of *Candidatus D. hongkongensis* showing multilayer cuticle with longitudinal ridges on the outer surface (LR), well-developed muscular layer (ML), lateral internal ridges (IR), a pair of uterine tubes filled with unsegmented eggs (UT), and an intestine (IN). The worm is surrounded by multinucleated giant cells forming a granulomatous reaction and intense inflammatory infiltrate including lymphocytes, plasma cells, histiocytes, neutrophils, and eosinophils. Scale represents 50 μm. This figure appears in color at www.ajtmh.org.

![Figure 3](image-url)  
**Figure 3.** Neighbor-joining phylogenetic tree inferred from the mitochondrial 12S rRNA sequences of filarial worm from the patient (circle) in comparison with other *Dirofilaria* species with their geographic origins and GenBank accession numbers. Bootstrap values more than 50% based on 1,000 replicates are shown along the branches. Scale represents nucleotide substitutions per site.
revealed about 3% of infection in stray dogs.\textsuperscript{,4} Furthermore, \textit{Candidatus} \textit{D. hongkongensis} has been identified in domestic and wild canine populations in South India.\textsuperscript{,8,16,17} Likewise, \textit{Dirofilaria} sp. closely related with \textit{Candidatus} \textit{D. hongkongensis} has been isolated from dogs in Thailand.\textsuperscript{6} Although the source of infection in our patient remains unknown, it is likely that domestic transmission could have occurred from canine reservoirs.

Clinical presentations of \textit{Candidatus} \textit{D. hongkongensis} infections include cervical lymphadenopathy, subcutaneous mass in various locations and subconjunctival lesion.\textsuperscript{,2,7,10} In our patient, the preceding lacrimal gland enlargement followed by the occurrence and persistence of an eyelid mass could suggest the route of migration of the worm with subsequent localization. In histopathological tissue samples, definite species identification of \textit{Dirofilaria} could be based on their characteristic cuticular ornamentations, characterized by conspicuous and sharp ridges in \textit{D. repens}, low and rounded with wavy pattern of external ridges in \textit{D. tenuis}, and tall, sharp, and narrow ridges in \textit{D. ursi}.\textsuperscript{3} Nevertheless, the shape, height, and distance between the ridges could be variable depending on the level of the worm body.\textsuperscript{1} Meanwhile, the cross-sectional features of the worm from our patient seem to be reminiscent of \textit{D. repens}. Therefore, the cuticular ridges of \textit{Candidatus} \textit{D. hongkongensis} and \textit{D. repens} seem not to be a discriminatory feature.

During the initial stage of infection, early nodule contains an abscess mainly composed of polymorphonuclear cells and eosinophils surrounding the dirofilarial worm.\textsuperscript{15} The presence of granulomatous reaction observed in the eyelid tissue of our patient was corroborated with a chronic stage of infection. In most circumstances, only a single worm has been observed in the lesion whilst the female worms harboring eggs or microfilarial larvae have been reported, suggesting that multiple infections with both male and female worms capable of mating and producing progenies could occur in some infected human cases.\textsuperscript{2,4} Meanwhile, the presence of unsegmented eggs in the uteri of the worm in our patient along with a solitary lesion could suggest a single worm infection.

The mitochondrial 12S rRNA sequence of the worm in our patient is perfectly identical with that of \textit{Candidatus} \textit{D. hongkongensis} from the left eyelid of a traveler to India.\textsuperscript{6} Although several gene members in the mitochondrial genome display microheterogeneity of sequences among different species of dirofilarial worms, some loci such as the internal transcribed spacer 1 and the 5.8S rRNA gene may not elicit phylogenetic differences between \textit{Candidatus} \textit{D. hongkongensis} and \textit{D. repens}.\textsuperscript{18,19} The mitochondrial 12S rRNA sequence spanning 575 bp deployed herein seems to be a useful genetic marker for the diagnosis of \textit{Candidatus} \textit{D. hongkongensis}. Meanwhile, the 12S rRNA locus displays differential sequence identity when compared with its closely related strain and other species in which 0.7%, 3.13%, and 11.13% of nucleotide differences have been observed in comparison with those of \textit{Candidatus} \textit{Dirofilaria} sp. "Thailand II," \textit{D. repens}, and \textit{D. immitis}, respectively.\textsuperscript{6,18,19} The occurrence of \textit{Candidatus} \textit{D. hongkongensis} reported herein has extended the geographic distribution of this putative novel species of zoonotic filariasis while molecular diagnosis seems to be mandatory.

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