A Novel *Dirofilaria* Species Causing Human and Canine Infections in Hong Kong


Research Centre of Infection and Immunology, State Key Laboratory for Emerging Infectious Diseases, and Department of Microbiology, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China; Department of Pathology, Queen Mary Hospital, Pokfulam, Hong Kong Special Administrative Region, China; Division of Otorhinolaryngology—Head and Neck Surgery, Department of Surgery, Queen Mary Hospital, Pokfulam, Hong Kong Special Administrative Region, China; Department of Medicine, Queen Elizabeth Hospital, Kowloon, Hong Kong Special Administrative Region, China; Pedder Clinic, Hong Kong Special Administrative Region, China; and Department of Pathology, St. Teresa’s Hospital, Hong Kong Special Administrative Region, China

Dirofilaria is globally the commonest manifestation of zoonotic filariasis. We report the detection of a novel canine species causing human and canine dirofilariasis in Hong Kong. Three human cases occurring over 10 months were identified, one presenting with cervical lymphadenopathy, one with an abdominal subcutaneous mass, and one with a conjunctival nodule. Transected worms recovered from the resected abdominal subcutaneous mass were morphologically compatible with *Dirofilaria*. The *cox1* gene sequences of the three human isolates were identical; however, they were only 96.2% and 89.3% identical to the *cox1* gene of *Dirofilaria repens* and *Dirofilaria immitis*, respectively. Sequencing of the 18S-ITS1-5.8S gene cluster was successful in the intact worm, and the nucleotide sequences were 94.0% and 94.9% identical to those of *D. repens* and *D. immitis*, respectively. Screening of the blood samples from 200 dogs and 100 cats showed the presence of the novel *Dirofilaria* species in 3% (6/200) of the dogs’ but none of the cats’ blood samples. Nucleotide sequences of the *cox1* gene and 18S-ITS1-5.8S gene clusters of the dogs’ samples were identical to those in the human samples. The sera of canines infected by this novel *Dirofilaria* species were negative when tested with the SNAP 4Dx *D. immitis* detection kit, except in the case of dogs with a mixed infection with *D. immitis* as detected by PCR. The results from this study suggest that this novel *Dirofilaria* species is a cause of filarial infections in humans and dogs in Hong Kong. We propose to name this *Dirofilaria* species “*Candidatus* *Dirofilaria hongkongensis*.”

There are eight known filarial nematodes for which humans are the natural definitive hosts. The human diseases caused by these filariae can be classified anatomically into three groups: lymphatic filariasis, subcutaneous filariasis, and serous cavity filariasis. *Dirofilaria*, a zoonotic filarial nematode with humans as accidental hosts, is most commonly associated with subcutaneous and ocular filariasis and is increasingly reported to cause human disease worldwide (2, 10, 28). The commonest *Dirofilaria* species causing human infections are *D. repens* and *D. immitis*, which are canine pathogens (27). Human *D. repens* infections are reported mainly in Europe and Asia, while human *D. immitis* infections are more prevalent in North America (6, 10). Other noncanine-associated species that occasionally cause human infections include *D. tenuis* (from raccoons), *D. ursi* (from bears), *D. subderrnata* (from porcupines), and *D. striata* (from bobcats) (4, 6, 27, 33).

The diagnosis of human dirofilariasis relies mainly on morphological features of the worm (27). *Dirofilaria* is characterized by a relatively large size, thick cuticle, and prominent musculature with muscle cells extending far into the body cavity (8). Different *Dirofilaria* species can be distinguished by their size, the thickness of the cuticle, and the presence or absence of longitudinal ridges. However, these features can sometimes be difficult to discern, especially in tissue sections (25). Definitive diagnosis can also be made by molecular methods in both human and animal infections (14, 34).

Recently, we have encountered three cases of human dirofilariasis. We have employed molecular techniques to identify the species. All three isolates had identical nucleotide sequences in the *cox1* (cytochrome oxidase subunit 1) gene and the ITS1-5.8S gene cluster, and the nucleotide sequences differ from other reported *Dirofilaria* sequences. In order to understand the potential zoonotic reservoir of this novel species, we have also screened asymptomatic stray dogs and cats for the presence of this novel *Dirofilaria* species.

**MATERIALS AND METHODS**

**Patients.** The patients were identified when their clinical specimens were sent to the Department of Microbiology or the Department of Pathology of Queen Mary Hospital. The specimens were either intact worms or tissue sections showing parasites compatible with *Dirofilaria*. This study has been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

**Specimens.** Patients’ clinical specimens were handled according to standard protocols (3, 32). Blood was collected from 200 asymptomatic stray dogs in the summer from 2009 to 2011 and from 100 asymptomatic stray cats in the summer of 2009 and 2010. These animals were euthanized by the Agriculture Fisheries and Conservation Department of Hong Kong as part of the routine policy for disposal.

Received 15 June 2012. Returned for modification 14 July 2012 Accepted 14 August 2012 Published ahead of print 22 August 2012 Address correspondence to Kwok-Yung Yuen, kyyuen@hku.hk. K.K.W.T. and S.S.Y.W. contributed equally to this article. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.01590-12
TABLE 1 Sequences of primers used in the study

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Target gene</th>
<th>Primer name and sequence</th>
<th>Target length (bp)</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filarioidea</td>
<td>cox1 (cytochrome oxidase subunit 1)</td>
<td>FIIL_COX1F, 5′-GCTTTRTGGTGCTTTTGGKTTACTTTT-3′&lt;br&gt;FIIL_COX1R, 5′-TAGTRTCAAAAGAGAATTTAAA-3′</td>
<td>321</td>
<td>Consensus primer designed by multiple alignment of the cox1 gene sequences of Filarioidea (available in GenBank)</td>
</tr>
<tr>
<td>D. immitis</td>
<td>18S-ITS1-5.8S</td>
<td>DS_ITSF, 5′-AACGCGATATTCGTTGGGTGT-3′&lt;br&gt;DS_ITSR, 5′-GCTAAGATTTAATATTGTTT-3′</td>
<td>376</td>
<td>Consensus primer designed by multiple alignment of the 18S-ITS1-5.8S gene sequences of D. immitis and D. repens (available in GenBank)</td>
</tr>
<tr>
<td>“Candidatus Dirofilaria hongkongensis”</td>
<td>ITS1-5.8S-ITS2</td>
<td>DI_ITSF, 5′-ATAGATGATTGCTCAATTAAGTAGAC-3′&lt;br&gt;DI_ITSR, 5′-GATAAGCTGATGGATTACGCCCT-3′</td>
<td>290</td>
<td>29</td>
</tr>
<tr>
<td>Wolbachia endosymbiont of D. immitis</td>
<td>16S rRNA</td>
<td>Wol_ITSF, 5′-AACCGCGAGCTTAATACGGTATA-3′&lt;br&gt;Wol_ITSR, 5′-ACGGTAGCCCTCTGTAATTA-3′</td>
<td>350</td>
<td>Multiple alignment of the 16S rRNA gene sequences of Wolbachia endosymbiont of D. immitis, D. repens, and D. ursi (available in GenBank)</td>
</tr>
</tbody>
</table>

Nucleic acid extraction. Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue (FFPE) and the worm using a DNA minikit (Qiagen, Hilden, Germany), and total nucleic acid was extracted from peripheral EDTA whole-blood sample using the EZ1 mini-fixed paraffin-embedded tissue (FFPE) and the worm using a DNA extraction kit (High Pure PCR Template Preparation Kit, Roche, Mannheim, Germany) as described previously (34).

PCR and DNA sequencing. Primer sequences used in this study are listed in Table 1. The specific primers used for the detection of the novel *Dirofilaria* species were designed by multiple alignments with CLUSTALW as specified in Table 1. The PCR mixture (25 μl) contained DNA, PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 3 mM MgCl2, and 0.01% gelatin), 200 μM each deoxynucleoside triphosphates (dNTPs), and 1.0 U Taq polymerase (Applied Biosystems, Foster City, CA). The PCR products were gel purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany). Both strands of the PCR products were sequenced twice with an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA), using the PCR primers. The sequences of the PCR products were compared with known sequences by BLAST analysis against the NCBI database. For the detection of Filarioidea species, the nucleotide sequences of *D. immitis* and *D. repens* were used as positive controls. The 16S rRNA gene sequences of *Filarioidea* (available in GenBank) were used as the outgroup for *D. immitis*. The sequences of the PCR products were compared with known sequences by BLAST analysis against the NCBI database. For case 3, PCR using the above-described protocol was repeated on the genomic DNA extracted from a previous study (T. H. Tang, S. Y. Wong, A. C. Cheng, R. W. Poon, and T. C. Wu, submitted for publication).

To evaluate the performance of the PCR for the detection of novel *Dirofilaria* species, plasmid was used as a positive control. It was prepared by cloning the cox1 gene and the 18S-ITS1-5.8S gene cluster into the plasmid vector pPCR®II-Topo (Topo TA cloning kit, dual promoter; Invitrogen). Each PCR run comprised two plasmid suspensions, 10 ng and 1 ng copies of extracted DNA per reaction, and a negative control (miniQ water). The detection limit was estimated at 10 copies of extracted DNA per reaction, and a negative control (miniQ water). The detection limit was estimated at 10 copies of extracted DNA per reaction, and a negative control (miniQ water). No cross-reactions were found between the *D. immitis* and *D. repens* (available in GenBank).

**RESULTS**

Case 1. A 50-year-old man presented in November 2011 with a 2-week history of a painful mass on the left side of the neck. He lived and worked on Hong Kong Island. His past health was unremarkable. One month before noticing the neck mass, the patient had an urticaria-like rash over upper and lower limbs, which subsided spontaneously. On physical examination, he was afebrile.

**Immunochromatographic assay.** Blood samples collected from the asymptomatic stray dogs were tested for antigen of *D. immitis* using SNAP 4Dx (IDEXX Laboratories, Westbrook, ME) as we previously described (34).

**Nucleotide sequence accession numbers.** Partial nucleotide sequences of the cox1 gene and the 18S-ITS1-5.8S and ITS1-5.8S gene clusters obtained in this study have been deposited within the GenBank sequence database under accession numbers JX187591 to JX187599 and JX290194 to JX290202, respectively.
nematode measured 260 μm by 930 μm. Longitudinal ridges were present on the surface. The multilayered cuticle measured 4 to 16 μm in thickness. There was a prominent inner muscle layer. An intestinal tract was present in the body cavity (isolate HKU1). The size and morphology are compatible with an adult worm. Three midnight blood samples were collected because of the initial suspicion of lymphatic filariasis, but microfilariae were not seen by microscopy or detected by PCR. Stool examination was negative for parasites. He was given one dose of ivermectin (900 mg) and a 6-week course of doxycycline, 100 mg once daily. At the time of writing, the patient remained well.

Case 2. In March 2012, a 37-year-old man presented with a 2-week history of right lower abdominal subcutaneous mass. His past health was unremarkable. He had traveled to India in February 2012 and to Thailand in December 2011. He did not have any close animal contacts at home or abroad. Ultrasound of the abdomen showed a 1.2- by 0.8- by 1.2-cm well-circumscribed complex cyst with multiple mobile echogenic foci along the right anterior abdominal wall (Fig. 2A). The mass lay along the deep subcutaneous fat layer with smooth margins. Complete excision of the mass was performed, during which nematodes compatible with *Dirofilaria* adult worms were found in the lesion (Fig. 2B to E) (isolate HKU2). A biopsy specimen of the subcutaneous lesion showed fibrofatty tissue with sparse lymphocytic infiltrate only. There was no increase in eosinophils (Fig. 2F). Three transected filarial parasites measuring 0.4 cm in width and 2.0 to 3.6 cm in length were recovered. The surface of the adult worms had an annular structure macroscopically (Fig. 2B and C). Numerous pear-shaped eggs were detected on the transected end of the adult worms when immersed in saline (Fig. 2D). The size of eggs was 32.5 to 37.5 μm by 15 to 20 μm. Microscopically, the worm measured about 350 μm by 460 μm in cross section. There were 99 longitudinal ridges that were present on the surface of the cuticle and were low and smoothly rounded. The multilayer cuticle measured 5 to 14 μm in thickness. There was prominent musculature, with lateral chords with four chord nuclei and internal ridges. An intestinal tract and a pair of uteri were evident in the body cavity. Taken together, this adult worm is compatible with a female *Dirofilaria*.

Case 3. The patient in case 3 has been described in a previous case report (T. H. Tang, S. Y. Wong, A. C. Cheng, R. W. Poon, and T. C. Wu, submitted for publication). Briefly, she was a 61-year-old woman who had a 2-week history of a rapidly growing left subconjunctival nodule after a mosquito bite below the left lower eyelid in May 2011. The excised nodule revealed a nematode associated with activated lymphoid cells, plasma cells, and eosinophils. The nematode has a thick cuticle with lateral internal ridges and prominent musculature (isolate HKU3). The patient was retired and had been living in the northern part of the New Territories of Hong Kong for over 20 years. She kept pet dogs at home.

**Molecular studies.** Isolates HKU1, HKU2, and HKU3 were PCR positive for the *cox1* gene specific for Filarioidea. The gene sequences of *cox1* genes from HKU1, HKU2, and HKU3 were identical. The sequence of the *cox1* gene of HKU1, HKU2, and HKU3 showed 96.2% nucleotide identity to that of *D. repens* and 89.3% identity to that of *D. immitis* (GenBank accession no. JF461458.1, AJ271614.1, DQ358814.1, AM749230.1 to AM749234.1, NC005305.1, DQ358815.1, AJ271613.1, and AM749226 to AM749229.1) (Fig. 3A). The sequence of the 18S-ITS1-5.8S gene cluster of HKU2 was 94.0% and 94.9% identical to those of *D. repens* and *D. immitis*, respectively (GenBank accession no. AF217800.1, AM621480.1, AM621481.1, AM621479.1, and AF217800.2) (Fig. 3B). Sequencing of the 18S-ITS1-5.8S gene.

![FIG 1](http://jcm.asm.org) Photomicrograph of excisional biopsy specimen of cervical lymph node from case 1. The nematode was surrounded by a granulomatous reaction with abundant eosinophils (hematoxylin and eosin staining; original magnification, ×100; scale bar = 100 μm).
cluster was unsuccessful for the specimens from cases 1 and 3, possibly due to the disruption of DNA in formalin-fixed specimens. Therefore, we designed a primer pair (DH_ITSF and DH_ITSR) that targeted the ITS1-5.8S gene cluster and produced a shorter gene product so that we could assess the nucleotide sequence in this region (Table 1). With this new primer pair, we were able to amplify the ITS1-5.8S gene cluster from HKU1, HKU2, and HKU3. Sequencing showed that the three isolates had identical ITS1-5.8S gene sequences. The sequences from these isolates were 97.4% and 98.0% identical to those of *D. repens* and *D. immitis*, respectively (GenBank accession no. AY621479.1 to AY621481.1 and AF217800.2) (Fig. 3C).

**PCR for Wolbachia 16S rRNA** was positive for the worm from case 2 but negative for the tissue sections from cases 1 and 3. The three midnight EDTA blood samples from case 1 were PCR negative for the *cox1* gene of Filarioidea and 16S rRNA gene of Wolbachia. Blood samples were not available from cases 2 and 3 for PCR tests.

Blood samples from 200 asymptomatic stray dogs and 100 asymptomatic stray cats were screened with PCR targeting the *cox1* gene. Six canine blood samples were positive (isolates HKU4, HKU5, HKU6, HKU7, HKU8, and HKU9). Sequencing of the *cox1* gene and ITS1-5.8S gene cluster showed that all six canine isolates were identical to the human isolates (Fig. 3A and C). The 18S-ITS1-5.8S gene cluster sequences from all the canine isolates were identical to the human isolate HKU2 (Fig. 3B). Among the six positive canine blood samples, two were also positive for *D. immitis* by PCR. All feline blood samples were negative for this novel *Dirofilaria* species by PCR.

**Antigen detection of *Dirofilaria* using SNAP 4Dx in canine blood.** Antigen testing for *D. immitis* was performed on canine plasma samples that showed a positive PCR result for *Dirofilaria*. The two samples found to be coinfected with *D. immitis* by PCR were positive for *D. immitis* antigen, while the four samples without *D. immitis* coinfection by PCR were negative for *D. immitis* antigen.

In view of the morphological, genetic, and antigenic differences of this novel *Dirofilaria* species with other known species, we propose to name it "*Candidatus Dirofilaria hongkongensis*.”

**DISCUSSION**

Conventional histological diagnosis of human dirofilariasis and identification of the causative organism to the genus level are usually possible. However, this requires considerable expertise, which may not be readily available in areas of nonendemicity. The typical morphology of the parasite in sections can be distorted in dead and degenerating parasites, by the host inflammatory response, or when the sections are cut at different levels of the parasite (21,27). Identification to the species level using histology is even more difficult because the microscopic features of some species have not been described in detail and the appearance of some features could be variable even within the same species. Even if an intact worm is removed from the patient, its morphological characteristics can vary with the stage of maturity, and the typical features of a particular species may not be apparent (8). The presence of the parasites in unusual locations such as lymph nodes may potentially cause confusion with other lymphatic filariases. In these difficult situations, molecular techniques are highly useful for making a
FIG 3  Phylogenetic tree showing the relationships of the patients’ isolates (HKU1, HKU2, HKU3) to the canine isolates (HKU4, HKU5, HKU6, HKU7, HKU8, HKU9) and related filaria species, inferred from cox1, 368 nucleotide positions (A); from 18S-ITS1-5.8S gene cluster, 446 nucleotide positions (B); and from ITS1-5.8S gene cluster, 262 nucleotide positions (C). The tree was constructed by the neighbor-joining method using Kimura’s two-parameter correction and bootstrap values calculated from 1,000 trees. *Ascaris lumbricoides* (GU326949.1) was used as the outgroup for panel A, and *Ascaris lumbricoides* (H721821.1) was used as the outgroup for panels B and C.
specific diagnosis of different filariases. In this report, we have used gene sequencing to assess one lymphatic, one subcutaneous, and one subconjunctival human infection due to “Ca. Dirofilaria hongkongensis” that were diagnosed over a period of 10 months in three patients who resided in different geographical areas in Hong Kong. This novel species is also morphologically distinct from other Dirofilaria species. Compared to D. repens, the histological section of the adult worm from case 1 showed less distinct longitudinal ridges (8). Longitudinal ridges seen in these three cases are not present in D. immitis or D. striata (8, 21), and the relatively long cross-sectional diameter and short surface ridges are not suggestive of D. ursi or D. subdermata. The cuticle of D. tenuis generally has a beaded appearance, which is not found in our cases. From case 2, it is apparent that the L3 larvae can mature into the adult stage and mate in humans, as eggs were detected on the transected end of the worms.

The partial nucleotide sequences of the cox1 gene and 18S-ITS1-5.8S gene cluster of the human and canine isolates were used in this study to delineate the species status of our Dirofilaria isolates. We chose the cox1 gene for genetic characterization of the Dirofilaria isolates in our study because this gene can reliably differentiate between different species of filariae, and it has also been used for the identification of Dirofilaria in previous studies (7, 9, 12, 20, 22, 30). The reported mean nucleotide distance of the cox1 gene within species ranges from 0% to 2% (9), and there is less than 1% difference between available D. repens sequences in the GenBank. Therefore, since our isolates have a 4% nucleotide difference from the reported D. repens sequence and an 11% difference from the D. immitis sequence, they belong to a separate species. The 18S-ITS1-5.8S gene cluster has also been employed to differentiate between filariae at the genus level (18, 19). A novel species, Acanthocheilonema ladakhii, was described based on the 18S ribosomal DNA (rDNA) gene sequence (15). In contrast to the cox1 gene, sequencing of the 18S-ITS1-5.8S gene cluster showed that our isolates have slightly higher nucleotide identity with D. immitis (94.9%) than with D. repens (94.0%). There have been no universally agreed criteria for defining new species among nematodes by using sequence similarity (1). For 16S rRNA sequencing for bacteria, many authorities recommend that the same species should have at least 97% nucleotide identity (36). Hence, based on the currently available GenBank sequences, we propose that these local Dirofilaria isolates, which were recovered from three different patients, belong to a new species, tentatively named “Ca. Dirofilaria hongkongensis.” We have further demonstrated that the antigen of “Ca. Dirofilaria hongkongensis” could not be detected by SNAP 4Dx in canine blood samples, which suggested that this novel species has different antigenic properties from those of D. immitis.

Wolbachia is an endosymbiont of many filariae, including Dirofilaria (11). Our previous study showed that D. immitis can be found in 10% of stray dogs in Hong Kong (34). All infected dogs also had detectable Wolbachia DNA in the blood. In this study, Wolbachia was detected in the intact worm from case 2 only. Wolbachia PCR was negative for the histological sections from cases 1 and 3, but this is likely related to the destruction of DNA due to formalin.

Dogs are considered to be the main reservoir for human D. repens and D. immitis infections because adult worms can survive in dogs for many years, resulting in high levels of microfilaraemia, which allows efficient transmission to mosquito vectors. Epidemic-
ological studies suggest that human dirofilariasis occurs in areas with high rates of canine infection (10, 13). Cats may also be infected by *D. repens* or *D. immitis*. However, cats are rarely considered to be a reservoir for human infection since microfilaremia is rare in cats (10). However, it is possible that the cats in this study may have been suffering from dirofilariasis without microfilaremia. In this study, we have shown that the nucleotide sequences of the *cox1* gene and 18S-ITS1-5.8S gene cluster of the three isolates from our patients and the six isolates detected in stray dogs in Hong Kong were identical, suggesting that these human and canine isolates belonged to the same species. The pathogenicity and pathology of “*Ca*. Dirofilaria hongkongensis” in dogs or other animal hosts is unknown. If this species is proved to be pathogenic in animals, new antigen detection tests or molecular techniques will be required for veterinary purposes. To the best of our knowledge, this is the first study that uses gene sequencing to demonstrate the relationship between the canine reservoir of *Dirofilaria* and human infection in the same geographical area at the molecular level. All *Dirofilaria* spp. known to date require an arthropod vector for transmission; it is likely that the life cycle of “*Ca*. Dirofilaria hongkongensis” also involves such vectors. However, we do not yet know the vector in transmitting this new *Dirofilaria* species. The absence of microfilaraemia in one of our cases (case 1) is also in line with the current knowledge that humans are accidental hosts in the life cycle of dirofilariae.

In humans, *D. repens* is typically associated with subcutaneous nodules and eye nodules, and rarely lymphadenopathy (23, 26). In Hong Kong, two cases of breast lesion due to *D. repens* based on morphological features have been reported (17). On the other hand, *D. immitis* is usually associated with pulmonary nodules (16). In our study, the patients presented with cervical lymphadenopathy (case 1), subcutaneous nodule (case 2), and subconjunctival nodule (case 3). With gene sequencing, accurate identification of *Dirofilaria* species is now possible, and the clinical spectrum of “*Ca*. Dirofilaria hongkongensis” will be clearer when additional cases are identified.

Management of *Dirofilaria* infection is straightforward for subcutaneous nodules, since surgical removal of the worm is usually sufficient. In the vast majority of cases, only a single parasite is present and systemic antiparasitic treatment is not necessary (24). However, for more invasive disease such as meningonephritis and pulmonary involvement, there are currently no standard recommendations for drug treatment. Both ivermectin (31) and albendazole (5) have been used. For cases 2 and 3 in the present report, resection of the nodules alone was curative. For case 1, antiparasitic treatment with ivermectin and doxycycline was given to the patient because it was uncertain if other cervical lymph nodes were involved.

There are several limitations to our study. First, intact worms were not identified in case 1 or case 3, and therefore, comparison of the morphological features of intact worms was not possible. The adult worms recovered from case 2 were also incomplete, and therefore, detailed morphological description was not possible. Second, since only one patient had blood samples examined for microfilaraemia, we cannot be certain whether microfilaraemia exists in humans. Testing on more human samples would be necessary. Third, comparison of HKU1 and HKU3 with other isolates was possible only by using a separate primer pair to amplify a shorter nucleotide sequence of the ITS1-5.8S gene cluster. This is presumably due to the effect of formalin on the specimens. Fourth, since the nucleotide sequences of the noncanine *Dirofilaria* species are not available at the moment, more detailed comparison with these rarer species is not possible. Fifth, another explanation for the negative antigen test in canine blood samples positive for “*Ca*. Dirofilaria hongkongensis” may be related to a low level of antigenemia below the threshold for detection. Further studies would be needed to further characterize the antigenic property of this novel *Dirofilaria* species. Finally, since the canine blood specimens in this study were originally used for the testing of *D. immitis* antigenemia by enzyme immunoassay for the comparison with PCR, routine blood smears for microfilariae were not performed (34). Because of the subsequent discovery of this novel *Dirofilaria* species in human specimens, we retrieved the archived blood samples to restet for *Dirofilaria* DNA using the specific PCR test.

Zoonotic filariasis is increasingly recognized in different countries (35). Climate change may contribute to the changing epidemiology and increasing incidence of the disease (10). As in the case of other vector-borne infections, accurate knowledge of the natural reservoir and vectors is crucial for control and prevention of the disease. With the rapidly decreasing cost of next-generation sequencing, future identification of novel species of parasites may be best achieved with complete genome coverage by sequencing, and more accurate identification of filariae to the species level and comparison between human and animal strains can be made. This will not only aid the clinical laboratories in diagnosing these infrequently encountered infections but also highlight the need to develop new veterinary diagnostic tests for locally important pathogens. Additional studies are needed to verify the status of this novel species and support the proposed nomenclature.

ACKNOWLEDGMENTS

We thank Alan Chi-Kong Wong, Siu-Fai Leung, Chik-Chuen Lay, Thomas Sit, K. F. Chan, Michelle L. Yeung, Byung Mo Hwang, Suet Yee Ng, Patrick I. T. Lau, and Steven D. Benton from the HKSAR Department of Agriculture, Fisheries, and Conservation (AFCD) for facilitation and support and members of the Animal Management Centres of AFCD. We are grateful for the generous support of Carol Yu, Richard Yu, Hui Hoy, and Hui Ming in the genomic sequencing platform on emerging infectious disease research.

This work is partly supported by the Tung Wah Group of Hospitals Fund for Research in Infectious Diseases, the HKSAR Research Fund for the Control of Infectious Diseases of the Health, Welfare and Food Bureau, the Providence Foundation Limited in memory of the late Lui Hac Minh, and Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Disease for the HKSAR Department of Health.

REFERENCES