CASE REPORT: UNUSUAL LOCATION OF *DIROFILARIA IMMITIS* IN A 28-YEAR-OLD MAN NECESSITATES ORCHIECTOMY

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Abstract. The fourth case of zoonotic *Dirofilaria immitis* infection in a California resident is reported. This case was unique in that it involved the spermatic cord, produced significant symptoms, and upon physical examination was suspected of being an incarcerated inguinal hernia. At surgery frozen sections were inconclusive regarding neoplasia and cord involvement precluded removal of the mass without orchiectomy, which was done. Histopathology revealed a well-preserved immature male *D. immitis*, the canine heartworm, in a branch of the spermatic artery. The morphological identification of the worm was confirmed by polymerase chain reaction (PCR) studies. This case illustrates that *D. immitis* infections can complicate differential diagnosis of extrapulmonary lesions and suggests that the public health significance of this vector-borne filarial worm needs to be evaluated through appropriate epidemiological studies in enzootic areas.

INTRODUCTION

The first three presumed autochthonous cases of canine heartworm, *Dirofilaria immitis* infections in human California residents were found in men, 67, 50, and 63 years of age.1–3 They were residents of Placer, Riverside, and Shasta Counties, respectively. The patients in the first two cases were symptomatic with cough and chest pain, while the patient in the third case was asymptomatic. All 3 had nodular pulmonary lesions demonstrated radiographically and were suspected of having either metastatic or primary lung neoplasia. Following extensive diagnostic efforts to determine the cause of the lesions, without any positive results, a thoracotomy was performed on each patient, with removal of the cause of the lesions, without any positive results, a thoracotomy was performed on each patient, with removal of the nodule for histopathological examination. The granuloma was unique in that it involved the spermatic cord, just inferior to the external inguinal ring. The swelling measured 5.2 × 3.1 × 2.9 cm. Cut sections of the mass revealed a granular, pale tan surface that appeared necrotic.

Gross pathological examination of the surgical specimen showed a fusiform, firm, pale tan swelling within the cord beginning 2 cm from the surgical margin. The swelling measured 3.1 m. Elsewhere, the diameter of the worm varied from 242.5 m to 396.7 m. Cut sections of the mass revealed a granular, pale tan surface that appeared necrotic.

Parasite identification. Histopathological examination of the fusiform mass showed extensive areas of necrotizing granulomatous inflammation. Large numbers of eosinophils were in portions of the necrotic tissue. Necrotizing vasculitis, involving both venous and arterial segments was also present. Longitudinal and transverse sections of a helminth could be seen within arterial channels in some of the levels of the sectioned mass. Some sections of the helminth were surrounded by a cellular reaction inside the vessel, while others were free in the lumen of the artery (Figures 1 and 2). There was a single worm whose cuticle was smooth on the external surface, and contained transverse striations and prominent internal longitudinal ridges at the level of the lateral cords. The cuticle measured 37.8 μm at the level of the lateral cords and the diameter of the worm at this level was 308.7 μm. Elsewhere, the diameter of the worm varied from 242.5 μm to 396.7 μm. The muscle bundles were of the coelomyarian type and filled the pseudocoelomic space. The muscle mass was divided into a dorsal and ventral component by prominent lateral cords. There was a single large reproductive tube which contained degenerating epithelial cells, but no spermatozoa, and a much smaller digestive tube located nearby. Some of the worm sections showed the reproductive tube to be completely empty. Based upon these morphological characteristics the worm was identified as an immature, male *Dirofilaria immitis*, the canine heartworm.4–6

Polymerase chain reaction confirmation. Using the out-
line of the positive histological section as a guide, the preserved, unsectioned portion of the spermatic cord that had harbored the positive sections was identified, and an examination of the wet tissue under a dissecting microscope permitted identification of the infected artery. Microdissection of the artery revealed a 1 cm piece of the intact worm free in the lumen of the vessel. This segment was removed and subjected to polymerase chain reaction (PCR) analysis using specific primers for *D. immitis* cuticular protein.7,8

Briefly, the worm sections were washed at room temperature in separate containers for 3 days using a glycine buffer, which was changed every 24 hr.9 The DNA from the worm was extracted using the QIamp tissue extraction kit according to the manufacturer’s protocol (Qiagen Inc., Valencia,
Figure 3. Polymerase chain reaction with *Dirofilaria immitis* primers. A. Scoles and Kambhampi, 1995; B. Watts and Courtney, 1999. **Lane 2**: DNA extracted from the worm sample. **Lane 3**: extraction blank. **Lane 5**: positive PCR control (DNA extracted from *D. immitis* microfilariae in the blood of a known infected dog). **Lane 6**: negative PCR control (water added, no DNA). **Lanes 7–10**: empty. **Lanes 1 and 4**: molecular ladder marker (100 bp Gibco, Life Technologies, Rockville, MD).
for 2 months was achieved. It has been reported that such D. immitis sequences. In this case, extraction and amplification were conducted in an “Easy Cycler” thermal cycler (EriComp, Inc., San Diego, CA). The reaction volume was 30 µl, containing 1.5 units of Taq polymerase (Perkin Elmer, Norwalk, CT) and 2 µl of DNA template. The reaction was covered with 40 µl of mineral oil. A positive amplification control consisting of a DNA sample extracted from D. immitis microfilariae recovered from a dog, and a negative control consisting of all the reaction reagents but no D. immitis DNA template were processed with the worm samples and the extraction blank.

The amplification products were electrophoresed on 2% agarose mini-gels. The gels were stained with SYBR Green I as per the manufacturer’s recommendations (FMC bioproducts, Molecular Probes, Inc., Rockland, ME) and the bands recorded by UV photography. The PCR product (Figure 3a) was sequenced (Davis Sequencing, Davis, CA) to confirm that the DNA generated by the primers coded for the D. immitis cuticular glycoprotein. The sequence corresponded to the expected fragment from D. immitis and was identical to that published by Culpepper and others10 and Poole and others.11 The second D. immitis primers reported on by Watt and Courtney8 produced the expected 438 bp fragment (Figure 3b), but we did not have this product sequenced. In this case, extraction and amplification of DNA from D. immitis tissue that had been fixed in 10% formalin for 2 months was achieved. It has been reported that such biopsy samples should not be stored in formalin.12 However, the length of time in formalin appears to influence the success in removing the inhibiting substance(s) by the glycine buffer wash. We were unable to obtain amplification from an adult D. immitis stored in 10% formalin for 2 years, even after washing the worm in the buffer.

DISCUSSION

Grass Valley, Nevada County, California, where the patient lived, is a highly enzootic area for D. immitis.13 The prevalence in dogs living outdoors, not on prophylaxis, with no travel out of their city of domicile varies between 42 and 174 per thousand in the foothill plant climate zone of the Sierra Nevada range, of which Grass Valley is a part.14 Exposure to infective mosquitoes is potentially high and two of the first three reported cases of human heartworm infection in California residents were in men who lived in Shasta and Placer Counties which contain foothill plant climate zones.13 This patient, however, had traveled through and been lost in an area of the United States that is also enzootic for heartworm.14,15 Since the stage of the worm in the patient’s tissues indicated that infection had occurred 5–6 months prior to the onset of clinical disease it is not certain that the infection was acquired in California. It may have been acquired while he was traveling. That possibility is of considerable epidemiologic importance because it is one thing to become infected in an area where one spends a lot of time, it is quite another to acquire the infection while just passing through an enzootic area. Transmission intensity in some areas may be high enough to make such transient exposure successful.

Published cases of human pulmonary dirofilariasis (HPD) indicate that this is a world-wide problem. Numerous individual cases and several reviews with new cases have been reported from the United States,1–3,16–28 Australia,29,30 Japan,31,32 and Spain.34,35 In addition, the dirofilaria worm, though misidentified initially, has been reported in the heart46 and the posterior vena cava.37 Infections involving the eye,38,39 subcutaneous tissues,40–42 abdominal cavity,43 and possibly the meninges44 have all been reported. From these cases, it should be apparent that the diagnosis of zoonotic D. immitis covers much more than just the respiratory system.

Serological studies on humans in western Spain have been conducted looking for IgG and IgM antibodies to D. immitis antigens. In enzootic areas of heartworm serologic tests in humans have demonstrated antibody in 9% of 395 surveyed.45 In the Columbian Amazon Basin, in a village where 7 of 13 dogs (54%) examined for D. immitis microfilariae were positive, serological studies on 18 Tikuna Indians showed that 9 of the Indians (50%) were positive with an enzyme-linked immunosorbent assay (ELISA) formatted test using adult somatic antigen and excretory-secretory antigens.46 In this study, Indians with and without Mansonella ozzardi infections had antibody to D. immitis.

Neither paper reported examination of the sero-positive humans for evidence of concurrent granulomas containing D. immitis. However, using a more purified 22 kiloDalton (kD) D. immitis cuticular protein fragment, the high false-positive rate seen with crude somatic antigen of D. immitis was greatly reduced.47 Furthermore, the specificity of the 22 kD based ELISA test was 90% and the sensitivity 100%.48 The positive predictive value of this test was 75%, while the negative predictive value was 100%.47 Two earlier papers,49,50 reported the successful application of serologic techniques using purified antigen from D. immitis. One of them51 used a 34 kD antigen that was immunodominant in microfilariae but not in later developmental stages of the worm. That a reliable serologic test for epidemiological investigations of D. immitis infections in humans is possible is indicated by these small studies.

Investigations in highly enzootic heartworm areas appear desirable because the public health aspect of D. immitis infections has not been explored systematically by either veterinary or human medical researchers. Such studies could serve to bring the differential diagnostic problem to the attention of physicians and emphasize the need to consider D. immitis in enzootic areas of heartworm. At the same time, such studies would clarify the need for organized control programs aimed at eliminating heartworm from the canine population.50–52

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REFERENCES


