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VERSITA

RESEARCH NOTE

Detection of *Dirofilaria immitis* in mid-western arid Argentina

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Abstract

Dirofilariosis, caused by *Dirofilaria immitis* and *D. repens*, is (re-) emerging worldwide. Dogs are the main reservoirs, while human infection has recently become an important focus of interest and attention. In Argentina, canine *D. immitis* infection has been described in eastern and northern subtropical and temperate humid regions, but never reported in mid-western arid regions so far. In this research note we report for the first time the occurrence of autochthonous human and canine *D. immitis* infection in the region.

Keywords

Dirofilaria immitis, conjunctival dirofilariosis, morphological analysis, molecular analysis, canine survey

Dirofilariosis, caused by *Dirofilaria immitis* and *D. repens*, is (re-) emerging worldwide (Genchi *et al*. 2011, Simón *et al*. 2012). Dogs are the main reservoirs, while humans are considered dead-end hosts, with 1782 cases reported all around the globe (372 and 1410 pulmonary and subcutaneous/ocular cases, respectively) (Simón *et al*. 2012). Recently, human disease has become an important focus of interest, due to a marked geographical expansion and rise of incidence, together with an increased severity, especially in subcutaneous/ocular cases (Simón *et al*. 2012).

In South America, about 50 human *Dirofilaria* infections have been described (Simón *et al*. 2012). The most frequent is pulmonary presentation due to *D. immitis* (Labarthe and Guerrero 2005, Simón *et al*. 2012), while subcutaneous and ocular presentations are scarce and attributed to *D. tenuis* or *Dirofilaria* sp. from wild mammals (Otranto and Eberhard 2011, Simón *et al*. 2012). Only five cases of human dirofilariosis (four pulmonary and one subcutaneous) have been reported in Argentina, but two of them could not be ascertained to be autochtonous (Vezzani *et al*. 2006). The four pulmonary cases were attributed to *D. immitis* based on histopathology of the excised pulmonary nodules (Caballer *et al*. 1994, Barcat *et al*. 1999, Riache *et al*. 2001), while in the subcutaneous case the *Dirofilaria* species was not defined (Abuin *et al*. 1998).

Regarding canine dirofilariosis (due to *D. immitis*) in Argentina, the infection has been described in eastern and northern subtropical and temperate humid regions (Vezzani *et al*. 2006, Rosa *et al*. 2008). National prevalence in dogs is considered to be around 3.5–5.1% (Labarthe and Guerrero 2005), varying from 0 to 74% at a local scale (Vezzani *et al*. 2006, Simón *et al*. 2012).

Despite a temperature-based predictive study that indicates that mid-western arid Argentina presents a low to moderate risk for transmission of *D. immitis* (Vezzani and Carbajo 2006), as far as we know, the infection has never been reported in the region. Only one survey carried out in Mendoza province reported that all dogs (n = 56) tested negative for *D. immitis* antigens (Albarracín and Serafín 2000).

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Human and canine case reports: morphological and molecular description

Samples of two unusual findings were analysed for identification: i) a filarial worm retrieved from the subcutaneous tissue of the anterior orbit of a middle-aged woman from a rural area of San Juan province (31°32′ S – 68°31′ W), preserved in 5% formalin, and ii) a heartworm recovered from the left pulmonary artery of a necropsied dog in the city of Mendoza (32°54′ S – 68°50′ W), preserved in 70° ethanol. After macroscopic identification, both worms were stained with Horen's trichrome stain, and paraffin-embedded transverse sections were stained with hematoxylin and eosin for microscopic analysis. Finally, a multiplex polymerase chain reaction (PCR) assay and sequencing were performed for simultaneous detection and discrimination of *D. immitis* and *D. repens* using the protocol described in Gioia *et al*. (2010).

At macroscopic examination, both filariid worms appeared with tapered ends and yellowish in color. The canine worm measured 260 mm in length and 1 mm in width, the human worm appeared broken with a length of 90 mm and a width of 0.5 mm. Microscopic examination showed pointed cephalic and posterior ends (the human worm lacked the caudal extremity), and a cuticle with smooth outer surface. The histological features of both worms were suggestive of *D. immitis*. The two helminthes had smooth cuticle lacking longitudinal ridges and a well-developed muscular layer. In the female worm the body cavity showed uterus and intestinal tube.

Regarding the molecular results, sequence analysis of both filarial worms showed a homology of 100 per cent with *D. immitis* 12S rDNA gene sequences deposited in GenBank (accession number EU169125).

Based on the size, morphological features (Furtado *et al*. 2010) and molecular analysis (Gioia *et al*. 2010), the worms were identified as a female (canine case) and a male (human case) of *D. immitis*.

Cross-sectional survey

To evaluate the prevalence of canine dirofilariosis in the city of Mendoza, between September 2011 and April 2012, 850 whole blood samples collected in EDTA (ethylene-diamine-tetra-acetic acid) were screened for microfilariae as described in Vezzani *et al*. (2011). Samples were submitted by veterinarians as part of their private practice, thus reliable epidemiological information was not available. Positive samples were processed with the modified Knott's technique and the microfilariae were identified according to morphological features (size, shape of anterior and posterior end, presence of cephalic hook) (Genchi *et al*. 2007). Confidence interval (CI α 0.95) for estimation of population prevalence and force of infection (FI = the probability of a susceptible individual becoming infected in one year) were calculated (Trotz-Williams and Trees 2003, Genchi *et al*. 2005).

Three blood samples, analyzed during summer, presented microfilariae (0.35%, 0.95 CI = 0–0.75%, FI = 0.00071). After concentration with the modified Knott's technique, microfilariae were morphologically identified as *D. immitis*. The three dogs were from the city of Mendoza and had never travelled.

Discussion

To our knowledge, and since neither the positive dogs nor the affected woman had traveled out of their place of residence, these findings represent the first evidence of dirofilariosis due to *D. immitis* in mid-western arid Argentina, an area geographically distant and ecologically different to the endemic regions previously known in the country.

In previous national reports of human dirofilariosis, species determination was assumed or achieved by morphological description, but never molecularly confirmed. This would be the first published molecular confirmation of autochthonous human dirofilariosis due to *D. immitis* in Argentina, but also, as far as we know, the first report of conjunctival *D. immitis* in South America. Five recent non-pulmonary cases in South America should be remarked: a *Dirofilaria* sp. subcutaneous infection in Chile (Pérez and Arce 2007), two subcutaneous and one intraocular infections attributed to *Dirofilaria* sp. in Peru (Beltrán *et al*. 2008), and an intraocular infection due to a species similar, but not identical, to *D. immitis*, in Brazil (Otranto *et al*. 2011).

This report confirms the risk predictions that suggest that the region is suitable for local transmission, at least during certain period of the year (Vezzani and Carbajo 2006). The concomitant presence of a minimum number of microfilaraemic dogs and about half dozen potential vector species in the region (Vezzani and Carbajo 2006), indicates the risk of a low, but stable, endemicity. Furthermore, unfamiliarity with diagnosis of heartworm disease by local veterinarians also suggests infection is underdiagnosed. Concluding, it is reasonable to presume that this is the "tip of the iceberg", with the real prevalence being completely underestimated.

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