Short communication

Two cases of subcutaneous dirofilariasis in Barcelona, Spain

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Abstract

In recent years, the number of reported cases of human dirofilariasis in Europe has increased and the circulation of *Dirofilaria* spp. in mosquitoes in several European countries has been proven. We report here two likely autochthonous cases of subcutaneous human dirofilariasis from Barcelona, Spain, caused by *Dirofilaria repens*. The potential for an increase in human infection is high given the number of cases published recently and the ability of vectors to spread through the Mediterranean basin.

Keywords: *Aedes albopictus, Culex pipiens, Dirofilaria repens,* dirofilariasis, heartworm, mosquitoes, subcutaneous nodules.

Introduction

Dirofilariasis is a mosquito-borne disease caused by an infection with the nematodes *Dirofilaria immitis* or *D. repens*. These parasites circulate naturally between mosquitoes and canids (Simón et al. 2012) but are occasionally able to infect humans, which are considered dead-end hosts (Simón et al. 2012; Otranto et al. 2013). *Dirofilaria immitis* has a worldwide distribution and causes benign pulmonary nodules (pulmonary dirofilariasis), while *D. repens* is limited to the Old World and leads to subcutaneous nodules and intraocular infections (Simón et al. 2012). The occurrence of dirofilariasis has increased in Europe in recent years, and has spread from the Mediterranean into northern and eastern European countries (Genchi et al. 2017) and human cases are now frequent in areas where dirofilariasis is endemic in dogs. This is thus evidence of the zoonotic nature of this infection (Otranto et al. 2011).

Current studies have revealed the presence of *Dirofilaria*-infected mosquitoes in most Mediterranean countries (Simón et al. 2012; Otranto et al. 2013). *Culex* mosquitoes (Culicidae), which bite mammals including dogs and humans (Martínez-de la Puente et al. 2012; 2016) probably play a central role in the transmission of *Dirofilaria* in Europe (Otranto et al. 2013). In addition, the spread of invasive mosquitoes may create novel epidemiological scenarios as in the case of the *Aedes albopictus*, which has been identified as a key vector of *Dirofilaria* in Italy (Cancrini et al. 2003).

As far as we know, to date, 10 cases of subcutaneous/ocular dirofilariasis and eight of pulmonary dirofilariasis have been reported in Spain (Simón et al. 2012; Rodríguez-Calzadilla et al. 2016; Ramírez de Ocáriz Landaberea et al. 2017). Here, we present information regarding two further cases of human subcutaneous dirofilariasis caused by *D. repens* in Barcelona, an area where the circulation of *Dirofilaria* is known to occur (Aranda et al. 1998; Montoya-Alonso et al. 2015).

Material and methods

Case reports

In March 2016, a 42-year-old woman (patient 1), resident in the Sant Gervasi district of Barcelona, was treated for a palpable swelling of the left cervical subcutaneous lymph node and a cervical edema. Two days later, the patient developed a left periorbital edema associated with itching and a foreign body sensation in the same eye. She visited an ophthalmologic clinic, where a 9-cm-long helminth was extracted from her left eye. Given suspicions of Loa-loa infection, she was referred to the International Health department of the Hospital Clinic, Barcelona. However, she had not previously visited any areas in

which Loa-loa is endemic (Zouré et al. 2011) but only the following places: Tanzania (2001), Thailand (2010), China but not Hong Kong (2010), Dominican Republic (2011), Lake Cuomo and Venice in Italy (2014), and Paris (France) and Malaysia (2015). In 2015, she also visited marshland in Catalonia (NE Spain). She had no pets or regular contact with either dogs or cats. Physical examination and blood tests showed no abnormalities, and she had $0.3x10^9$/L eosinophils. After the clinical diagnosis of dirofilariasis, she took albendazole 400 mg twice a day. After finishing the treatment, the patient noted a subcostal nodule that was extracted and submitted for analysis. A 5-cm helminthic parasite was found inside the nodule and was identified as a *Dirofilaria* sp.; histopathologic examination revealed lympho-eosinophilic inflammation. A computed tomography scan found no pulmonary nodules. Two weeks later, however, the patient noted a second tender and moderately painful nodule with 0.5-cm gross axis in her left gluteal area and was prescribed ivermectin for two days plus albendazole for two weeks (Böckle et al. 2010). The nodule was not removed due its deep position. After the course of ivermectin, the patient was found to be asymptomatic after a follow-up visit one month later.

Four months later, a 40-year-old woman (patient 2) from the same neighbourhood was referred to the International Health department of the Hospital Clinic after the detection of a helminth parasite surrounded by eosinophilic infiltrate inside a subcutaneous nodule on her left arm. The nodule appeared after a pruriginous wheal that the patient linked to an insect bite. The patient cohabited with a dog but had no contact with cats. Her only overseas journey had been to Botswana (2015). Her blood tests showed no abnormalities and she had an eosinophilic count of $0.2x10^9$/L. No further treatment was needed and the patient was found to be asymptomatic after a follow-up visit a month later.

The parasites from each patient were maintained refrigerated in saline solution (patient 1) or paraffin-embedded (patient 2) until subsequent molecular analyses (see below).

Entomological surveillance

The residences of the two patients included in this study were located in the same area of Barcelona (1.4–1.6 km apart). Mosquito surveillance was performed in the area where patient 2 normally goes walking. Mosquitoes were trapped using four Biogents (BG) Sentinel traps. Additional captures were performed using aspirators in mosquito resting places (e.g. vegetation, sculptures) in five capture sessions. Mosquitoes were identified to species level using the morphological criteria in Schaffner et al. (2001).

Molecular analyses

Genomic DNA was isolated from the parasites using the Qiagen DNeasy® Tissue and Blood Kit (Qiagen, Hilden, Germany). Prior to DNA extraction, the paraffin-embedded sample was treated with xylene for five minutes. Genomic DNA from mosquitoes was isolated using the DNA Kit Maxwell® 16LEV (Promega, Madison, WI). Molecular detection of *Dirofilaria* parasites was conducted based on the protocol in Bataille *et al.*(2012). Samples showing positive amplifications were re-amplified with primers ColintR and ColintF. PCRs were resolved in 1.5% agarose gels. Amplicons were sequenced bi-directionally according to BigDye 1.1 technology (Applied Biosystems, Carlsbad, CA, USA) using an ABI 3130xl automated sequencer. Sequences were edited using the software Sequencher™ v4.9 (Gene Codes Corp, © 1991–2009, Ann Arbor, MI, USA) and were blast-compared with those deposited in public databases (GenBank and the Barcode of Life Data Systems).

Results

The sequences obtained from the filarial worms isolated from the two patients were identified as *D. repens*. The parasite isolated from patient 1 showed a perfect match (100% identity) with a 648bp *D. repens* sequence isolated from a 30-year-old woman from Italy (Genbank accession number: KT899073; Fontanelli Sulekova et al. 2016). The sample from the patient 2 shared 99% identity with deposited sequences from *D. repens*. The two Barcelona sequences isolated were deposited in GenBank (accession numbers: patient 1: MH780816; patient 2: MH780817).

In total, 1 *Culex pipiens*, 1 *Culiseta longeriolata* and 11 *Aedes albopictus* mosquitoes were captured during the five trapping sessions. Filarial parasites were not detected in any mosquitoes. A blood sample from the second patient's dog was analysed using an antigenic test to *Dirofilaria* spp. and gave a negative result.

Discussion

Here, we report two cases of human infection by *D. repens* in Barcelona, Spain. There is strong evidence to support the local circulation of the parasite in this area, although the travel history of both patients raised suspicions and so these cases of human dirofilariasis were initially reported as imported parasitosis (Rodríguez-Calzadilla et al. 2016). However, neither of the patients had travelled to a *D.*

repens area outside Europe where dirofilariasis circulates in humans (Simón et al. 2012). Current studies, however, demonstrate a high prevalence of *Dirofilaria* spp. in pets in Europe (Montoya-Alonso et al. 2014; Fuehrer et al. 2016) and confirm parasite infection in mosquitoes (Otranto et al. 2013), which raises the possibility of autochthonous transmission. This may be especially the case of areas where parasites are commonly found infecting dogs, thereby providing evidence of their role as reservoirs of *Dirofilaria* parasites affecting humans (Otranto et al. 2011). Despite the fact that only a few human cases have ever been reported, Spain is regarded as an endemic area for *D. repens* (Simón et al. 2012).

The two simultaneous cases described here, which were reported in patients living close by, were probably autochthonous infections as suggested by the increasing number of cases reported in Europe in recent years. Nevertheless, none of the mosquitoes tested positive for the parasite, which could be due to the small number of mosquitoes that were captured. Further studies are necessary to corroborate the role that native and invasive mosquito species cohabiting in the area play in the transmission and potential spillover of *D. repens* between dogs and humans.

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