



Subconjunctival human dirofilariasis by *Dirofilaria repens* in the Mediterranean Basin

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ABSTRACT

Purpose: Human dirofilariasis cases have increased in Europe for the last few decades. We document an autochthonous case of dirofilariasis infection in the subconjunctival space of a 60-year-old Spanish man.

Observations: The patient presented with pain in his right eye, which was diagnosed as *epi-scleritis*. In spite of the treatment administered, no improvement was reported. An external exam showed conjunctival congestion on the temporal part of the right eye bulbar conjunctiva. The anterior chamber showed no flare or cells in both eyes and dilated fundus exam was normal. Nevertheless, a long whitish vermiform mobile mass was detected under the conjunctiva in the temporal part of the right eye. The worm was surgically removed and diagnosed morphologically and also molecularly as *Dirofilaria repens*. This species uses dogs and wild canids as definitive hosts and principal reservoirs, while mosquito species are transmitters. Humans act as accidental hosts, and clinical manifestations depend on the location of the worm in the organs and tissues.

Conclusions and importance: The correct diagnosis and control of subcutaneous/ocular dirofilariasis by *D. repens* require several professionals under the One Health approach to deal with this zoonotic disease, which poses a serious public health problem, at least in the Mediterranean Basin.

1. Introduction

Dirofilariasis is a zoonotic infection caused by filarioid nematodes of the genus *Dirofilaria* (Spirurida, Onchocercidae). Among the different species, *D. repens* uses dogs and wild canids as definitive hosts and principal reservoirs, and mosquito species as vectors. Humans act as accidental hosts and, in most cases, a single worm develops, without microfilaremia.¹ Herein, we report an autochthonous subconjunctival case of *D. repens* infection treated at the Ophthalmology Service of Hospital General Universitario de Castellón, Castellón, Spain.

2. Case report

A 60-year-old man from Nules (Castellón, Spain) attended the emergency unit of Hospital General Universitario de Castellón, complaining of a pain in his right eye which he had suffered for a week. Initially, the patient had consulted his general practitioner a week before because of red-eye but without discharge. He was diagnosed with *epi-scleritis*. Following treatment with dexamethasone eye drops, no improvement was reported. After the standard practice, we carried out a full exploration of the patient. Best-corrected visual acuity was 20/20 in both eyes. An external exam showed conjunctival congestion on the

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¹ Reference URL: <https://www.sciencedirect.com/science/article/pii/S2451993622003164?via%3Dihub>. Please add the ORCID number for each of the users upon clicking on their name alongside the author info. Here, Fernando Simón (ORCID: 0000-0002-8177-6083).

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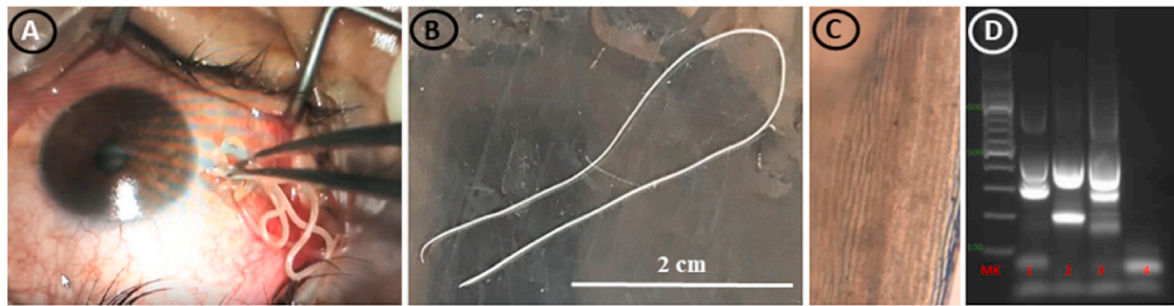


Fig. 1. (A) Worm of *Dirofilaria repens* removed from the subconjunctiva of the patient eye; (B) entire worm isolated in the present case; (C) aspect of the ridges of the cuticle under an optical microscope; (D) Multiplex PCR amplification of the DNA extracted from the worm surgically removed from the patient. Lane 1: sample obtained from the worm removed from the patient; lane 2: *D. immitis* positive control (500 pb and 204 pb); lane 3: *D. repens* positive control (500 pb and 327 pb); lane 4: negative control. MK: molecular marker, 100–1000 pb.

temporal part of right eye bulbar conjunctiva. Following slit-lamp examination, the anterior chamber showed no flare or cells in both eyes, although there was a long whitish vermiform mobile mass under the conjunctiva in the temporal part of the right eye. Dilated fundus exam was normal. Emergency surgery was performed under local anesthesia. A small conjunctival incision was made using Westcott scissors to expose the worm. The entire parasite was removed while alive (Fig. 1A). The translucent parasite was stored in physiological saline and sent to the Microbiology Unit of Hospital General Universitario de Castellón, and afterwards to the Department of Parasitology of University of Valencia for identification. Thereafter, we referred the patient to the internist for further examination to ensure there were no other worms in the rest of the body. The patient had no history of similar episodes in the past, living in the countryside with different animals. He had no history of international travel. Within one month, the excision site healed uneventfully, without recurrences.

The worm was morphologically identified as an immature female worm, thin, cylindrical, white, 0.470 mm thick and about 110 mm in length (Fig. 1B). Anterior end was rounded and of greater diameter (213 μ m) than the posterior end (163 μ m), with 105 μ m tail. Under the microscope, after clarification with Lactophenol, the outer surface of the nematode's cuticle was found with external longitudinal cuticular ridges (Fig. 1C). Some features prompted us to consider a filariform appearance, assigning it as *Dirofilaria repens*. Since other nematodes could be implicated in the parasitism of the eye, and to reaffirm the morphological diagnosis, the worm was sent to Laboratory of Parasitology of University of Salamanca in order to be molecularly processed. Crude DNA preparations were obtained from the isolated worm following the instructions of the Nucleospin® Tissue Kit (Macherey-Nagel). Then, the extracted DNA was resuspended in 15 μ l of PCR-grade water and amplified by multiplex PCR according to the protocol described by² with minor modifications. The results showed a pattern of bands compatible with *D. repens* (Fig. 1D).

3. Discussion

Dirofilaria repens causes both canine and feline subcutaneous dirofilariasis, although it is also able to cause human subcutaneous/ocular dirofilariasis. The transmission of this nematode takes place through a mosquito vector with species belonging to different genera, i.e. *Anopheles*, *Aedes*, *Culex* and *Coquillettidia*,³ which have been detected in the autonomous region of Valencia, particularly in Castellon province.⁴

This species is an endemic nematode of southern Europe (Italy, Spain, France, and Greece), and particularly of the Mediterranean Basin.^{1,5} However, *D. repens* prevalence is higher in humans than that of others species of *Dirofilaria*, possibly due to the easy detection of subcutaneous nodules and sub- and conjunctival infections.⁶ In any case, this endemic disease is gradually spreading to West-Central (Germany, Austria, Poland, and Hungary), Balkan (Serbia, Croatia, and Bulgaria),

Eastern (Slovakia, Romania, Ukraine, Rostov region, and in various territories of Russia), Baltic (Lithuania and Latvia) and the Nordic (Finland) European countries.^{3,7,8}

During *D. repens* migration, and from a clinical point of view, different body areas and tissues have been reported to be affected, with the symptoms usually being limited to edema, local irritation, erythema and pruritus, or presenting with no symptoms at all. When reaching the eye, the worm can be observed through the subconjunctiva, although it is able to migrate in the intra-, peri-, or retro-ocular space. *Dirofilaria* can cause lid and lacrimal gland swelling, uveitis, glaucoma, *epi*-scleritis, and intraocular dirofilariasis.^{1,3}

The diagnosis of this zoonotic filariasis is based on the microscopic identification of the worm in a histological section of a biopsy or the extraction of the worm from the lesion. Serological diagnosis does not usually make sufficient interspecific discrimination. Molecular techniques are a complementary tool, particularly in those cases where the host's immune response may alter the parasite morphology, or where the parasite's microhabitat may lead to specific confusion.^{9,10}

The best treatment option in this type of filariasis is the surgical removal of the worm, as pharmacological treatments are usually not necessary. Measures to control this infection are focused on dogs and humans as well as mosquitoes.

In summary, multidisciplinary collaboration among entomologists, parasitologists, veterinarians, clinicians and public health experts, under the One Health approach, is needed in order to deal with this public health issue caused by *D. repens* infection, which is relevant, above all, in the Mediterranean Basin.

Patient consent

Consent to publish the case report was not obtained. This report does not contain any personal information that could lead to the identification of the patient.

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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Ethical approval

Approval was not required.

Declaration of competing interest

The authors declare no conflict of interest.

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