

# Subconjunctival Infection With *Dirofilaria repens*

## Serological Confirmation of Cure Following Surgery

José M. Ruiz-Moreno, MD; Fernando J. Bornay-Llinares, MD, PhD; Guadalupe Prieto Maza, MSc; Magali Medrano, MD; Fernando Simón, PhD; Mark L. Eberhard, PhD

Cases of zoonotic dirofilariasis infection, caused by *Dirofilaria repens*, occur widely throughout European, African, Middle Eastern, and Asian countries. The reports of this infection in humans in Spain are limited, and we herein report the case of a 43-year-old man from Elche (Alicante), Spain, who was seen with acute hyperemic reactivity of the temporal limbus of the right eye. A large nematode was visualized on examination and the intact worm was surgically removed. The parasite was identified as a mature but infertile female *D repens*. The level of serum antibodies against *D repens* was monitored for 6 months after surgery using immunoenzymatic assays. Serological results confirmed, as expected, the presence of a single worm and the parasitological cure after the surgical removal of the parasite. To our knowledge, this is the fourth autochthonous case of *D repens* infecting humans in Spain and also the first autochthonous case of subconjunctival localization. *Arch Ophthalmol.* 1998;116:1370-1372

Zoonotic filarial infections in humans are relatively common and most of these are due to parasites in the genus *Dirofilaria*. Cases of subcutaneous dirofilariasis were originally called *Dirofilaria conjunctivae* because many of them localized in and around the eye and eyelids. In the United States it was established that *Dirofilaria tenuis*, a parasite of raccoons, was the primary agent and that in Europe, Middle Eastern countries, Africa, and Southeast Asia the parasite most often responsible was *Dirofilaria repens*.<sup>1</sup> *Dirofilaria repens* is a natural parasite of carnivores, primarily dogs, foxes, and cats.

*Dirofilaria repens* infections are particularly common and often reported from European countries surrounding the Mediterranean, particularly Italy (168 cases), France (53 cases), and Greece (21 cases).<sup>2</sup> There are 3 published reports of *D repens* infecting humans in Spain. The earliest of these cases, reported in the early 1950s, was from the eastern coastal region of Levante, and the most recent case was from the is-

land of Ibiza.<sup>2,3</sup> In addition, Spain was cited as the possible country of origin of infection in 3 additional cases diagnosed in Norway, the United Kingdom, and The Netherlands.<sup>4-6</sup>

We report here a case of subcutaneous dirofilariasis acquired in Elche (Alicante), Spain. An intact nematode was extracted from the subconjunctival tissues of the eye of a man and was identified as a female *D repens*.

### REPORT OF A CASE

In April 1997, a 43-year-old male resident of Elche (Alicante, Spain) came to the Instituto de Oftalmológico de Alicante for ophthalmic evaluation. He had a complaint of swelling and redness of the right cheek and lower eyelid, which began 1 week before. The day before admission, the patient developed an intense hyperemic tumefaction in the outer part of the right eye. The patient had lived in a rural area for the past 40 years. He had a pet dog at home and had never traveled out of Spain.

Results of the ophthalmologic examination were as follows. Best-corrected visual acuity was 20/20 OD and 20/20 OS. In the right eye, an avascular swelling and

From the División de Oftalmología y IOA (Dr Ruiz-Moreno) and División de Microbiología (Dr Bornay-Llinares), Universidad Miguel Hernández, Alicante, Spain; Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain (Ms Maza and Dr Simón); Centro de Salud Santa Faz, Alicante (Dr Medrano); and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Ga (Dr Eberhard).

marked conjunctival hyperemia were noted (**Figure 1, A**). Slitlamp examination showed a pale yellow, highly active worm in the temporal limbus in the subconjunctiva. Ophthalmoscopy disclosed a normal ocular fundus. The left eye was normal.

Using local anesthesia, the conjunctiva was incised over the site where the worm was located. The worm was surgically removed and preserved in 10% formaldehyde for identification.

The results of blood and biochemical analytical tests were normal (including eosinophils,  $0.005 \times 10^9/L$ ); no microfilaremia was detected in blood samples. Serum samples were obtained at the time of surgery and then 1, 3, and 6 months later. The detection of IgG anti-*D repens* antibodies was performed as described previously by solid-phase enzyme-linked immunosorbent assay<sup>7</sup> and enzyme-linked immunoblot analysis<sup>8</sup> using *D repens* somatic antigens extracted from adult worms obtained from naturally infected dogs according to published methods.<sup>7</sup>

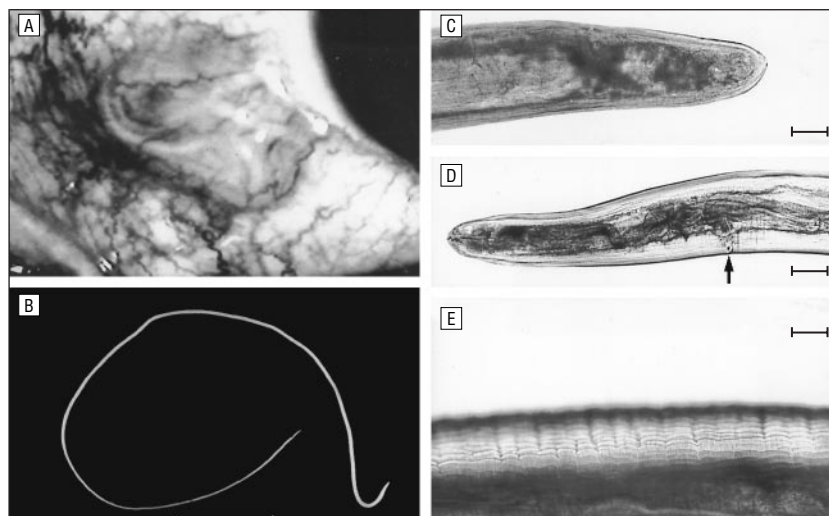
## PARASITOLOGIC FINDINGS

The parasite was recovered intact (**Figure 1, B**) and measured 8.5 cm in length with a maximum diameter of 480  $\mu m$ . The anterior end (**Figure 1, D**) was bluntly rounded and of greater diameter than the posterior end (**Figure 1, C**). The cuticle of the worm, beginning about 2 mm from the anterior end (at the level of the ovejector) and extending to the tail, had marked longitudinal ridges with transverse striations (**Figure 1, E**). The specimen was a female worm; the esophagus measured 525  $\mu m$  in length, the ovejector was located 1.7 mm from the anterior end, and the tail was 100  $\mu m$  long. The worm was unmated and the paired uterine tubes were full of infertile eggs. The reproductive tubes at all levels were highly coiled and looped.

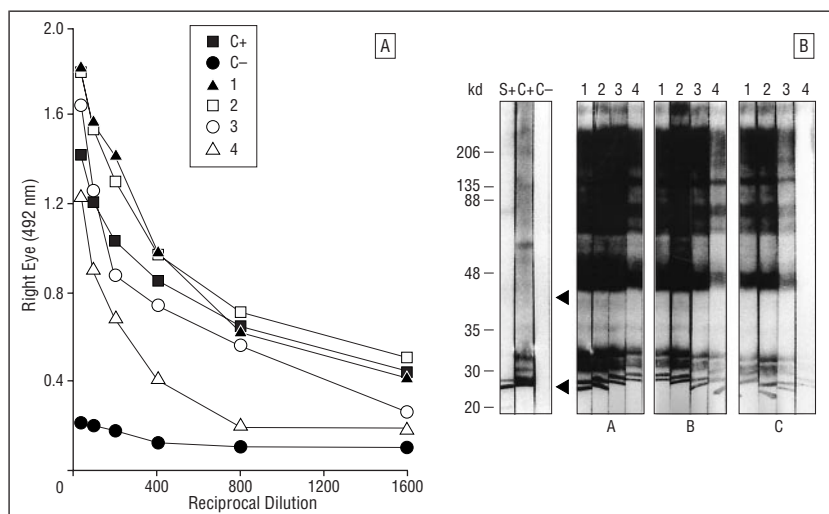
Based on the size and cuticular and internal morphologic features, the specimen was identified as a mature but infertile female *D repens*.

## SEROLOGICAL FINDINGS

The serum samples were tested using dilutions ranging from 1:50 to 1:



**Figure 1.** A, *Dirofilaria repens* adult worm under the conjunctiva. B, Photomicrograph of female *D repens* as removed from subconjunctiva. Anterior end is to the right. C, Photomicrograph of posterior end of worm illustrated in part B, cleared in glycerin. Bar = 100  $\mu m$ . D, Photomicrograph of anterior end of worm illustrated in part B, cleared in glycerin. Position of the ovejector is marked with an arrow. Bar=200  $\mu m$ . E, Photomicrograph of surface of cuticle of worm in part B, cleared in glycerin, illustrating the nature of ridges at the level of midbody. Bar = 50  $\mu m$ .



**Figure 2.** A, Serological analysis by enzyme-linked immunosorbent assay; 1 through 4, patient serum samples. 1 indicates serum sample obtained at the time of the surgery; 2, 3, and 4, serum samples obtained 1, 3, and 6 months after surgery, respectively. C+ indicates positive control; C-, negative control. B, Immunoblot analysis of the same sera studied by enzyme-linked immunosorbent assay. Lanes 1 through 4, patient serum samples as described for the left figure. Lane A indicates 1:40 dilution of the sera; lane B, 1:80 dilution; and lane C, 1:160 dilution. C+ indicates positive control from a patient with subcutaneous dirofilariasis; S+, positive control from an infected patient without subcutaneous involvement; and C-, negative control. The region between arrowheads shows the specific markers for subcutaneous dirofilariasis.

1600. Reactivity of the samples obtained during surgery and at 1 and 3 months after surgery was similar to that of the positive control. However, the sample obtained at 6 months after surgery showed a significant decrease in reactivity at dilutions greater than 1:200 (**Figure 2, A**).

Western blot analysis was carried out at sera dilutions 1:40, 1:80, and 1:160 to show the reactivity pattern of antibodies present in the

samples. A wide range of *D repens* antigens were strongly recognized by the patient sera at the 1:40 and 1:80 dilutions. However, a decrease in the intensity of the reaction was observed in the sample obtained 3 months after surgery when tested at the 1:160 dilution. This trend became more evident in the sample obtained 6 months after surgery that only weakly recognized the antigens located between molecular weight 26

and 40 kd that seem to be specific markers for subcutaneous dirofilariasis (F. Simón, unpublished data, 1997). Therefore, the serological follow-up was able to demonstrate a decrease in the level of *D repens*-specific antibodies, confirming the presence of a unique parasite.

## COMMENT

Although there are only a handful of reports describing zoonotic *D repens* in people from Spain,<sup>2</sup> it probably occurs much more frequently and is either unrecognized or misdiagnosed. The parasite is known to be prevalent in dogs and other carnivores in Spain.<sup>9,10</sup>

Based on the size of the worm, it is likely that the patient had been infected at least 6 months, probably much longer, and had not exhibited symptoms until the worm entered the conjunctiva of the eye. Once in that site, the symptoms were typical and representative of what others have reported. The symptoms quickly resolved on removal of the worm, and because only a single worm is present in most cases there was no need for further treatment.

Surgical removal of the parasite did not present any difficulty and use of a cryoprobe was not necessary, as described previously.<sup>11</sup> Other

surgical techniques for removing the parasites from the anterior chamber also offer no major problems.<sup>12</sup>

There is little information about the serological profiles in cases of subconjunctival dirofilariasis. A recently published article<sup>13</sup> reported that no *D repens* antibodies could be detected. In our case, however, we found high levels of specific antibodies. These antibody levels decreased significantly in the third month and became negative approximately 6 months after surgery. To our knowledge, this is the first communication showing the use of serological methods to confirm the parasitological cure in a *D repens* infection.

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Corresponding author: José M. Ruiz-Moreno, MD, Departamento de Oftalmología Facultad de Medicina, Universidad Miguel Hernández, Campus de San Juan, Apartado 18, 03550 Alicante, Spain (e-mail: upr@expres.es).

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## IN OTHER JOURNALS

### NEW ENGLAND JOURNAL OF MEDICINE

#### Treatment Of Cytomegalovirus Retinitis With A Sustained-Release Ganciclovir Implant

**Background:** Sustained-release, intraocular implants that deliver ganciclovir are an alternative method for the treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome (AIDS).

**Methods:** We conducted a randomized study of 188 patients with AIDS and newly diagnosed cytomegalovirus retinitis. The patients were randomly assigned to treatment with an implant delivering 1 µg of ganciclovir per hour, an implant delivering 2 µg of ganciclovir per hour, or intravenous ganciclovir. The primary outcome we studied was progression of cytomegalovirus retinitis.

**Results:** The median time to progression of retinitis was 221 days with the 1-µg-per-hour implant (75 eyes), 191 days with the 2-µg-per-hour implant (71 eyes), and 71 days with ganciclovir administered intravenously (76 eyes;  $P < 0.001$ ). The risk of progression of retinitis was almost three times as great among patients treated with intravenous ganciclovir as among those treated with a ganciclovir implant (risk ratio, 2.8;  $P < 0.001$ ). However, the risk of disease in the initially uninvolved eye was lower with intravenous ganciclovir than with a ganciclovir implant (risk ratio, 0.5;  $P = 0.19$ ). Patients treated with intravenous ganciclovir were also less likely to have extraocular cytomegalovirus infections (0, vs. 10.3 percent in the two implant groups;  $P = 0.04$ ).

**Conclusions:** For the treatment of cytomegalovirus retinitis, the sustained-release ganciclovir implant is more effective than intravenous ganciclovir, but patients treated with a ganciclovir implant alone remain at greater risk for the development of cytomegalovirus disease outside of the treated eye. (1997;337:83-90)

David C. Musch, PhD, MPH, et al, Department of Ophthalmology, University of Michigan, Ann Arbor