Case report

Subcutaneous Human Dirofilariosis By D. Repens In South Italy: A Case Report.

Gaetano Brindicci1, Carmen Rita Santoro2, Fabio Signorile1, Armando Leone1, Giuseppe Di Ciaula1, Laura Monno1, Gioacchino Angarano1.

1Operative Unit of Infectious Diseases, Hospital-University Polyclinic of Bari, Italy
2Operative Unit of Infectious Diseases, San Giuseppe Moscati Hospital of Taranto, Italy

Running title: Human dirofilariosis in Apulia.

SUMMARY

Human dirofilariosis is a zoonosis caused by different Dirofilaria species: D. repens, D. immitis, D. tenuis and D. ursi, thin nematodes belonging to the Onchocercidae family, whose larval stages are generally found in the natural (felines and canids) or accidental (human) definitive host.

In Europe, human infection is rare, even in areas considered endemic such as Spain or Italy.

In this paper we describe the case of an 82-year-old woman living in Modugno (Bari municipality), who came to our observation for a subcutaneous nodule on her right thigh that had appeared in the previous two weeks and gradually became necrotic.

The woman lived in an apartment with a dog. An adult worm, white, thin, about 140 mm long, came out of the necrotic area spontaneously. After microscopic examination, the worm was identified as D. repens. In Apulia, a South-Italy region, human dirofilariosis is a rare disease and since 1885 only 11 cases have been reported. In recent years we have witnessed an increase in the number of diseases transmitted by vectors at all latitudes, and in our region an increase in the Aedes albopictus population has been reported, so it is reasonable to expect an increase in dirofilariosis cases in humans.

Key words: subcutaneous human dirofilariosis, Dirofilaria repens

Corresponding author: Gaetano Brindicci

Operative Unit of Infectious Diseases, Azienda Consorziale Universitaria-Ospedaliera Policlinico, Piazza Giulio Cesare, 11 - 70124 Bari.

Phone Number: +39 3403682909 - E-mail: gaetanobrindicci@gmail.com
INTRODUCTION

Human dirofilariasis is a zoonosis caused by some species of Dirofilaria: *Dirofilaria repens*, *D. immitis*, *D. tenuis* and *D. ursi* (CDC, 2018). *Dirofilaria*, nematodes of the *Onchocercidae* family, affect both domestic and wild carnivores living in tropical and temperate regions of the world, where they are transmitted at the end of a mosquito blood meal when infectious larvae L3 leave the insect and penetrate into the skin (Service and Ashford, 2001).

In Europe, human cases are caused by two species of this Nematode, which belong to two distinct subgenres. *Dirofilaria repens* belongs to the subspecies *Nochtiella* and is generally located in the subcutaneous tissue of canines or felines, infecting man accidentally. *Dirofilaria immitis*, on the other hand, belongs to the *Dirofilaria* subtype and is generally found in the pulmonary artery and in the right ventricle, in the lung and cervical artery of dogs and cats but rarely in man (Genchi et al., 2011). Mosquitoes of the genus *Culex*, *Aedes* and *Anopheles* are the intermediate host and vectors of these parasites, transmitting the microfilaria they introduce with the blood meal when biting an infected host. Larvae in the third stage of maturation (L3), which in animals become adult worms (developing microfilaria that circulate in the bloodstream) rarely reach that stage in humans, and *microfilariae* have rarely been detected in blood (Eberhard, 2006, McCall et al., 2008, Genchi et al., 2009, Blaizot et al., 2018).

In humans, penetration of active larvae is usually followed by a significant antibody response that delimits the site of penetration. The adult worm measures about 1-15 cm in length and reaches the final location after a long migration. Although the infection is often asymptomatic (Eberhard, 2006) both species can determine cutaneous and visceral localization. Generally, *D. immitis* is responsible for the visceral form, especially pulmonary, and *D. repens* for the subcutaneous and subconjunctival infection, hesitating about 2 to 12 months after penetration into a single subcutaneous nodule, often accompanied by local erythema, itching or urticarial manifestations. Usually, it only hosts an immature female, while the nipple with a male worm, much shorter and slender, is rarely detected. In both cases, the infection is devoid of specific features, so deeply positioned nodules are often incorrectly identified as malignant tumors, at least until a skin biopsy is performed (Simón et al., 2012).

In Europe, human infection is rare, even in areas considered endemic, such as Spain or Italy (Pampiglione et al., 2000, Muro et al., 1999). In Italy, the most important review dates to 2001, with a description of about 60 cases collected between 1990 and 1999, reported in Piedmont, Emilia-Romagna, Sardinia, Sicily, Tuscany, Apulia and Lombardy (Pampiglione et al., 2001). Recently, several biology and epidemiology studies regarding the increase in
cases of human dirofilariosis have been published (Fuehrer et al., 2016, Arbune et al., 2015). In particular, in 2013 two different papers pointed out the risk of an increase in cases of human dirofilariosis in Apulia, due to the increased population of Culex pipiens (Capelli et al., 2013, Giangaspero et al., 2013).

**CASE REPORT**

In this paper we describe the case of an 82-year-old woman, (residing in Modugno, a town of about 40,000 inhabitants in the metropolitan area of Bari), who came to our observation with a subcutaneous nodule in the medial right-thigh region that had appeared in the previous two weeks. The nodule was initially not painful, and was without any signs of phlogosis. The patient reported being treated for arterial hypertension and did not report any other disease; she lived in an apartment and owned a dog. Gradually, in the next two weeks, the nodule increased in size and showed a hard-elastic consistency to palpation, overlaid by reddish and warm skin with a central necrotic region (figure 1). Furthermore, excision was not required because a thin, mobile, cylindrical white worm spontaneously exited the skin while the patient was in our clinic for specialist advice, as required by the Emergency room doctor. The patient also underwent blood examinations during the same access, without evidence of eosinophilia or other major alterations. One tube was stored and then analyzed by veterinarians without the presence of microfilaria. After about two weeks, there was complete regression of the skin lesion.

**Morphology analysis**
The worm was thin, cylindrical, white, 0.5 mm thick and about 14 mm in length (figure 2). The anterior end was rounded and of greater diameter than the posterior end (figures 3A and 3B). Under the microscope, after clarification with xilolo, the outer surface of the nematode’s cuticle was found to have fine transverse striations and prominent longitudinal ridges (figure 4). Each longitudinal ridge was separated from the others by a distance that was larger than the width of the actual ridge itself. Based on size and cuticular morphologic features, the worm was identified as a *Dirofilaria repens*. It was not possible to extract DNA from the adult worm since it was stored in formalin. The search for micro-filariae circulating in the blood was negative.

**DISCUSSION**

In Apulia, human dirofilariosis is a rare disease and only 11 cases have been reported since 1885. In the latest paper published in a review regarding 60 Italian cases in 2001, only one
patient contracted the pathology in Apulia. Nevertheless, Italy ranks above other nations for the highest number of registered cases of dirofilariasis in man. The relationship between the canine *D. repens*-infested population and the prevalence of the disease in man has been demonstrated. Furthermore, several publications have highlighted not only how *Aedes albopictus* is an efficient vector for Dirofilariasis, but also how this vector is becoming increasingly present in south and north Italy.

Dogs are in fact a prime breeding tank for mosquitoes, as Webber showed in 1955, hosting microfilaria in their blood for some months (even up to 3 years in some cases). Furthermore, female adult worms produce around 5,000 microfilariae per day.

From a clinical viewpoint, diagnosis in the case of subcutaneous nodules is difficult because many pathologies, both infectious and non, occur with the presence of such nodules. Differential diagnosis should be made not only with insect bites, but also with infections, neoplasms or immune system mediated disorders.

As far as the epidemiological aspect is concerned, human disease by *D. repens* is present only in the Old World and is quite rare (about 3500 cases in the last 45 years), although it is considered endemic in Italy (Genchi and Kramer, 2017). It should be noted that the mosquito prefers to feed on the blood of other animals, not humans.

Clinical manifestations of dirofilariasis tend to crop up only at a local level, as with our case, also owing to man being an occasional host and hence not an ideal habitat (as shown by the generally smaller sizes of the adult worm found in man). A surgical operation to remove it is preferable with such infections, although in our case it wasn’t required.

In recent years we have witnessed a rise in the number of diseases transmitted by vectors at all latitudes, though in the near future there is likely to be an increase of dirofilariasis in man. Bearing this in mind, it is vital for a clinic (above all in countries where such vectors are rarer) to be aware of what diagnostic procedure to adopt.

Finally, we wish to point out that, as far as we know, the described case is the first in the literature with spontaneous emission of the worm through the skin. The age of the patient (and therefore more damaged skin) and the location of the nodule (easily approachable by the patient herself) could explain this unique event.

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**Conflict of interest**

The authors declare that there is no financial/personal interest.
REFERENCES


Figure 1. The cutaneous nodule from which the worm has come out
Figure 2. The adult worm
Figure 3. Anterior (A) and posterior (B) end of the worm.
Figure 4. The outer surface of the nematode’s cuticle