



## HISTOPATHOLOGICAL DIAGNOSIS OF HUMAN *DIROFILARIA REPENS* INFECTION: REPORT ON THREE CASES WITH UNUSUAL SITES OF INVOLVEMENT

### Pathology

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### ABSTRACT

Human zoonotic filariasis due to *Dirofilaria repens* is a world-wide infection occasionally transmitted to man by a zooanthrophilic bloodsucking mosquito belonging to the genera *Culex*. *Dirofilaria* live in the subcutaneous tissue of their hosts causing the formation of nodules which normally requires surgical removal and an histological examination. Rare localizations have also been reported in deep tissues like the lung, spermatic cord and uterine round ligament.

Since epidemiological data suggest that human zoonotic filariasis represents an Italian emergent zoonosis, the aim of these case report is to better characterize *dirofilaria* endemic zones in order to develop a general view of this problem entity and its changing in geographical distributions, giving histopathologists and parasitologists new useful data concerning the nematode features and diffusion. We report on three cases of human infections in unusual sites of involvement for this parasitosis, diagnosed in Southern Italy.

### KEYWORDS

*dirofilaria repens* . nematode . infection

### INTRODUCTION

*Dirofilaria (Nochtiella) repens (Nematoda, Onchocercidae)* is a mosquito-transmitted filarioidea whose life cycle consists of five developmental larval stages which take place in vertebral hosts like dogs, cats and other carnivores and an arthropod intermediate host and vector represented by mosquitos of the genera *Aedes*, *Anopheles* and *Culex*<sup>1</sup>. Adult female worms produce thousands of first-stage microfilariae that are subsequently ingested by the vector during feeding. Development in the mosquito requires about two weeks. Microfilariae undergo to two developmental changes in the mosquito and as third-stage larvae, are inoculated back into the host during the act of feeding, where they will infiltrate into the subcutaneous tissue. The sexual maturity in their natural definitive hosts can be accomplished after several months. Humans represent accidentally hosts, being patient infections extremely rare<sup>2</sup>. Since the human body is an unusual environment for the parasite, the larvae's development is inhibited leading to a retardation of sexual maturity<sup>3</sup>. Human subcutaneous tissue of exposed anatomic sites, e.g. head, eye, neck, trunk, and lower extremities of the body represent the common site of involvement of this kind of infection, as a result of a mosquito bite. The nematode slowly migrates through the subcutaneous tissue until the activation of the host immune system. A foreign body cell response is then observed, due to the parasite's death<sup>4</sup>, thus leading to the formation of a subcutaneous nodule composed by the dead worm and a massive inflammatory infiltrate. Deep tissues like lung<sup>5,6</sup>, spermatic cord<sup>7,8</sup> and uterine ligament have also been reported as extremely rare localizations<sup>9</sup>. Infections in humans are usually asymptomatic but acute symptoms as local tenderness feeling, itching, redness and swelling can be observed as well. Surgical excision represents the gold standard treatment<sup>4,10</sup>. Up to now, nothing has been reported about the advantage of an antifilarial medication, performed alone or in association with surgical therapy.

Endemic foci for *Dirofilaria repens* exist in Southern and Eastern Europe, Asia Minor, Central Asia, Sri Lanka and south eastern United States. Italy represents the European country with the highest prevalence in the world<sup>11</sup>.

The authors herein report on three new cases of unusual filarioidea localizations occurred in the Apulia Region (*Southern Italy*).

### CASE REPORT

A 45-year-old Caucasian man, who lived in the countryside working as dog charmer, presented with a few months history of inguinoscrotal swelling. Clinical examination revealed a left inguinoscrotal hernia, thus leading the patient to a hernioplasty. Accidentally, a solid fatty mass within the spermatic cord of the left testis has been discovered by

the surgeons during the hernioplasty. In the suspicion of a malignant process, an orchiectomy was immediately performed<sup>19</sup>.

A 51-year-old Caucasian man, who lived in the countryside, complained of a swelling and painful mass in the inguinal area. A lymphadenomegaly due to lymphadenitis process of unknown aetiology was suspected.

A 69-year-old Caucasian man presented a subcutaneous abdominal swelling mass. A clinical diagnosis of foreign body granuloma after skin-penetrating trauma was performed.

Histologic sections of surgical specimens were fixed in 10% buffered formalin, paraffin embedded and 5 µm thick sections stained with Hematoxylin-Eosin. Then histochemical studies were performed for PAS and connective tissue Masson Goldner trichrome stains. Measurement of microscopic lengths was carried out using a digital microscope.

Grossly, in the first case, the didymus diameter was proved to be 50 mm while the lengths of epididymis and funiculus were 90 mm. At 20 mm from one of the resection margins, the funiculus presented a whitish area of fibrous consistency, of 20,5 mm in-diameter. Histological examination of the specimens revealed any pathological alterations of the didymal and epididymal structures. Funiculus soft tissues showed a severe granulomatous chronic inflammation characterized by a lympho-histiocitary reaction with an eosinophilic infiltrate around cross sections of a nematode identified as *Dirofilaria repens* (Fig.1).

In the second case a yellow-brown specimen measured 70 x 35 mm was examined. Histological findings showed a reactive lymph nodes hyperplasia with widespread soft tissues inflammatory infiltrate mainly composed by lymphomononuclear and granulocytic eosinophils around cross sections of a nematode identified as *Dirofilaria repens* (Fig.2).

In the third case, the excisional biopsy nodule measured 15 x 4 mm, and histology showed a subcutaneous tissue with a conspicuous inflammatory infiltrate by lymphomononuclear and granulocytic eosinophils with a granulomatous reaction around cross sections of a nematode identified as *Dirofilaria repens* (Fig.3).

### DISCUSSION AND CONCLUSION

*Dirofilaria* is a worldwide distributed disease with the most endemic areas in the Mediterranean countries like Italy which has the highest prevalence in the world<sup>9</sup>. The most important risk factors regarding human infections are mosquito density, warm climate, extended

mosquito breeding season, outdoors human activities and the abundance of microfilaraemic dogs.

The incidence of human dirofilariasis has been increasing over the last five decades<sup>4 13 16 17 18</sup>, due to an increased opportunity for travels and outdoors living, environmental changes and diffusion of the mosquito vectors, introduction of new mosquito species and the improved recognition of neglected infections<sup>4</sup>.

The authors herein reported on three cases of *Dirofilaria repens* diagnosed in Italian patients living in Apulia Region (Southern Italy), occurred in the last years. Both patients reported any travel outside the Apulia area. The first case was found by chance during an inguinoscrotal hernioplasty: a solid fatty mass was noticed within the spermatic cord, and an orchiectomy was performed in the suspicion of a malignancy of the testis.

The second case presented as a painful inguinal mass which was evaluated as a lymphadenitis with lymphadenomegaly of unknown aetiology. According to the literature, this seems to be the first case of a deep inguinal soft tissue localization of *Dirofilaria repens* human infection.

The last case presented a subcutaneous abdominal swelling mass with a clinical diagnosis of foreign body granuloma after skin-penetrating trauma.

It should be underline that deep tissues infiltration could be observed in some *Dirofilaria* species, e.g. *Dirofilaria immitis* and *Wuchereria bancrofti*<sup>14</sup>, as a result of a lymphatic flow or active movement only, but it has never been reported in a human *Dirofilaria repens* infection. Histopathological features revealed, in all cases, sections of the nematode enclosed in the surgically excised nodules, surrounded by a granulomatous lympho-histiocitary and lympho-mononuclear reactions with several eosinophils sited around cross sections of the nematode. The correct identification of a *Dirofilaria repens* nematode relied upon histological examination of its microscopical features<sup>13</sup> including a thick laminated cuticle with external longitudinal ridges and the presence of a well-developed circumferential musculature interrupted by two lateral cords. Each longitudinal ridge is separated from the others by a distance that is larger than the width of the actual ridge itself.

The morphology of the nematodes was well preserved, even though the nematodes' size represents a further useful characteristic in the differential diagnosis between the several *Dirofilaria* species. The external longitudinal ridges of the parasites observed are typical of those species living in the subcutaneous tissues of their natural hosts. Usually the worm cross section has a diameter of 220 to 600 µm. The maximum diameter of the two nematodes body, observed in our patients, was 340 µm and 460 µm with a cuticle thickness of 8-10 µm respectively. The presence of a uterus and oviducts with ovocytes in different evolutions phases lead us to confirm that our parasites were all females. Both patients revealed a normal blood cell count, without eosinophilia. Due to the lack of specificity and inability to distinguish between exposure and active infection<sup>4 13 14</sup>, serologic tests were not performed.

Because of its unusual locations in deep tissues, differential diagnosis should include other granulomatous diseases and malignant neoplasms<sup>4 15</sup> thus, a radical surgical excision of the mass is mandatory, even though it could expose the patients to high operatory risks and invalid results (orchiectomy).

The aim of this study is to better characterize dirofilariasis endemic zones in order to develop a general view among this entity and its changing in geographical distributions, giving histopathologists and parasitologists new useful data concerning the nematode features and diffusion.



FIG.1

FIG.1 Case 1. A thick laminated cuticle with external longitudinal

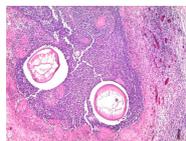


FIG. 2

ridges and the presence of a well-developed circumferential musculature interrupted by two lateral cords of the nematode (H/E, 40x magnification).

FIG.2 Case 2. Severe granulomatous chronic inflammation characterized by a lympho-histiocitary reaction with an eosinophilic infiltrate around cross sections of a nematode identified as *Dirofilaria repens* (H/E, 5x magnification).



FIG.3

FIG.3 Case 3. A nematode identified by an histochemical stain for PAS (10x magnification).

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