Case Report: Molecular Detection of *Dirofilaria repens* in an Italian Patient after a Stay in Tanzania

Donato Antonio Raele,1,4 Nicola Pugliese,2,1 Gianfranco LaBella,1 Agata Calvario,3 Maria Scarasciulli,4 Ilaria Vasco,1 Giovanna La Salandra,1 and Maria Assunta Caﬁ2

1Istituto Zooproﬁlatico Sperimentale della Puglia e della Basilicata, Foggia, Italy; 2Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Italy; 3Laboratorio di Virologia U.O.C. Microbiologia e Virologia AOU Policlinico, Bari, Italy; 4Laboratorio di Virologia U.O.C. Microbiologia e Virologia, Dipartimento Interdisciplinare di Medici, Università degli Studi di Bari, Valenzano, Italy

Abstract. A 35-year-old man was admitted to a hospital in the south of Italy because of a periocular nodule and subpalpebral edema. The patient reported having been stayed in Tanzania ﬁve months before. Hematologic parameters were within the normality range, the *Acanthocheilonema vitaeae* ELISA did not detect signiﬁcant levels of antifilarial IgG, and no further symptoms were described. The surgical inspection of the nodule led to the isolation of two filarial larvae, whereas loop-mediated isothermal ampliﬁcation and PCR detected *D. repens* DNA. The patient was treated with doxycycline, and he was found no more positive at the follow-up.

INTRODUCTION

Human diroﬁlariasis by *Dirofilaria (Nochiella) repens* (Nematoda, Onchocercidae) is a mosquito-borne parasitic zoonosis, which mostly affects dogs in the Old World.1,2 However, the disease is becoming a matter of growing concern because several factors, such as the frequent lack of clinical signs in both humans and dogs, the limited range of treatments, and the climate changes, are contributing to its wide circulation in Europe.3 In fact, an increasing number of reports are being recorded not only from Italy and other European countries bordering the Mediterranean Sea, where diroﬁlariasis is considered endemic,7,4 but also from Eastern Europe, in particular Russia and former Soviet countries,5 Northern Africa, and Middle and Far East.3 Microfilaricidal domestic dogs are considered the most important *Dirofilaria repens* reservoir, despite wild dogs and cats being involved, too.4,6 Microfilariae produced by female worms may invade the bloodstream of the natural reservoir of the parasite and be ingested by mosquitoes, where they molt and develop into the infecting stage (larva L3), which, in turn, can be released or transmitted by bite to other hosts.7 In humans, diroﬁlariasis is often asymptomatic, and the parasite usually does not develop into the adult form,7 but larvae may spread through the bloodstream up to reach the subcutaneous tissues, often forming nodules.8 The golden standard for the diagnosis of canine or feline diroﬁlariasis is the Knott’s test and its variants,9,10 based on the microscopic detection and morphological identiﬁcation of microfilariae from blood. Considering that expert operators are needed to correctly recognize and identify microfilariae, molecular tests have been developed to selectively amplify by PCR11 and loop-mediated isothermal ampliﬁcation (LAMP)12 species-speciﬁc regions of the *D. repens* genome. However, the intermittence of microfilaria, along with the low concentration in blood, makes challenging the prompt diagnosis of diroﬁlariasis by *D. repens* in dogs.7 The issue is even more critical for humans because the microfilarial stage is absent or, at least, very rare.13 In those cases, diagnosis may be achieved by combining anamnestic data (especially those about travels or lifestyle) and the clinical picture, with ultrasonography14 or morphometric examination.15

CASE HISTORY

In March 2019, a 35-year-old man, resident in Apulia, a region in the south of Italy, presented to a local hospital because of a subpalpebral edema surrounding a periocular nodule, about 1.5 cm2 large, localized under the left lower eyelid (Figure 1). The anamnestic record did not evidence relevant pathologic events, but the patient reported a two-week stay in Tanzania ﬁve months before the hospital admission. He also declared no further movements outside Apulia after having been returned to Italy. The hematologic parameters were within the normality range, and the hemochromocytometric proﬁle was regular (Table 1). A sample of serum underwent *Acanthocheilonema vitaeae* ELISA (Bordier Afﬁnity Products, Crissier, Switzerland), a pan-filarial IgG-detecting immunoenzymatic assay,16 which returned a value of 0.46, thus below the positivity threshold, established at 1.

The nodule was surgically inspected, and two adult ﬁlarial parasites, 10 and 5 cm long, respectively, were removed (Figure 2A). The SEM analysis of the specimens revealed morphological features compatible with *D. repens*, including longitudinal ridges on the external cuticle, typical of *D. repens,*17 Candidatus *Dirofilaria hongkongensis,*18 and *Dirofilaria ursi,*19 and two large lateral chords, considered *D. repens* speciﬁc (Figure 2B).17

The Knott’s test resulted negative, but LAMP12 and PCR17 detected *D. repens* DNA from a specimen of blood EDTA. Total genomic DNA was also extracted and puriﬁed from a section of the removed worms and used as a template in the PCR17 to conﬁrm the identiﬁcation. The nucleotide sequences of the ampliﬁcation products from blood (GenBank accession number MT683121) and worm (MT683122) were 100% identical between themselves, and one of them was aligned by ClustalO with a representative panel of corresponding sequences from *D. repens* present in GenBank.

* Address correspondence to Donato Antonio Raele, Istituto Zooproﬁlatico Sperimentale della Puglia e della Basilicata, Via Manfredonia, 20, Foggia 71121, Italy; E-mail: donatoantonio.raele@izspb.it

† These authors contributed equally to this work.

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Abstract. A 35-year-old man was admitted to a hospital in the south of Italy because of a periocular nodule and subpalpebral edema. The patient reported having been stayed in Tanzania five months before. Hematologic parameters were within the normality range, the *Acanthocheilonema vitaeae* ELISA did not detect significant levels of antifilarial IgG, and no further symptoms were described. The surgical inspection of the nodule led to the isolation of two filarial larvae, whereas loop-mediated isothermal amplification and PCR detected *D. repens* DNA. The patient was treated with doxycycline, and he was found no more positive at the follow-up.

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The maximum likelihood phylogeny was inferred by the mean of PhyML 3.0 by applying the GTR model, selected by FindModel. The sequence of the isolate was part of a heterogeneous cluster (Supplemental Figure 1) that included sequences from D. repens isolated in Germany, Italy, and Croatia, 100% identical among themselves. Conversely, the sequence from this study was slightly distant \((p\text{-distance} = 0.0015)\) from them.

After surgical removal of the adult worms, the patient was treated with doxycycline, and no resurgence was reported after one year.

The diagnosis of human dirofilariasis mostly relies on the morphologic identification of the worm after biopsy or surgical removal.\(^2\) Preventive analyses such as ultrasound examinations or pan-filarial IgG tests might be useful to better address surgery, but they cannot provide a definite diagnosis before the morphometric inspection. The currently available ELISA test may be relatively beneficial, but it lacks specificity as it may detect IgG produced after infestation by several species, such as Brugia malayi, Loa loa, Mansonella sp., Onchocerca volvulus, and Wuchereria bancrofti, other than Dirofilaria immitis and D. repens.\(^16\) On the other side, morphologic identification may be time- and labor-consuming, and it requires operators with specific expertise, not always available in all diagnostic laboratories. Nevertheless, the here reported case also poses a matter of sensitivity. Molecular assays managed to detect and identify D. repens DNA from the patient’s blood, but no microfilariae, or even L3 larvae, were observed by the Knott’s test, as expected because of the infrequency of microfilariaemia in humans.\(^13\) In addition, the case evidenced the absence of potential hematological markers for dirofilariasis because no parameter was significantly out of range. The absence of eosinophilia and the low level of IgE, along with the negative results gathered by the IgG-detecting ELISA, reflect the poor efficacy of hematologic or serological diagnostic tools, already known.\(^20\) Conversely, the case highlights the possible interest of molecular tools due to their sensitivity and specificity, already proved in vitro and, less frequently, in field. It is tempting to speculate that, in humans, the circulation of larval stages of D. repens in the patient’s blood occurs at very low concentration, insomuch that it can be detectable only by highly sensitive methods, such as the molecular ones. Further studies might be aimed to clearly ascertain which stages are vesiculated by the bloodstream and their concentration.

The value of molecular tests is also enhanced by its potential contribution to the differential diagnosis between loiasis and dirofilariasis, especially for those patients declaring stays in tropical regions considered endemic for L. loa. The latter infestation has usually worse outcomes than dirofilariasis, but it is often (but not always) associated with eosinophilia.\(^21\) In fact, despite uncommon, periocular localization of L. loa has been reported.\(^22\)

Finally, the present report adds further considerations about the possibility to import cases of dirofilariasis from Africa. The infestation has already been diagnosed in patients returning from Malaysia and Botswana to Spain\(^23\) or from Senegal to France\(^24\) and Belgium (the latter case with the evidence of microfilariaemia),\(^25\) but no clear pieces of evidence were provided about the acquisition of the infection in foreign countries. Unfortunately, to date, no sequences of D. repens collected from Central and Southern Africa are available for comparison, despite it being isolated or detected from dogs in Tanzania\(^26\) and Cape Verde.\(^27\) Despite the scarce clonality of D. repens population, they could offer very useful information about the worldwide circulation of the parasite, and a potential starting point to assess the international transmission routes, which could be directed to, or even originating from, Europe.
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Authors’ addresses: Donato Antonio Raele, Gianfranco LaBella, Ilaria Vasco, Giovanna La Salandra, and Maria Assunta Cafiero, Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy, E-mails: donatoantonio.raele@izspb.it, gianfranco.labella@izspb.it, ilaria.vasco@izspb.it, giovanna.lasalandra@izspb.it, and mariaassunta.cafiero@izspb.it. Nicola Pugliese, Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Italy, E-mail: nicola.pugliese@uniba.it. Agata Calvario, Laboratorio di Virologia U.O.C. Microbiologia e Virologia AOU Policlinico, Bari, Italy, E-mail: agata58calvario@gmail.com. Maria Scarasciulli, Laboratorio di Virologia U.O.C. Microbiologia e Virologia, Dipartimento Interdisciplinare di Medicina, Università degli Studi di Bari, Valenzano, Italy, E-mail: maria.scarasciulli@uniba.it.

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The following are supplemental files and will be available online only

Supplemental Figure 1