RESEARCH LETTER

Multiple-site subcutaneous *Dirofilaria repens* infection in a Polish patient with a history of rheumatic disease

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Introduction Dirofilariasis is a disease that occurs all over the world. In Europe, it is endemic in Ukraine and around the Mediterranean (Italy, France, Greece, Spain), but also in Germany, Austria, and Hungary.[1,2] From 2007 to 2012, a total of 18 cases of *Dirofilaria repens* infection in humans were documented in Poland, all of which manifested as subcutaneous nodules.[3] The first case of human dirofilariasis in Lithuania was reported in September 2011.[4]

Dirofilariasis is a zoonotic disease. Two species of nematodes are responsible for its development in humans: *D. immitis* and *D. repens*. Human infection occurs accidentally (accidental host), because the natural and reservoir hosts of *Dirofilaria* are domestic and wild carnivores, especially dogs and cats, but also wolves, coyotes, foxes, and muskrats. The high incidence of dirofilariasis in dogs, the main reservoir hosts, may be the cause of increasing transmission to humans.[2] Adult females of *Dirofilaria* worms can produce up to 5000 microfilariae per day.[1] However, in the human body, the parasites rarely reach adulthood, and usually no offspring, that is, microfilariae, are formed. The majority of larvae that enter the human body die.[4,5]

The life cycle of a *Dirofilaria* worm consists of 5 stages that take place in vertebrate hosts and various species of mosquitoes (*Aedes*, *Ochlerotatus*, *Culex*, *Culiseta*, *Coquillettidia*, and *Anopheles*), which serve as intermediate hosts / vectors (carriers). The bacterial endosymbiont *Wolbachia* sp. plays a specific role in the course of *Dirofilaria* infection, enhancing the inflammatory response induced by the parasite. Infection with *D. repens* is the most common and widespread dirofilariasis in the world. In humans it most commonly occurs in 2 clinical forms: as subcutaneous / submucosal nodules or as interstitial lung disease. In many cases, the disease proceeds asymptomatically or with nonspecific symptoms, which can delay the correct diagnosis. Involvement of the eyeball is possible, and the parasite is then visible under the conjunctiva. A common manifestation of the disease is also a breast lump, which may be misdiagnosed as a neoplastic process. The biggest challenge associated with diagnosing dirofilariasis is its initial misdiagnosis as a primary tumor or lung metastases. Serological blood tests, the Knott's test, imaging examinations (chest X-ray, ultrasound examination, computed tomography, magnetic resonance imaging), as well as biopsy and histopathologic examination may be helpful in diagnosing dirofilariasis. Molecular biology methods are also used increasingly often to confirm the disease.[6]

Patient and methods **Patient** A 47-year-old woman with a history of hypothyroidism and degenerative joint disease was admitted to the Department of Infectious Disease and Neuroinfection at the University Clinical Hospital in Białystok on December 20, 2019, with migratory skin pain and nonspecific subcutaneous nodules. Initially, in March 2019, and subsequently in October 2019, the patient had the subcutaneous nodules removed from the right upper limb. After the procedure, the patient underwent diagnostic workup for rheumatologic, hematologic, and dermatologic disorders. One of the extracted nodule was subjected to a histopathologic examination on October 31, 2019, which revealed a necrotic focus with a cluster of parasitic nematode larvae. The histopathologic material was sent to

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the Department of Parasitology of the National Institute of Public Health – National Institute of Hygiene for the parasite species identification using the polymerase chain reaction (PCR) method. On December 5, 2019, an abdominal and right axillary ultrasound examination as well as chest X-ray were performed, both of which were unremarkable. Laboratory tests and echocardiography were carried out. During a differential diagnosis an examination was conducted to detect antibodies against *Toxocara canis*, *Taenia solium*, and *Echinococcus granulosus*, but the results were negative. Laboratory investigation showed an increase in the percentage of eosinophils up to 11% and a prolonged activated partial thromboplastin time of 43.6 seconds. During the hospitalization, antiparasitic treatment (albendazole) was administered with good tolerance.

Microscopic evaluation Identification of the parasite species was based on the assessment of a stained histopathologic specimen obtained from the nodule in the subcutaneous tissue, which contained cross-sections of the nematode. The evaluation took into account morphologic features of the worms, such as maximum body diameter, cuticular striations, and uterine structure.

Molecular examination Three sections from the paraffin block containing the lesion / nodule were deparaffinized, and DNA was subsequently isolated from the samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).[7] The PCR method was used to amplify a fragment of the first subunit of the cytochrome oxidase gene of *D. repens* using specific primers RepIm-F0 5′-TCAGATTAGTATGTTTGTTTGAACTTCTTATT T-3′ and RepIm-R0 5′-ACAGCAATCCAAATAGAA GCAAAAGT-3′.[8] The amplification was performed under the following conditions: 95 °C for 3 minutes, followed by 45 3-step cycles (95 °C for 15 seconds, 61 °C for 20 seconds, and 72 °C for 40 seconds). The amplified products were sequenced using the Sanger sequencing method. The obtained sequences were compared with the sequences deposited in the GenBank database using the CLC Main Workbench 20.0.2 software (Qiagen, Redwood City, California, United States) and the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST).[9]

Results **Microscopic evaluation** In the histologic specimen, cross-sections through a female nematode were observed. They had an oval shape with approximate dimensions of 320 μm × 630 μm. On the surface of the nematode, longitudinal ridges were visible, arranged at a distance of approximately 10 μm from each other. Inside the nematode, an intestine and a double uterus filled with oocytes were visible. Microfilariae were not detected. Based on the morphologic features of the nematode, it was recognized as a female *D. repens* (FIGURE 1A–1D).

Molecular examination The amplification product sequence of 250 bp (309 bp including primers) showed a high degree of similarity (99.6%) to 23 *D. repens* sequences reported in the GenBank database. In the database, there were also 5 *D. repens* sequences showing slightly less similarity to the sequence obtained in our study (Supplementary material, *Table S1*). Sequences of the other nematode species reported in the GenBank database, such as *Dirofilaria* sp. 'hongkongensis', *D. ursi*, and *Onchocerca* spp., showed a similarity below 96% to the obtained sequence. Based on the PCR method, the patient was diagnosed with *D. repens* infection. The sequence obtained in this study was reported to the GenBank database (accession number, OR016125).

Discussion We report a new case of dirofilariasis (*D. repens*) in a patient from Poland. The disease manifested as subcutaneous nodules and migratory skin pain. The initial symptoms were nonspecific, and the patient was initially diagnosed for rheumatic, hematologic, and dermatologic conditions, which delayed the proper diagnosis. The diagnosis was ultimately reached based on the assessment of a stained histopathologic specimen from the subcutaneous nodule, which contained cross-sections of a female nematode, and confirmed by a molecular examination. The presented case is exceptional due to a female *D. repens* being discovered in a human host (accidental host), whereas it is typically expected to be encountered in definitive hosts.

Until 2012, approximately 2000 cases of human dirofilariasis (*D. repens*) have been diagnosed and documented in Europe. The highest number of cases was reported in Ukraine, followed by regions surrounding the Mediterranean (eg, Italy), as well as in Hungary, Austria, Serbia, and Slovakia.[1-4,8,10,11] In Poland, the first case of *D. repens* infection in a human was described in 2007,[12] and in 2011, further 3 cases of dirofilariasis were diagnosed and documented (3 women from the Mazowieckie province).[3] Subsequently, cases of dirofilariasis in various locations of the body have been described in the literature, including the conjunctiva, subcutaneous tissue of the torso (abdomen, back, side, clavicle), thigh, head (forehead, chin, eyebrow arch, occiput), and others.[5,6,13,14] In 2019, the first case of dirofilariasis affecting the oral mucosa was described in Serbia.[15]

The increasing number of dirofilariasis cases in Europe is associated with significant climate change (global warming), the growth of mosquito populations (intermediate host / vector), as well as the global migration of people and animals (especially dogs), all of which are considered the most important factors in the expansion of the disease.[4,8]

Typically, the parasite needs to be surgically removed, and in some cases, it is necessary to excise the parasite along with the surrounding

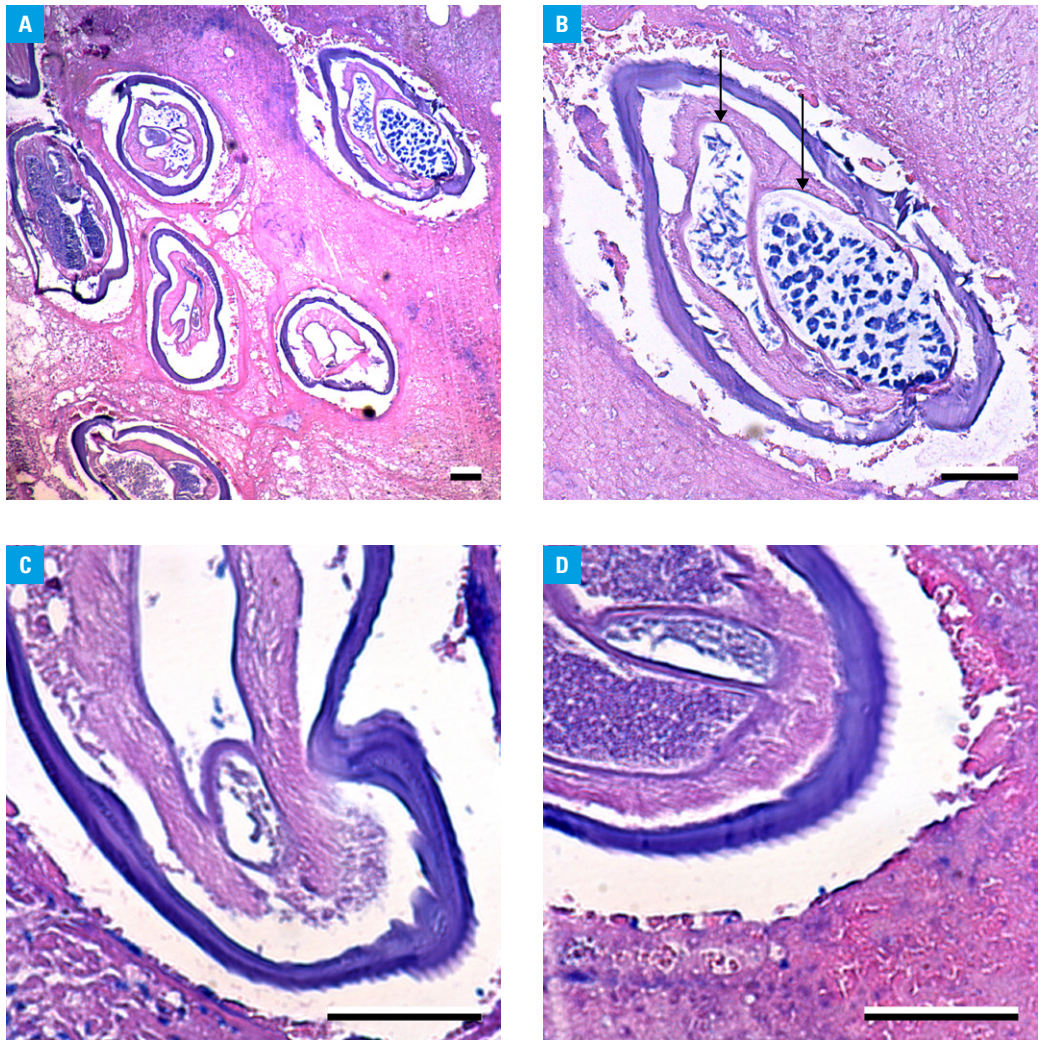

FIGURE 1 Cross-sections of a female *Dirofilaria repens* nematode found in the subcutaneous nodule of the patient; hematoxylin and eosin staining; scale, 0.1 mm. **A** – cross-section image showing 6 sections through the female *D. repens* nematode (objective 4×); **B** – cross-section image showing a double uterus (arrows; objective 10×); **C**, **D** – close-up views of the cuticle of *D. repens*, clearly showing the external ridges (objective 20×)

affected tissues. On histopathologic examinations, besides the parasite, clusters of granulocytes and eosinophils, as well as clusters of macrophages and plasma cells, can be observed, like in our case. Molecular methods are being increasingly used in the diagnosis of dirofilariasis to confirm the presence of *D. repens* infection[7]; such methods were also used in our study.

Conclusions The presented case of dirofilariasis underscores the need for raising awareness about the increasing risk of this zoonotic disease, its atypical symptoms, as well as diagnostic standards that should be based on molecular biology methods. Furthermore, it is important to spread the knowledge among veterinarians and pet owners about possible infections in dogs, symptoms that may suggest an infection, and the need to monitor cases in both human and animal populations.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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ETHICS APPROVAL AND PATIENT CONSENT The authors claim that all procedures contributing to this study were approved and in accordance with ethical standards of the respective country and institutional committees on human experimentation. The patient provided her written consent to allow for anonymous reporting of the results of this study.

CONFLICT OF INTEREST None declared.

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