

CASE REPORT

Intramuscular human *Dirofilaria repens* infection of the temporal region – case report and review of the literature

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Abstract

Dirofilariasis is a zoonotic worldwide-distributed disease, especially in regions with warm to temperate climate, where it recently recorded a significant increase of prevalence. A 61-year-old female, a dog owner, residing in the Southwest of Romania, near a swamp, developed a painless nodule in the left temporal region. The contrast-enhanced computed tomography (CT) scan revealed a temporal intramuscular cystic lesion. The surgical exploration confirmed the lesion as with intramuscular localization. The histopathology and biochemistry investigations established as a cause of the lesion an infestation with *Dirofilaria repens* species. The patient underwent an anthelmintic treatment with Diethylcarbamazine and the clinical and radiological follow-up did not revealed any recurrences within a period of 15 months after surgery. This is the second case reported in Craiova (Romania), the seventh worldwide reported case with localization in the temporal region, and the second one with intramuscular development in this region. The existence of such cases should alert the clinician to include parasitosis in the differential diagnosis of atypical space-occupying lesions of the head regions.

Keywords: *Dirofilaria repens*, histopathology, intramuscular, temporal, zoonosis.

Introduction

Human dirofilariasis is a zoonotic, worldwide-distributed disease caused by parasites of the genus *Dirofilaria*, of the family *Onchocercidae* [1, 2]. Typically, the natural hosts for these filarial nematodes are dogs and wild members of the genus *Canis*, but the infections may occur in a variety of species (spp.), including humans [1, 3]. There are more than 40 species described, but the human infections mainly involve the *Dirofilaria immitis* and *D. repens* [1, 2, 4]. Throughout the life cycle, these parasites undergo five developmental forms and the cycle starts in the animal host by releasing from adult female *Dirofilaria* spp. of microfilariae (live larvae) into the host's blood [1, 2]. Then the vectors, which are hematophagous insects (especially female of mosquito species of the *Culicidae* family), ingest microfilariae during feeding on an infected host [5]. In these vectors, the microfilariae evolve by undergoing a series of molts to the infective third larval stage. In this stage, during feeding on another host (inclusive human), they penetrate into the host and then migrate *via* blood to the pulmonary arteries and more rare in the right ventricle of the heart for the *D. immitis* [3], and respective to the subcutaneous tissue and subconjunctiva for the *D. repens* species [2]. Here they develop into fertile macrofilariae, the sexual maturity being reached within six months post-infection. Humans are less suitable hosts and therefore the *Dirofilaria* larvae

do not reach maximum development, remaining infertile, and consequently in the human peripheral blood there are no circulated microfilarias [1, 2].

Not so long ago, dirofilariasis was considered to be a rare disease, but data from recent years proves that it is a worldwide-distributed zoonotic disease, especially in regions with warm to temperate climate, where the disease becomes endemic [6]. In Europe, most of the cases were reported in Mediterranean countries [2, 7–9], with the tendency of increasing the reported cases from central and northeastern European countries [1, 4, 10]. In Romania were reported a few dozen of human dirofilariasis cases but without knowing the precise prevalence of *Dirofilaria* spp. [11–14].

We report a case of human subcutaneous *D. immitis* infection from Dolj County and we discuss the clinical and morphological peculiarities of this case in the context of the other similar cases reported in Romania.

Case presentation

A 61-year-old female (B.I.), retired, resident of Craiova, Dolj County (a southwestern County in Romania), in an urban area, but close to a swamp, with no travel history, a dog owner, presented in July 2015 to our Institution (Department of Oral and Maxillofacial Surgery, Emergency County Hospital, Craiova, registration No. 32250, 22/06/2015) with a nodule on the left temporal region. The

patient noted the onset of symptoms about six months ago, period during which she complained about two episodes of widening and growing in size of the left temporal swelling, without pain, itch, fever or arthralgia. During these episodes, she followed treatment with antibiotics (Augmentin – 1000 mg daily for five days) prescribed by her family doctor and the symptoms have remitted each time. Her past medical and history family records were not significant for the pathology in question. The patient recalled multiple mosquito bites during dog walks in the swamp area that was close to her house.

Local physical examination

Physical examination confirmed a single, firm, painless, rounded mass with about 3.5 cm in maximum diameter, into the left temporal region. The overlying skin was unaltered and moving freely on the surface mass, but the pseudotumoral mass was fixed to the underlying structures.

Paraclinical investigations

Her lab data showed eosinophilia (7%, normal less than 3%), elevated erythrocyte sedimentation rate (ESR) (35 mm/1 h, normal less than 30 mm/1 h), and sign of urinary infection; all other routine clinical laboratory tests being normal. The contrast-enhanced computed tomography (CT) scan was performed and revealed a cystic lesion with inhomogeneous ring enhancement in the left temporal muscle (Figure 1, A and B). A chest X-ray and abdominal ultrasound revealed no pathology.

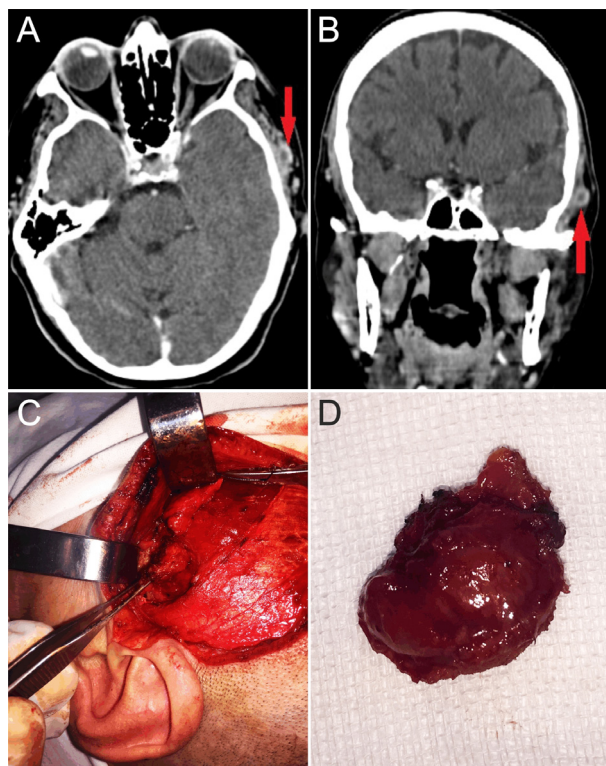


Figure 1 – (A and B) CT scan shows left temporal muscle with a cystic lesions bordered by inhomogeneous ring enhancement (A: Axial section; B: Coronal reconstruction). (C) Intraoperative image reveal an intramuscular tumoral mass in left temporal region. (D) Gross aspect of the surgical specimen.

Initial management

Based on history, clinical, laboratory and imaging findings, a provisional diagnosis of left intramuscularly temporal tumor was considered. At this point, we took into account as differential diagnosis a wide range of injuries, ranging from malignancies to benign neoplasm and other pathologies such as fungal infections, hamartomas and abscesses. With the informed consent of the patient, we decided removal of the tumor for histopathological examination, after the adequate preoperative work-up. An incision of about 5 cm was made in the left temporal region above the tumoral mass, by cutting after identification the temporal fascia. Thus was revealed a round tumoral mass of about 2.5×2.5 cm², clearly bordered by the temporal fascia but adherent to the temporal muscle (Figure 1C). The lesion was removed together with an area of the adjacent temporal muscle. The wound was closed by primary intention and the healing was uneventful. After surgery, the patient received treatment with Sulcef (2000 mg daily) and Dexamethasone (vial of 6.6 mg, twice daily) for three days and the temporal swelling disappeared.

Morphological examination

Gross examination

The surgical specimen measured $3 \times 3 \times 1$ cm³, had elastic consistency, a red-brown color and on cut section a cheesy material oozed out (Figure 1D).

Histopathological examination

After fixation and paraffin embedding, histological sections were stained using Hematoxylin–Eosin (HE), Masson's trichrome, van Gieson's trichrome, Periodic acid–Schiff (PAS) and Giemsa. Within the connective tissue and adjacent skeletal muscle were observed several granulomas with suppurative necrosis, surrounded by concentric scar tissue (Figure 2A). At low magnification, inside the granulomas were noted longitudinal and several oblique and cross-sections of worms and even dead worms (Figure 2B). At higher magnification, the worms presented thick multilayered cuticle with longitudinal ridges (Figure 3, A and B), lateral chords (Figure 3C), well-developed musculature (Figure 3C), paired uterus and a digestive tract (Figure 3D). All these features suggested a dirofilariasis infestation with a non-gravid female *D. repens*. The parasites were located 0.546 mm to each other as measured by an Axio Imager D2 microscope equipped with an AxioCam 503 camera (Carl Zeiss Microscopy, Jena, Germany).

Around the worms, it was observed an inflammatory reaction consisting of macrophages, epithelioid cells, lymphocytes, plasma cells, neutrophils and numerous eosinophils (Figure 4A). Some eosinophils were attached to the cuticle and infiltrate the dead dirofilarias. The adjacent fibroadipose and skeletal muscle tissue exhibited lymphomonocytic infiltrates.

Immunohistochemical findings

Immunohistochemically, in the inflammatory infiltrate

we noticed the presence of both T-cells (CD3 positive) and B-cells (CD20 positive) (Figure 4B) in nearly equal ratios, with the CD4+ T cell subset (Figure 4C) being better represented than the CD8+ T cell subset (Figure 4D) around the parasites. Furthermore, the most numerous inflammatory cells were the macrophages (CD68 positive) (Figure 4E) followed by plasma cells (CD138 positive)

(Figure 4F) and eosinophils (especially highlighted in Giemsa staining).

A diagnosis of left intramuscularly temporal dirofilariasis with *D. repens* was suggested based on the above-mentioned findings.

The specimen was not further processed for molecular tests to detect the species.

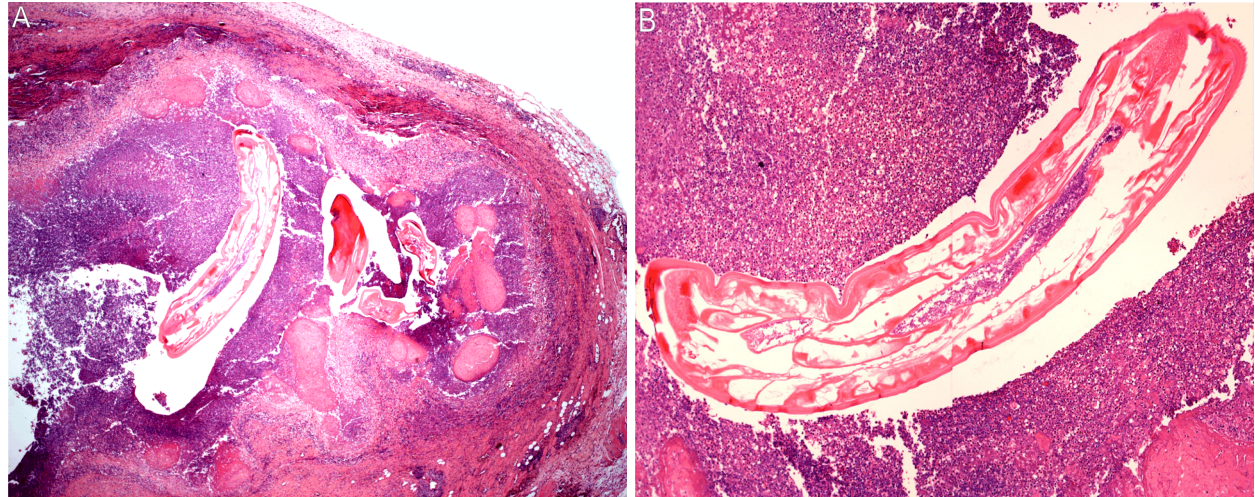


Figure 2 – HE staining: (A) Suppurative granulomas with longitudinal and several oblique and cross-sections of worms ($\times 25$); (B) Longitudinal sections of a *Dirofilaria repens*. Panoramic image composed of $\times 100$ images.

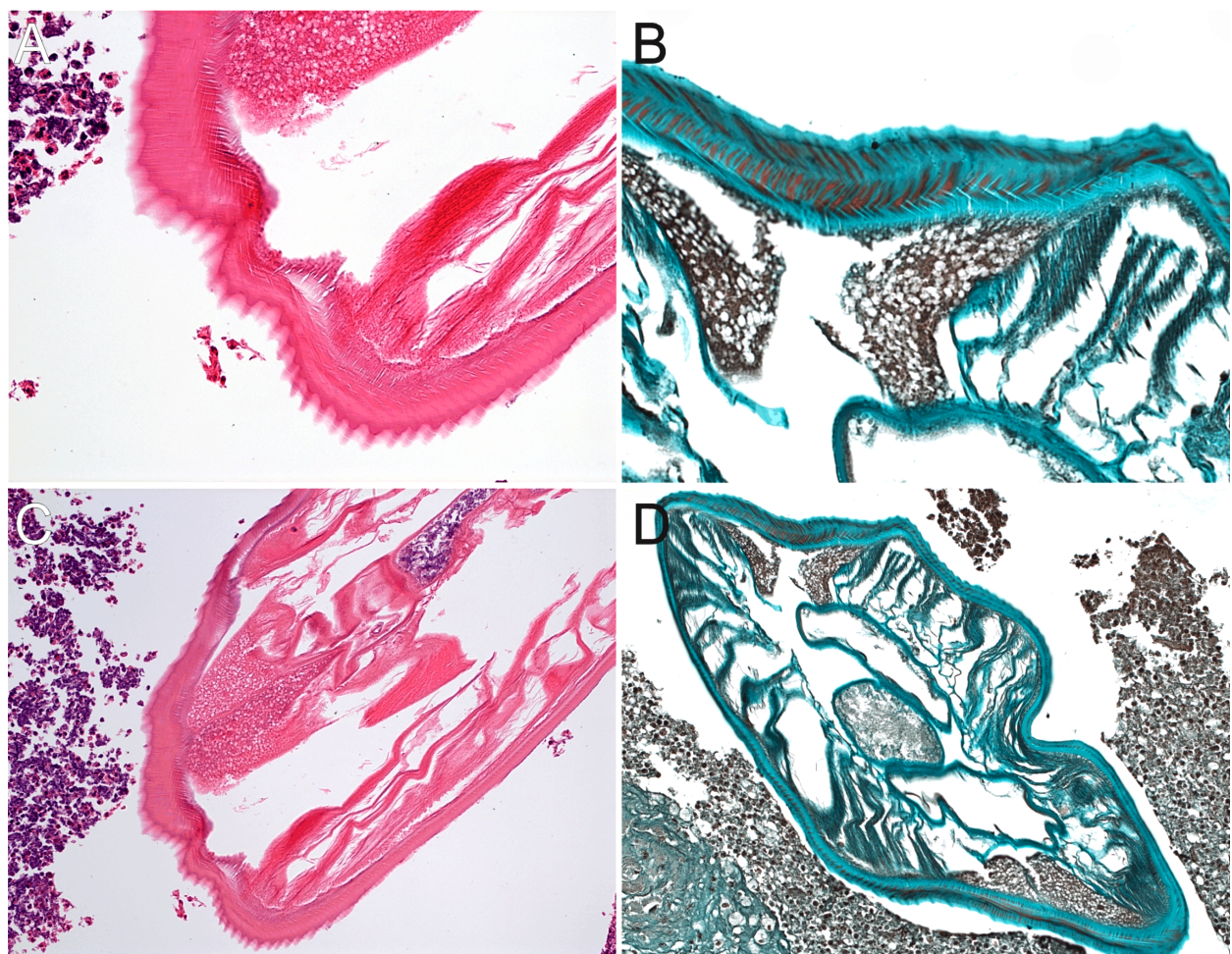


Figure 3 – (A and B) *Dirofilaria repens* – multilayered cuticle with longitudinal ridges; (C) *D. repens* – lateral chords and well-developed musculature; (D) *D. repens* – paired uterus and a digestive tract. HE staining: $\times 200$ (C); $\times 400$ (A). Masson's trichrome staining, $\times 200$ (D); $\times 630$ (B).

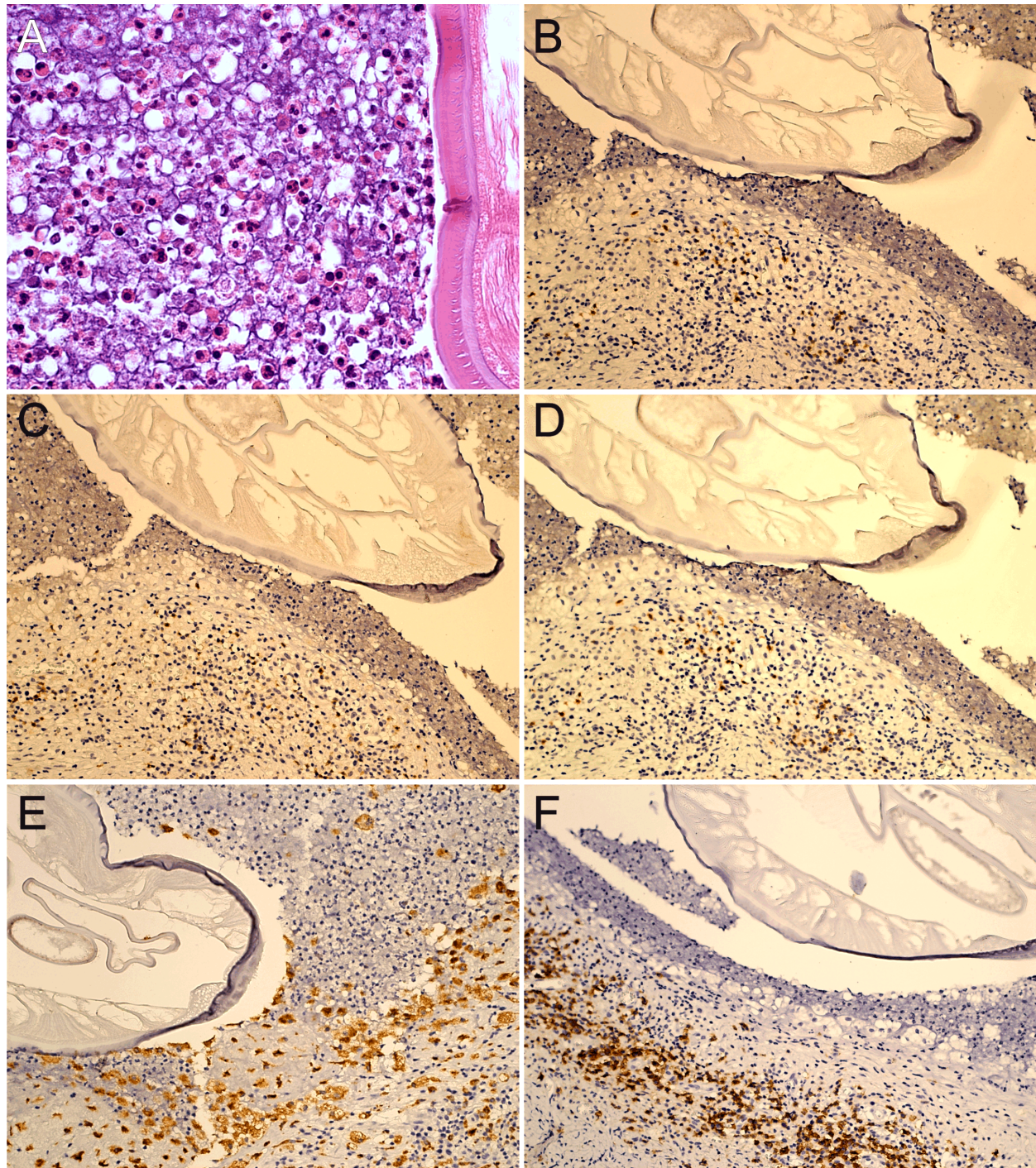


Figure 4 – (A) Suppurative granulomas consisted of macrophages, epithelioid cells, lymphocytes, plasma cells, neutrophils and numerous eosinophils (HE staining, $\times 630$); (B) CD20+ B-lymphocytes (brown) surrounding parasites and infiltrating the adjacent fibroadipose and skeletal muscle tissues (Anti-CD20 antibody immunostaining, $\times 200$); (C) CD4+ T-cells (brown) surrounding parasites (Anti-CD4 antibody immunostaining, $\times 200$); (D) CD8+ T-cells (brown) surrounding parasites (Anti-CD8 antibody immunostaining, $\times 200$); (E) CD68+ macrophages (brown) surrounding parasites (Anti-CD68 antibody immunostaining, $\times 200$); (F) CD138+ plasma cells (brown) surrounding parasites (Anti-CD138 antibody immunostaining, $\times 200$).

Post-operative management

The patient was discharged as cured from the surgical point of view and was guided toward a specialized laboratory for the diagnosis and treatment of parasitic diseases. The serological enzyme-linked immunosorbent assay (ELISA) for the evidence of exposure to *Ascaris lumbricoides*, *Echinococcus* spp., *Taenia solium*, *Toxocara canis*, *Toxoplasma gondii*, and *Trichinella spiralis* was

negative, but the serum immunoglobulin E (IgE) level was elevated (902.6 IU/mL, the normal values being less than 100 IU/mL). However, the definitive diagnosis was supported by the serodiagnosis assay of filarial infections based on an enzyme-linked immunosorbent test (EIN), which screen for the antibodies against the main filarial species: *Brugia* spp., *Dirofilaria* spp., *Loa loa*, *Mansonella* spp., *Onchocerca volvulus*, and *Wuchereria bancrofti*. This test proved high serum level of antibodies

against *D. immitis* antigen. Thus, correlating the biochemical data with morphological ones the final diagnosis was established as left intramuscular temporal dirofilariasis with *D. repens* species.

An anthelmintic treatment was established with Diethylcarbamazine (100 mg tablets; one tablet in the first day, two tablets in the second day, and three tablets in the third day for seven consecutive days). After the treatment, the serological tests have been normalized and the patient did not present any clinical or radiographic evidences of recurrence within a period of 15 months of follow-up.

Discussion

In recent years, there has been a significant increase in the number of human dirofilariasis cases. Thus, if in 2009 the worldwide reported number of cases was of 1200 [10], in 2015 there were recorded over 4000 cases [15]. In addition, in the last decade it was noticed an increasing trend of both canine and human infections extension from the endemic southern Europe areas towards the northern and northeastern regions [1, 6, 16–18]. To this fact, several factors seem to contribute, such as: (i) global warming, which influence the spreading of dirofilariasis vectors; (ii) increasing prevalence rates of *Dirofilaria* infections in domestic and wild animals, alongside movement and relocation of these infections hosts in popular travel destinations throughout the Europe continent; (iii) introduction of new, competent vectors; (iv) increasing parasite resistance to chemoprophylaxis; and (v) increasing human tourism and migration in or from the endemic areas [1, 6, 16–19].

Regarding the prevalence of *Dirofilaria* infections both in humans and animals in countries from the Balkan Peninsula, the data are scarce and inconsistent. Since 1996, during the VIIth European Multicollloquium of Parasitology, it was reported a prevalence of *Dirofilaria* in dogs ranging from 3% to 35% in countries from this part of the European continent [20]. Data from the first half of the twentieth century do not specify which *Dirofilaria* spp. are prevalent in Romanian regions and barely in 2012, a nationwide serological screening reported a high prevalence of *D. immitis* species in Tulcea County (from 6% to 14%), followed by south, southwest and southeast Romanian districts (3% to 3.3%) [21]. Moreover, other researchers reported an even higher incidence for these species, respective of about 24% in different areas of Romania [22]. Nevertheless, more recent data indicated a 7.1% seroprevalence of *D. immitis* in the east and the south Romanian Counties [23] and respective 8.9% in dogs from the Romanian eastern part, with the highest values in Galați County (60%), followed by the Counties of Vaslui (12.0%) and Iași (7.7%) [24]. Regarding the Romanian prevalence of *D. repens*, data are more limited, this species being identified in the northeastern [25] and southern [26] regions. More recently, Ionică *et al.*, found an overall prevalence of 6.92% for *D. repens* and a 23.91% incidence for coinfections of *D. immitis* and *D. repens* in dogs in Romania [23]. The authors revealed the highest prevalence rates of both *Dirofilaria* spp. as being recorded in the districts that include the Danube's floodplains, where the climate is suitable for the development of some vector species for these parasites. Also, if

for *D. immitis* it was stated that the main foci are located only in proximity of major rivers, for the *D. repens* the geographical distribution seems to be wider [23]. In the eastern Romania, more exactly in Galați County, both the serological and preventive chemotherapy (PCT) tests proved the presence of *Dirofilaria* spp. coinfections in only four dogs [24]. Furthermore, *D. repens* was confirmed by molecular methods in four dogs exported from Romania to Germany [27].

In Romania, throughout time, were reported very few cases of human dirofilariasis. One of the first reported cases was described in 2009, in a 29-year-old woman, which was addressed to the Department of Ophthalmology from the Emergency County Hospital of Craiova, with a bulbar conjunctival nodule at the right eye [13]. After incision, a live worm was extracted and the microscopic investigation identified it as being an immature *D. repens*. In 2012, Popescu *et al.* reported a case of human *D. repens* infection developed in a 31-year-old male, who lived in a suburban area (near Bucharest), which clinically present a subcutaneous nodule of the lower right abdominal region [14]. Both the histopathological examination and the PCR tests confirmed the *D. repens* infection. Rășcanu *et al.*, reported in 2014 a series of eight cases of human dirofilariasis diagnosed between September 2012–November 2013, at the “Matei Balș” National Institute for Infectious Diseases, Bucharest, Romania [28]. Patients' age ranged from 16–74 years, with a male/female ratio of 1:3; six cases were from southern Romania, and two cases were from eastern regions. In four cases, patients presented with skin nodules and in the remaining cases with conjunctival nodules. In six cases, the morphological examination identified *D. repens* but without further analysis being done for species identification [28]. In 2015, it was published a case of a recurrent subcutaneous human dirofilariasis due to *D. repens* diagnosed in the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania [29]. A 65-year-old woman, living in Bihor County was presented in August 2012 to the infectious disease doctor for a left shoulder subcutaneous nodule. Histopathology and molecular tests confirm the *D. repens* infection. After surgical removal of the nodule and seven days treatment with Albendazole 400 mg/day, the symptoms were remitted until December 2012, when the patient returns with a left breast subcutaneous nodule. After the surgical removal, it was again confirmed the existence of *D. repens* infection. The patient was treated with a single dose of Ivermectin 150 µg/kg and no further nodules developed [29].

The most affected human maxillofacial regions by the *D. repens* infestation seem to be the subconjunctival tissue and subcutaneous skin around the eyes [18]. Reviewing the literature data regarding the subcutaneous temporal region location of the human dirofilariasis, we found no more than six cases. The first reported case belongs to a 31-year-old man resident in Monfalcone (located on the coast of the Gulf of Trieste, Italy), who presented a nodule in the right temporal region, from which a nematode from the *Dirofilaria* spp. was morphological identified but without being able to specify what subspecies it was [30]. In 1996, Pampiglione *et al.* reported

a case of human *D. repens* infection localized in the left temporalis muscle, in a 43-year-old woman resident in southwestern Sicily [31]. In a case series of 19 human *D. repens* cases reported between 2001–2008 in Serbia, one was located in the temporal region; the diagnosis being established by histopathological and molecular tests [32]. In 2015, an infection with the *D. repens* was diagnosed in a German citizen, resident in a village close to the river Elbe, who addressed to the doctor with a subcutaneous nodule on the right temple. After surgical removal, the infective subspecies was established by histopathological and molecular exams [33]. In the same period, in northern Germany there was reported another case of human dirofilariasis, developed in a 40-year-old patient as a painless swelling in the left zygomatico-temporal region [34]. Histopathology and molecular investigation demonstrated *D. repens* infection. In 2015, it was reported a cases of imported dirofilariasis in a Japanese woman, who was infested during her trip in Sardinia Island [35]. Two years after infestation, she developed a subcutaneous nodule of the right temporal region. Based on its morphological features and presence of the mitochondrial 12S rRNA gene sequence, it was established that the *D. repens* was the infesting agent. Moreover, the 12S rRNA gene sequence of this parasite was 100% homologous with that of *D. repens* isolated from an Italian man confirming the imported character of this human dirofilariasis case. It seems that our case it is the seventh case with temporal region localization and the second one with intramuscular development.

Very often, the patient does not remember being bitten by mosquitoes, and for many months, the patient is asymptomatic as we recorded in our case. When the *D. repens* infestation becomes clinically visible, the patient commonly presents a single painful subcutaneous or subconjunctival nodule, which may raise suspicion of a malignant tumor or other non-tumoral lesions (tuberculosis or fungal infection) [8]. Therefore, usually the diagnosis of human dirofilariasis relies on the microscopic and morphological identification of the *Dirofilaria* spp. isolated from tissue lesions [36].

In our case, presence of thick, multilayered cuticle with longitudinal ridges, large lateral chords and large muscle cells, confirm *D. repens* infection. Actually, the *D. immitis* infection was excluded by the fact that unlike *D. repens*, this *Dirofilaria* spp. do not show longitudinal ridges and transverse striations [37]. The differential diagnosis could include other human zoonotic *Dirofilaria* spp. or nematode with similar subcutaneous location. In this regard, we must consider the possibility of human infection with *D. tenuis*, *D. ursi* and *D. striata*, which could be imported by traveling in other countries from outside of Europe [38, 39]. However, these *Dirofilaria* spp. differ in respect to the body dimensions and a smaller number of ridges. Other nematodes with human subcutaneous location that must be taken into account, particularly among travelers which go to tropical countries, could be *Onchocerca volvulus* (in which the cuticle shows circular stripes), *Loa loa* (in which the cuticle is smooth and with knob-like structures) [38, 40], *Onchocerca jakutensis* [41], *Onchocerca lupi* [42, 43] and *Setaria labiatopapillosa* [44] (in all these last three species the

cuticle have a circular and not longitudinal configuration as it is in *Dirofilaria* spp).

The results of histopathology examination could be in some measure confirmed by the serologic study; such was in our case, where the EIN test detected significant antibody titer of anti-*D. immitis* antigen. However, we must keep in mind that in 30% of cases this test can be false-positive because of cross-reactions with other nematode antigens, such as *Toxocara canis*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Ancylostoma duodenale*, or *Necator americanus* [45]. As in our case this test was negative, the EIN test may be interpreted as positive and may be considered specific for the diagnosis of human dirofilariasis. Better results could be obtained by using ELISA tests specifically developed for the detection of *Dirofilaria*-specific antibodies based on purified somatic antigen of immature *D. repens* removed from human cases [16]. Another diagnostic method is based on the immunohistochemical or ELISA detection of *Wolbachia* antigens (e.g., WSP – *Wolbachia* surface protein) [6], considering that *Wolbachia* is a symbiotic bacteria that are essential for larval development in vertebrate hosts and for the long-term survival of adult worms [46]. Nevertheless, such methods have proven to be limited since *Wolbachia* was recently found in new species of filariae from the *Onchocercidae* family [47].

When the conventional morphology could not discriminate between the *Dirofilaria* spp. (the nematodes could be altered by the inflammatory response or by surgical manipulation), the polymerase chain reaction (PCR) assay, which can be done inclusive on paraffin-embedded sample, has proven its usefulness [48]. As target for PCR amplification were used regions of mitochondrial (cox1) [49] or nuclear genes (12S rDNA, 5S rDNA) [50, 51]. Recently were developed loop-mediated isothermal amplification (LAMP) assays for the diagnosis of *D. immitis* [52] and *D. repens* [53], which proved to be more specific and sensitive than conventional PCR based on the same gene. Also, being a rapid and less expensive diagnostic method, this could serve as a screening tool for these *Dirofilaria* spp. infections in endemic regions [53].

In the present case, after surgical removal of the lesion, the patient underwent an anthelmintic treatment with Diethylcarbamazine and the clinical and lab data normalized and no evidence of recurrence was recorded within a period of 15 months of follow-up. The treatment of choice in human dirofilariasis consists in surgical excision of the lesions with a follow-up period, during which the patient will be monitored for other symptomatology characteristic of nematode infestation [54]. Anthelmintic chemotherapy is not routinely administered prior or after the surgical removal of dirofilarial lesions. However, an antifilarial medication based on a Ivermectin and Diethylcarbamazine or Ivermectin and Doxycycline/Tetracycline could be use in recurrent cases [12], cases with deep located lesions in the body to prevent further invasive surgery or as prophylaxis in patients after heavy exposure to hematophagous insects in endemic areas for dirofilariasis [55]. Moreover, in cases with suspected co-infections and in those cases with multiple foci, an adjuvant antibiotic therapy should also be considered [14, 34].

✉ Conclusions

We reported a case of human dirofilariasis with *D. repens* species, which proved to be the seventh case with temporal region localization reported worldwide and the second one with intramuscular development. The diagnosis was a challenging task requiring thorough morphological and biochemical investigations to exclude other lesions that could develop in such location. Such case attracts attention to the need for timely information of doctors to increase awareness for this disease having in mind that the true burden of such zoonosis in Romania is unknown.

Conflict of interests

The authors declare no conflict of interests.

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Received: October 17, 2016

Accepted: July 11, 2017