Review

HUMAN DIROFILARIASIS: CURRENT SITUATION AND POSSIBILITIES FOR DIAGNOSIS

B. Chakarova¹, M. Mitev^{2*}

¹Department of Hygiene, Epidemiology, Microbiology, Parasitology and Infectious Diseases,
Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

²Department of Medical Physics, Biophysics, Roentgenology and Radiology, Faculty of Medicine,
Department of Diagnostic Imaging, University Hospital 'St. Kirkovich', Trakia University,
Stara Zagora, Bulgaria

ABSTRACT

Dirofilariasis in humans is a rarely diagnosed vector-borne zoonotic helminthic invasion. It is caused from filarial nematodes genus *Dirofilaria* & Railliet Henry, 1911 (Onchocercidae, Nematoda). Usually, the life cycle of the parasite takes place between mosquitoes (Culicidae) and carnivores. The human is an accidental host for the dirofilaria wherefore microfilariae in his blood circulation are almost always absent. The pathology of dirofilariasis results from the accidental localization of immature worms in the human body. *Dirofilaria immitis* caused heart and pulmonary dirofilariasis, but *Dirofilaria repens*, *D. tenuis*, and others can be found in different parts of the human body. The spread and *Dirofilaria*-invasive rates are undergone significant modifications affected by global climate change. Surgical extraction of the parasite in humans usually has a therapeutic effect, after which an etiological diagnosis is possible.

The aim of the study is to review the current situation of human dirofilariasis in clinical and epidemiological aspects and possibilities for diagnosis.

Key words: human dirofilariasis, clinical and epidemiological aspects dirofilariasis, diagnostic methods

INTRODUCTION

Dirofilariasis in humans (heartworm disease in dogs) is a rarely diagnosed vector-borne zoonotic helminthic invasion. It is caused from filarial nematodes genus *Dirofilaria* & Railliet Henry, 1911 (Onchocercidae, Nematoda). The first observation of *D. repens*-invasion in humans was probably reported in 1566 by Amato Lusitano, a Portuguese physician. He describes a worm nestled in the inner corner of an eye in a 3-year-old girl (1). In 1626 Birago incorrectly described the larval forms of roundworm specimens as *Dioctophyme renale*. In 1856 the genus was renamed as *Filaria* by Leidy and in 1911 as *Dirofilaria* by Railliet & Henry (2). In the scientific literature, about 27 species of the genus *Dirofilaria* have been reported. Only three species of them are

diagnosed as parasites in humans: *D. Immitis* (Leidy, 1856), *D. repens* (Railliet & Henry 1911) and *D. tenuis* (Chandler, 1942). Autochthonous *D. repens* infections have been found in dogs in most European countries, from Portugal to Russia (1). The main reservoir for *D. repens* in many countries in the world such as the United States, Southern Europe, Australia and Japan is the dog. (3). The life cycle of the parasite takes place between mosquitoes, which are intermediate hosts, and domestic and wild carnivores (such as foxes, wolves, raccoons) acting as definitive hosts. Adult nematodes give birth to microfilariae, which are transmitted by blood-sucking through intermediate hosts - over 70 potential species of mosquitoes of the family Culicidae (2, 4-12). *D. immitis* caused heart and pulmonary dirofilariasis, but *D. repens*, *D. tenuis*, and others can be found in subcutaneous nodules, or freely migrating (L3) filaria in the subconjunctiva, in the large vessels of the mesentery, peritoneum, the spermatic

*Correspondence to: Mitko Mitev, Department of Diagnostic Imaging, University Hospital "St. Kirkovich", Trakia University – Stara Zagora, 6000
Mobile: +359 88 770 6079, E-mail: mitev.mitko69@gmail.com

cord in the lungs and liver (13, 14). In Bulgaria only *Dirofilaria repens* is now found in humans - the people are sporadically infected. The dirofilariasis are distributed mainly in regions with warm climates and nowadays, the infection raises public health concerns in a number of countries (6, 15). *D. repens*-invasion in humans is accidental and the diagnosis of the disease creates problems for public health in a number of countries (6, 15, 16).

The pathology of dirofilariasis results from the accidental localization of immature worms in the human body (8). The worms do not reach adulthood in human wherefore microfilariae in his blood circulation are almost always absent, which makes the etiological diagnosis extremely difficult. The distribution and *Dirofilaria*-invasive rates are undergone significant modifications affected by global climate change. Preliminary clinical diagnoses of patients with dirofilariasis mostly vary and the final diagnosis is staged after the surgical removal of the granuloma or the adult filaria. In most cases, the definitive diagnosis is obtained after the worm removal. Surgical extraction of the parasite in humans usually has a therapeutic effect, after which an etiological diagnosis is possible.

The aim of the study is to review current situation of human dirofilariasis in clinical and epidemiological aspects and possibilities for diagnosis.

Current trends in the epidemiological characteristics and clinical manifestations of dirofilariasis

From the three species of *Dirofilaria*, found as parasites in humans, in Europe, *D. immitis* has been reported from Southern Europe; *D. repens* - mainly from the countries of Mediterranean region and Eastern Europe (among dogs in Italy - 66%, France - 22%, Greece - 8%, Spain - 4%, etc.), (17). Georgieva et al. (1992), Georgieva et al. (2001), Kirkova et al. (2011); Radev et al. (2016); Iliev et al. (2017) explore wild animals, stray dogs and cats, from some regions in Bulgaria and establish among them invasion with *D. immitis* and *D. repens* (18-22). Where heartworm disease is widespread in dog populations, there is a high risk for human infection, which creates serious public health problems in countries with growing populations of stray dogs (3, 23). *D. tenuis* was

reported for the first time as a parasite in humans by Pistey (4). Over 70 potential species of mosquitoes of the genera *Anopheles*, *Aedes*, *Culex* and *Coquillettidia*, are vectots for *Dirofilaria sp.* In Europe, the most powerful vector for the transmission of *Dirofilaria sp.* (according to the literature) is *Culex pipiens*. The spread and *Dirofilaria*-invasive rates are undergone significant modifications affected by global climate change. Dirofilariasis is prevalent mainly in areas with warm climates - at an average daily temperature above 14.8⁰C (below it the development of *Dirofilaria* stops) for 30 days (life expectancy of the vector) and a time interval of 130 days with increasing day (24). In the second half of the last century, more than 800 cases have been described in about 30 countries, with the age of the patients varying from 4 months to 100 years. People of working age are most often affected - around 40 years of age (3, 6, 16). In North and South America, Australia, and parts of Africa, Japan, and Oceania, only *D. immitis* is widespread among dogs. In Northern Europe, China, Saudi Arabia and South Africa - only the species *D. repens*, and in Southern and Central Europe, Russia and the former southern Soviet republics, as well as in India are found as *D. immitis* and *D. repens*. (3, 16, 25).

Vutchev et al. (2003 2004); Daskalova et al. (2006); Harizanov et al. (2010, 2014); Chakarova et al. (2013, 2014); Neichev et al. (2014); Kaftandjiev et al. (2016); Velez et al. (2019-a,b,c) are reported *D. repens*-invasion with varying location in humans in Bulgaria (26-37). Harizanov et al. (2010), Harizanov et al. (2014) presented a retrospective analysis of the reported 47 cases of human dirofilariasis, for the period 1973 - 2011. In 78.47% of cases, the subcutaneous invasion was observed, followed by subconjunctival (21.27%) and genital (4.25%) infection. The authors reported a dominance of the upper body localization (80.85%) over the lower body (19.14%). A feature of ophthalmic dirofilariasis, according to the analysis made, was the subconjunctival location of the parasite and that it was not covered by the host response. The authors also found and reported of cases of localization of the parasite in the vitreous, lens and ocular space, etc. (29, 30). For 10 years before 2003, Vutchev et al. (2003), Vutchev et al. (2004) described 12 cases with subcutaneous localization of the parasite, and for 20 years - 22 cases (26, 27). Daskalova et al. (2006) reported a patient with cutaneous left lower

eyelid dirofilariasis (28). Chakarova et al. (2013), Chakarova et al. (2014) and Kaftandjiev et al. (2016) described a rare localization of human dirofilariasis - in the epididymis (31, 32, 34). Velev et al. (2019^a) described epididymal dirofilariasis in a child as the first case report from Bulgaria (35). Harizanov et al. (2010) presented 9 cases of *D. repens* infection in only one year - 2009 in Bulgaria (29). Chakarova et al. (2013), Chakarova et al. (2014), and Neichev et al. (2014) described 3 cases with subcutaneous localization of the parasite: in the area of the right mammary gland, the eyelid of the right eye, in the right epididymis and two with subconjunctival localization, all cases were in patients from South-eastern Bulgaria (31, 32, 33). According to Chakarova et al. (2014) the average duration from the beginning of the clinical symptoms to the parasite extraction was 168 days (5 months and two weeks), (32). Velev et al. (2019^b) presented a case of a 51-year-old male with *D. repens*-invasion, localized in the tongue (36). We did not find studies with analyzes risk factors using a multivariate approach, but we believe that the dynamics of the spread of *Dirofilaria*-invasion is related to climate and social factors. The growing number of infections in Bulgaria, as well as worldwide, due to global climate change and the active migration of people and

their accompanying pets, suggests the need for better diagnosis and registration of cases (4, 6, 8).

Possibilities for diagnosis dirofilariasis

According to many authors, including from Bulgaria, due to the nature of the disease and the small number of cases, diagnosis is difficult to be defined. The most common diagnoses are neoplasms, lymphadenitis with unknown aetiology, hydrocele, lipoma, dermoid cyst, fibroadenoma, neurofibroma, allergic conjunctivitis. The authors indicate that serological diagnosis does not significantly contribute to the detection and identification of the parasite in humans, and therefore could only be used as a screening method (30, 31).

The lack of diagnostic tests to determine the dirofilariasis invading humans causes the unreliability of the facts about the spread of dirofilariasis caused by either *D. repens* or *D. immitis* (6). The females of *D. repens* are viviparous and after mating, microfilariae are released in the peripheral blood (diagnostic stage in the definitive hosts). In filarial infections, the immunological response is mainly caused by microfilariae which rarely develop into sexually mature specimens in humans.

Figure 1a. *D. repens* 70 mm long (Imagine Chakarova B.)

Figure 1b. Microphotography *D. repens* – can be seen longitudinal ridges of the cuticle (Imagine Chakarova B.)

If the infection occurs as larva migrans, especially in the subconjunctiva, and the patient was not exposed to other potential causes of larva migrans, the clinical picture is highly suggestive of *D. repens*. The anamnesis should exclude the visit of the patient to endemic areas of other filaria, such as *Loa loa* in Africa. In case of intraocular cysts or subcutaneous nodules, the diagnosis is more difficult, but a live moving worm can be seen using a pre-operative high-resolution ultrasound (1, 38, 39). In humans, *D. repens* usually reaches in its biological development to larvae of the third stage (L3) with dimensions usually 60-120 mm long by 350 µm wide (**Figures 1a, b**). The most distinctive features of are the longitudinal ridges of the cuticle, which are not present in any other filarial worm infecting humans (**Figures 2a, b**).

In most cases, in people infected with *D. repens* antibodies against the filaria are not found or very low titres can be found (40). Such low titres can be observed in other nematode infections due to cross-reacting antibodies (1). Development of diagnostics has been reported, based on the purified somatic antigen of immature female *D. repens*, removed from humans, and the application of their results have ambiguous diagnostic value (41, 42). Serodiagnosis can be a good alternative to invasive diagnostics since surgery is avoided; hidden invasions can be detected, facilitating therapeutic and epidemiological studies (43). However, serology has its drawbacks: in the first place it is not possible to determine the different level of the positive and negative predictive value of the test performed; these tests are still in the experimental field and are not yet commercially available. The problem is the existence of higher seroprevalence in humans exposed to mosquitoes - possible vectors in endemic areas. This must always be taken into account before interpreting the results of immunodiagnostics (40, 43-45). Molecular and biological techniques can be applied as modern methods (46). These methods (PCR) are included in the diagnostic kit in cases of uncertain diagnosis, especially in endemic areas (47). In the case of intraocular cysts or subcutaneous nodes, diagnosis is more difficult, but a living moving worm can be seen using high-resolution preoperative ultrasound (38, 39). In the microscopic examination, female specimens of *D. repens*

do not usually contain microfilariae. Distinctive and most characteristic features of *D. repens* are the longitudinal ridges of the cuticle, which are not found in any other member of the filariae family diagnosed in humans (48). Serological studies are not useful in human cases. Therefore, in most cases of people with *D. repens* antibodies against the filaria are not found, or very low titres can be found (40, 43). However, such low titres are also observed in other nematode infections due to cross-reacting antibodies. Analysis of blood samples by microscopy or PCR is not useful for the same reason. Szenasi et al. (2008) provided an overview of about 2,000 cases identified so far in Hungary, as well as 16 new cases (8 men and 8 women) with an average age of 60, of which 8 cases with ocular localization, 7 with subcutaneous localization. In one case, dirofilariasis was diagnosed histologically after removal of an axillary lymph node in a patient with lymphoid leukaemia. Patients had not travelled abroad. According to the authors, most cases of dirofilariasis were the result of autochthonous invasions. The authors suggested that with increasing the number of cases, this parasitic disease would become more important in Central Europe, which would increase with the improvement of the clinical diagnosis (49). Ilyasov et al. (2013) presented *D. repens* as a worm causing subcutaneous dirofilariasis, characterized by the development of benign subcutaneous nodes resembling skin tumours and ocular dirofilariasis with orbital, eyelid, conjunctival, retroocular and intraocular locations. According to the authors, intraocular and retroocular dirofilariasis caused significant damage and discomfort to patients because of the presence of worms and their surgical removal. They described a case of a 20-year-old woman in north-western Russia. She visited an ophthalmologist for pain and redness of the skin, as well as swelling of the inner corner of the left upper eyelid. The swelling sequentially migrated to the temporal zone, the lower eyelid and the inner corner of the lower eyelid. The results of the study were within normal limits. After about four subsequent relapses (about four weeks after the initial symptoms and signs), CT scan of the paranasal sinuses and orbits was performed. The scan revealed a 12x13x14 mm soft tissue structure behind the left eyeball and adjacent medial tear to the optic nerve. No other abnormalities in

the visible area of the brain and sinuses were detected (50).

Figure 2a. Front end of *D. repens*

Figure 2b. Tail end of *D. repens*

A month later, magnetic resonance imaging (MRI) confirmed the presence of a cystic structure with irregular rounded shape; smooth borders closely adhered to the eyeball and the

optic nerve (50). On T2w images, the lesion was found to have a high-density core and low-density surrounding tissue. It was found that the adjacent retrobulbar tissue was slightly swollen, the optic nerve was displaced medially and downward, and the adjacent upper muscle was displaced medially and upward (50). A retroocular cystic lesion was proven in the left orbit with a well-shaped capsule and high density but heterogeneous nucleus structure. Through high-resolution ultrasound, a well-defined 3mm wall was found resembling a cyst containing fluid and a tightly wound internal structure that appeared to move actively. The diagnosis was retroocular parasite *Dirofilaria* spp. The worm was removed by a transpalpebral orbitotomy. A live roundworm was removed from the cyst with dimensions 80x0.6 mm (50). By conventional PCR, a roundworm of the *Dirofilaria repens* species was identified. CT and MRI have been used as successful diagnostic methods for dirofilariasis by other authors (50-53).

CONCLUSION

The data on the cases of dirofilariasis in Bulgaria are incomplete and inaccurate, as at this stage the national legislation does not require the registration of cases of dirofilariasis in humans.

With the global climate changes, the increased migration flow and the accompanying problems, prognostic hypotheses are formed for expanding the areas of vector-transmitted zoonotic infections and invasions.

The combination of serological, histological, molecular, CT and MRI methods and techniques increases the diagnostic possibilities in human heartworm disease.

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