

## VORMS DIROFILARIASIS INCREASING EMERGING ZONOSIS IN MONTENEGRO

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### ABSTRACT

#### Summary

*Dirofilariasis belongs to the group of parasitic vector-borne zoonoses. These systemic helminthiases have been widespread distributed in the world, with over 50 million registered cases. Two main filarial species (spp.) have adapted to canine, feline and human hosts: *Dirofilaria repens* and *Dirofilaria immitis*. Human dirofilariases caused inflammatory changes in lymph nodes, lung, irregular and prolonged fever. Different species of infected animals and humans are reservoirs of microfilariae, for blood-sucking arthropod (mosquitoes, flies, ticks). In the vector phase (6 – 10 days), through the process of metamorphosis microfilariae become invasive. In the next phases, feeding arthropods are donors of the microfilariae to sensitive individuals of the humans. The geographical distribution and infectiveness of these vector-borne parasites undergone modifications influenced by global environment and climate changes. Mediterranean area is an endemic region for filarial diseases. Montenegro is a small country in the Balkan region. First three cases of autochthonous dirofilariasis were registered in 2014 / 2015. In Montenegro, there are not data of dirofilariasis in veterinary pathology. In our cases, identification of causes based on surgical extirpation of granulomas and morphologically identification the worms has been performed in three cases.*

**KEYWORDS:** *Dirofilariasis, Diagnosis, Epidemiological, Clinical Aspects*

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### INTRODUCTION

In the Mediterranean area and in our country dirofilariasis represent increasing emerging zoonosis. (1, 2). Among much known *Dirofilaria* (D) species, the most prevalent are two main filarial species: *D. immitis* and *D. repens*. (3, 4, 5)

Blood-feeding arthropods can be transmitted these worms parasites to humans. In most of the cases the infective larvae (*microfilariae*) has injected through mosquito bites. Many species of mosquitoes are participating in transmission (genera *Culex*, *Aedes*, *Anopheles* and *Culiseta*).

(6, 7, 8) Some species of fleas (*black flea*), lice, and ticks are also resubmitted act as vectors. (9, 10)

In the past few years, the incidence of human filariasis has been reported to be increasing in many parts of the world, putting the disease in the group of emerging zoonoses. (11 - 20)

The infection caused by *D. repens* is the most widely reported dirofilariasis with endemic foci in Eastern and Southern Europe and Asia. (3, 7, 18, 19). In the Mediterranean area, the incidence of human dirofilariasis has increased especially subcutaneous and pulmonary forms of diseases (Italy, Romania, Serbia, Germany, and France). (10 - 22) In our country, human dirofilariasis was first diagnosed in 2014. (2)

Only immature worms cause human infections. Those conclusions based on the data obtained in the last 10 years. It is not rare to find worms of both species in anatomical localization distinct from those commonly associated with each species. Human dirofilariasis is typically manifested as either subcutaneous nodules or lung parenchyma disease. In many cases (60%) are asymptomatic. (23 - 26)

Patients infected with *D. repens* notice a subcutaneous lump (13, 15, 16) in the affected area which most commonly includes face and conjunctiva of the eye and sometimes chest wall, upper arms, thighs, abdominal wall, and male genitalia. Ocular involvement is usually a periorbital, orbital, subconjunctival, or subcutaneous infection. (11, 27, 28, 29). *D. repens* worms were found in the lungs, scrotum, penis, spermatic cord, epididymis and female mammary glands (30 - 32). Subcutaneous/ocular dirofilariasis caused by an adult and preadult *D. repens* worms in subcutaneous tissues, present nodules that grow gradually over a period of weeks or months. Histology reveals four types of nodules with diverse contents and characteristics (2, 26). The highest incidence of subcutaneous dirofilariasis occurs in an individual's age 40 – 50 years. In contrast to pulmonary dirofilariasis women are seen to be more susceptible to subcutaneous dirofilariasis than men (55, 4%: 44, 6%). Human *D. immitis* infection has been associated with the human pulmonary dirofilariasis and is usually asymptomatic, and associated with hepatic, intraocular and mesenteric adipose tissues, testicular arteries, and conjunctiva (27, 28, 29, and 32). Pulmonary dirofilariasis is characterized by the formation of pulmonary nodules around immature adult worms. When the larvae reach a small or medium branch of the pulmonary artery, they block its passage, causing embolism and localized inflammation. The disease often remains undiagnosed or misdiagnosed as a malignant lesion. Single nodules appear most frequent. Multiple lesions have also been describing. Histological studies of lung nodules caused by *D. immitis* have shown that cellular reaction and inflammatory changes comprise eosinophils, lymphocytes, and plasma cells, accompanied by a histolytic reaction and inflammatory changes in the tissues surround capillaries. Necrotic regions with pulmonary artery disruption due to the existing worms have also been observed (32).

The Mediterranean region has to be endemic for dirofilariasis. In Montenegro, since January 2014, the first case of human dirofilariasis was diagnosed. In May 2015, two cases were diagnosed. (2). Veterinary service in Montenegro does not have data on the prevalence of infections in dogs. Numerous human cases have been reported for the European Union (27). Until 1999, most of the reported cases originated from the Mediterranean area, (9, 11, 12) where *Dirofilaria* spp. is traditionally endemic (Italy, France, Greece, Spain, Serbia), with sporadic reports of small outbreaks of subcutaneous/ocular infections caused by *Dirofilaria* in Germany, Netherlands, United Kingdom, and Norway. Canine dirofilariasis has not been reported earlier in Central and Northern Europe. (27)

Drastic changes of ecosystem (33, 34, and 35) present basis for epidemiological changes for this group of infectious diseases. Agent's adaptability coverage and expansion cover the spectrum of natural hosts and vectors thanks to their easy and quick transition from enzootic in zoonotic transmission cycles. This has enabled significant expansion and has given new importance to co-transmissible and co-infective forms of the diseases, with consequent hardships diagnosis, therapy and prognostic assessment (36 - 39). Identification of the species causing *Dirofilaria* nodules (34, 35) can be altered the structure of parasites or to the decomposition of worms inside nodules.

In humans, intact worms have been collected from subcutaneous nodules or ocular conjunctiva in some infection by *D. repens* (26).

Some of collected *D. repens* worms were mature females carrying intrauterine embryos or microfilariae (26, 27). In human cases with subcutaneous dirofilariasis, circulating microfilariae was reported. These data demonstrate that the full development and fertilization of *D. repens* worms in human host are feasible, contradicting the commonly accepted belief that *Dirofilaria* worms cannot fully develop in human patients.

In contrast, *D. immitis* pulmonary nodules are internal and most are asymptomatic. The worms that caused the infection are being undifferentiable. A recent analysis of *D. immitis* and *D. repens* showed that *D. immitis* stimulates more vigorous antibody productions against its energy metabolism and detoxication machinery from *D. repens*. These specific antibodies can block the activity of parasitic enzymes and limited capacity of survival of *D. immitis* larvae were the infection time in human patients due to worm metabolism blockage and consequently could account for a lack of worm maturation in the host (38, 39).

Dirofilariasis has to be considered as a differential diagnosis in patients with subcutaneous or pulmonary disturbances (pneumonia).

Effective therapy is possible by surgical removal of the adult worms. The surgical intervention is much more complex for pulmonary, ocular, retroocular and other internal locations. Chemotherapy is not recommended for human dirofilariasis. The symbiotic relationship between *Wolbachia* and various species of filariae, including *D. immitis* and *D. repens*, has provided promising new option for the treatment of filariasis using *Wolbachia* as a therapeutic target. Symbiotic bacteria *Wolbachia* are susceptible to tetracyclines, a finding that has inspired research on the effects of these antibiotics alone and in combination with other drugs on human and animal filariasis. Oral treatment with *diethylcarbamazine is usually used* (DEC) (2 mg per kg t.i.d.), only oral *ivermectine* (150 mgr per kg) promising over a period of 4 weeks was added to surgical treatment. (40)

## **METHODOLOGY**

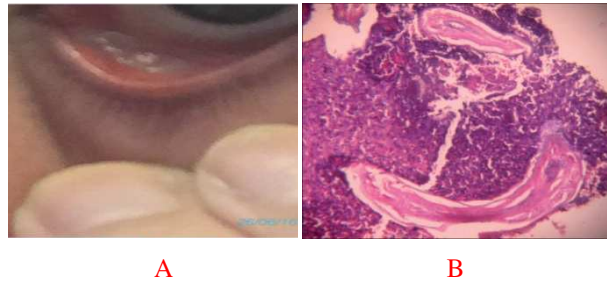
In this study, there were presented first diagnostic cases of human dirofilariasis in Montenegro, with difficulties in diagnosis, therapy, and prognosis.

In diagnostics procedures of human dirofilariasis, in the absence of microfilariae in the blood, detection is usually performed via biopsies that determined the presence of worms in nodules. After the biopsy is performed, successful worm identification is possible. Molecular and immunological diagnosis is currently viable as an alternative or complementary to morphology-based diagnostic techniques. For cases in which parasites exhibit altered morphologies due to the host's reaction, parasite detection by Polymerase Chain Reaction (PCR) is an invaluable technique with high sensitivity and specificity. Positive reactions are obtained with minimal quantities of parasite DNA. Immunohistochemical staining is used to confirm the existence of *Wolbachia* or its molecules; positive reaction indicates the prior presence of dirofilaria.

## **RESULTS**

In our investigation, dirofilariasis is diagnosed in 3 patients from 2014-2015. The patients were residents of Montenegrin coastal region: Two patients from Kotor, and one patient from Tivat. All 3 patients were civil and have never left Montenegro. Two patients were male and one female, ages 43, 29 and one girl 18 years old. In the first case, the

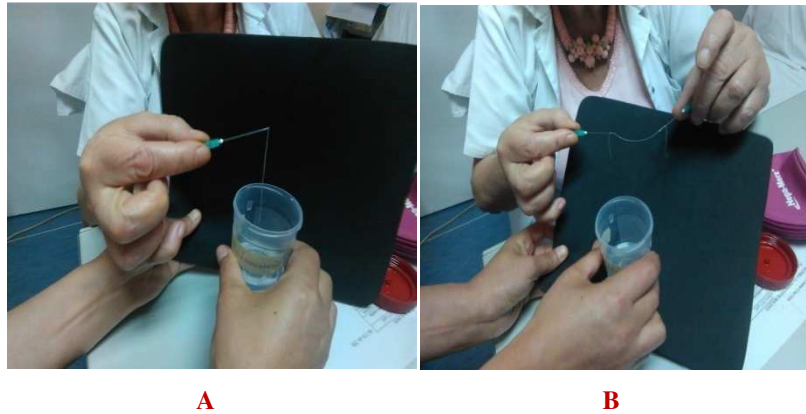
polymorphic symptoms occurred in January 2014. The ocular disturbances occurred later in the form of pain and short light flash, abdominal pain, and dry cough, lasted for long and resulting with pneumonia. After conducting an extensive search, he was admitted in the surgical ward of the General Hospital of Kotor with suspected impacted epigastric hernia. On January 10, 2014, the surgeon using intraoperatively method succeeded to extirpate entirely solid fibrous granuloma site epifascial, the midline supraumbilical. There was indeterminate thread parasite 9.7 cm long in the excised granuloma. The pathological findings showed a granulomatous tissue with new blood vessels and giant cells of foreign body-type cells and conclusions were made that such images can be found in infections with a filarial parasite. (Figure 1 A and B)



**Figure 1: Human Infection with *D. Repens*: A – Conjunctivitis (Original Photo Documentation of PhD Bogdankaandric Full Professor, 2015). B - Predominantly Subcutaneous Nodes most often Evolved into a Granuloma (Thanks to Harish S. Permi, Department of Pathology KS Hagde Medical Academy, India, 2011, Vol.3, Issue: 2, P 199 - 201)**

A multi-pattern blood test of microfilaria was negative. Histological examination of the worms has identified dirofilaria, based on the morphological exclusion of *Wuheria bancrofti*, *Loa loa*, and *Onchocerca volvulus*. Serological examination of antibodies of *Toxocara*, *Trichinella* proved to be negative. After the surgical procedure patient was treated with oral *ivermectin* (150 mg per kg), and dovicine 2x100 mgr. He feels good so far.

In another case, a patient is a hunter. He was sent to the Clinic for Infectious Diseases in Podgorica for the appearance of subcutaneous nodes with occasional itching, redness on his right forearm that appeared 6-7 months prior to arrival at the clinic, with local pain and itching, and occasional leather generalized cutaneous allergic manifestations. At the first examination on the left forearm were found solitary nodus size 4 cm, inflamed, itching and pain. A Patient complains of feeling squirming something alive under the skin, and occasional generalized skin allergy. Microbiological and serological investigations have not confirmed the etiologic suspicion of larva migrans, *Trichinella spiralis*, *Toxocara canis* and *Toxocara catis* infection. The next step was a surgical extirpation of subcutaneous nodes. After surgical intervention, an extirpation of nodes, there was found three ads like worm longer than 10, 5 cm, which in veterinary laboratories, based on morphological characteristics was identified as *D. ripens*. (Figure 2 A and B)



**Figure 2: A And B.: Worm, Longer then 10,5 Cm, Morphologically Identified as *Dirofilaria Repens* to Veterinary Laboratory in Podgorica (Original Photo Documentation of Prof.Dr.Bogdanka Andric, 2015)**

The third case was a student from Tivat, sent by a surgeon because of the appearance of nodus and strong itching changes in the right forearm. The change occurred three to four months before surgical intervention, And in the beginning with sub febrile temperature, general weakness, and exhaustion. (Figure 3)



**Figure 3: After Mosquito Bite, a Manifestation of Erythema and Inflamed Nodus in Region Antebrachia L. Dex., Ask the Medical Intervention (Original Foto Documentation by Prof.Dr.Bogdanka Andric, 2015)**

After surgical incision, threadlike a worm, of 11cm length, was pulled out from the wound, which, in veterinarian laboratory in Podgorica, was identified as *D. repens* (Figure 4)



**Figure 4: In Granulomatous Solitary Nodes, Surgeon Pulled Out Thread Like Worm 11 Cm Long from the Wound, Which in the Laboratory in Podgorica, was Identified as *D Repens*. (Original Photo Documentation by Prof.Dr.Bogdanka Andric, 2015)**

*Dirofilariasis* has to be considered as a differential diagnosis for patients with subcutaneous or pulmonary disturbances (pneumonia). Effective therapy is possible by surgical removal of the adult worms. Oral treatment with *diethylcarbamazine* (DEC) (2 mg per kg t.i.d.) over a period of 4 weeks was added to the surgical treatment of patients, only oral *ivermectine* (150 mg per kg).

## DISCUSSIONS

Dirofilariasis is considered as a group of Parasitic Vector-Borne Diseases (PVBD) caused by species of the genus *Dirofilaria* transmitted by vectors. (3). Among all *Dirofilaria* species in human pathology, the most relevant are *D. immitis* and *D. (Nochtiella) repens* (4, 5) due to their severe pathological effects and their high prevalence and incidence in the world. *D. immitis* produces both canine and feline cardiopulmonary dirofilariasis, whereas *D. repens* causes both canine and feline subcutaneous dirofilariasis. In addition, *D. immitis* and *D. repens* are responsible for human pulmonary and subcutaneous/ocular dirofilariasis, respectively, throughout to the world (3, 13, 15, 16, 17, 18, 27).

A comparison of the historical and epidemiological data in a 10-year period shows that changes in the distribution and prevalence of dirofilariasis are occurring in the World. These changes could be partially attributed to the growing interest of the dirofilariasis, especially with respect to human infection, and to climate change, which has increased the range of specific vectors of *Dirofilaria* spp. (21, 34, 35, and 36)

The Culicidae mosquitoes are highly diverse, more than 3000 species have adapted to a range of habitats that extend from coastal areas to mountain ranges. The studies present enzymatic activity in recovered larvae or Polymerase Chain Reaction (PCR) based filarial DNA replication (38) has demonstrated vector activity for *D. repens* by *Anopheles maculipennis*, *Aedes aegypti*, *Mansonia uniformis*, *Mansonia annulifera*, *Aedes albopictus*. (6 - 9) All the species that have been implicated in *D. immitis* transmission belong to the genera *Culex*, *Aedes*, *Anopheles*, and *Culiseta*.

Transmission of dirofilariasis is influenced by two factors that affect each of the two components of the worms life cycle: human behavior with regarding pets and climatic factors that allow the presence of competent vector population and *Dirofilaria* spp. Larval development in these vectors. (10, 14, 21)

In Europe, dirofilariasis is characterized by the presence of *D. immitis* and *D. repens* the coexistence of both species in some countries and their expansion within countries where they are traditionally endemic. (8, 27). Epidemiological studies and recent clinical reports on the expansion and emergence of dirofilariasis described a significant prevalence of autochthonous infections of dogs with *D. repens* and/or *D. immitis* in central and northern European countries where canine dirofilariasis was previously not reported (14).

Until 1999 most of the reported cases originated from Mediterranean countries where *Dirofilaria* spp. is traditionally endemic (Italy, France, Greece, Spain) (11, 17, 19, 27). Over the following decade, more cases were reported in Mediterranean countries (1, 3) and at the same time, a series of cases were described in Turkey, Serbia, Croatia, Hungary and Austria.

In each of these hosts, *D. immitis* and *D. repens* exhibit specific developmental patterns, each with distinct biological and clinical implications. (4) At the same time, both *D. immitis* and *D. repens* are themselves hosts to symbiotic bacteria of the genus *Wolbachia*. *Wolbachia* is a type of *Rickettsia*, and are symbiotic with filarial nematodes. (39, 40) These bacteria are largely responsible for the pathogenesis of filarial disease in that they are the principal initiators of innate inflammation and their release correlates with a severe adverse inflammatory reaction. (23, 24, 26) The study which has resulted in a profound shift in the understanding of filarial biology, the pathogenic mechanisms of infection in their hosts, and issues related to the treatment of this parasitic disease (23, 24). Traditionally, the involvement of vectors in the parasite life cycle makes dirofilariasis transmission and distribution susceptible to global climate change, and rates have undergone rapid and significant changes in defined geographic regions in recent years. (36, 37, 38) The knowledge that *D.*



repens and *D. immitis* have different hosts, explains the expansion and disease severity (39, 40)

## CONCLUSIONS

Parasitic transmissible zoo-noses in Montenegro belong to the group of emerging infection since it is growing public health problem. Considering the fact that there hasn't been dedicated enough research space to this group of infectious diseases, the consequences would be reflected in the future.

From an epidemiological perspective, dirofilariasis is considered emerging human parasitic disease. Rapid and significant changes in the distribution and prevalence of canine and feline reservoirs are being reported around the world. These changes, in turn, change the epidemiological parameters for human dirofilariasis. More frequent reports of human dirofilariasis in Europe, show severity, especially in cases of subcutaneous/ocular dirofilariasis, has raised concern about and interest in this disease and heightened vigilance in recent years among physicians and researchers. Knowledge of the genome, proteome, biochemistry, metabolism, molting, pathogenesis, and survival mechanisms of *D. immitis* and *D. repens* is limited to date. Further in-depth studies of these topics may provide information on novel therapeutic and infection control targets and explain the differences in the relationships between these parasites and their different hosts.

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## **ETHICAL CONSIDERATIONS**

All experiments were carried out in compliance with the relevant laws and guidelines, in accordance with the ethical standards of the Declaration of Helsinki.

