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DIAGNOSTIC DIFFICULTIES OF TRANSMISIVE WORMS ZOONOSES DIROFILARIJASIS AND THELASIASIS IN HUMAN AND VETERINARY PATHOLOGY

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ABSTRACT

Dirofilariasis(1) and Thelasiasis(2) diseases are transmissible parasitic zoonoses, previously considered to be accidental and rare human diseases. In the last 10 years, the increased number of recorded cases in human and veterinary pathology worldwide has been correlated with the geographical expansion and increased prevalence of these diseases in the hosts to which they have been adapted. The dramatic global changes in ecosystems (3 - 7), which have been largely influenced by human activity, have been the basis of epidemiological changes in this group of infectious diseases. The high adaptability of the parasites and the increasing spectrum of natural hosts and vectors, contributed to their transition from enzootic to zoonotic transmission cycles, without the need for longer-term evolution in enzootic cycles.

Clinical diagnostics are not easy to conduct due to the registered multietiological co-transmission of infectious agents across to the same vector and co-infectious or symbiotic forms of the disease, with consequently more severe forms. In 60% of those infected, the disease progresses asymptomatically. In manifested forms of disease, the similarities of the clinical presentation with other diseases and numerous complications complicate the clinical diagnosis. In co-infection forms, species identification is difficult because of the similarity of clinical presentation (D. imitis and D.repens, and Thelasiasis) (8,9). These problems are compounded by the problem of immunological evaluation, which is important for the etiologic diagnosis and therapy, especially in cases of frequent findings of co-infectious and symbiotic forms of the disease. (10)

In relation to therapeutic treatment, the problems are related to the frequent need for operative resolution of the most frequent manifestations (subcutaneous and ocular dirofilariasis / thelasias), worm extirpation and morphological identification. Serological identification of specific antibodies is very complex and uncertain due to the exceptional abundance of the causative antigens and the host immune response, respectively.(11). The method of choice is PCR, which detects the presence of the smallest amounts of parasites` DNA. (12)

All of our patients were treated with oral administration of ivemectin (150 mg / kg) + dovicine 2 x 100 mg + melarsomine / pro die. The results of the treatment proved to be satisfactory. However, worldwide there is a need is for new and more effective antibiotics.(13,14)

The Mediterranean area is endemic to numerous parasitic diseases. The first cases of diagnosed Dirofilariasis in Europe originate from the Mediterranean area (15,16). From 2015, the number of infected animals and humans with D.repens in Montenegro is constantly increasing. Between 2014 and 2017, at the Clinic for Infectious Diseases in Podgorica, tests for dirofilariasis (D. imitis and D. repens) began to conduct, and during 2017, Thelasiasa was diagnosed for the first time. In our investigations during this period, a total of 18 + 1 cases of dirofilariasis disease were registered: 5

cases of pulmonary disease, 6 cases of subcutaneous disease, 7 cases of ocular dirofilariasis, and 1 case of ocular thelasias. During the same period, there were conducted targeted trials in the private veterinary clinic "Grandov" in Bijelo Polje, Montenegro. Subcutaneous dirofilaria was diagnosed in 11 dogs, 1 case of ocular thelaziasis in cattle, and 2 cases in dogs, using operative methods, worm identification, serological IFA test and PCR method.

KEYWORDS: Dirofilariasis, Thelasiasis & diagnostic difficulties

INTRODUCTION

Dirofilariasis and Thelasiasis are diseases in the group of parasitic transmissible zoonoses in expansion, based on an increase in the number of reported cases in the last 10 years (17 – 20). This is in contrast to earlier understandings that these are accidental and rare diseases of humans. An increased number of reported cases in the human population correlates with geographic expansion and increased prevalence of these diseases in hosts to which they are adapted (vertebrates: dogs, cats, humans) (21 – 23) and haematophagic vectors (mosquitoes, ticks of the flea, some flea species (black flea , flies) (24 - 29).

The asymptomatic character of these parasites (which occurs very often) and the inability of clinical recognition are the reason for their quiet spread in the animal population, in parallel with the increase in the number of human cases. In manifest infections, difficult diagnosis is due to a number of factors such asepidemiological, morphological, immunological and microbiological, etc.(32)

Dirofilariasis has been known in animals since the 17th century. Lombardy nobleman Francesco Birago has published the first known reference to canine filariasis, describing the presence of adult worms Dirohilariae immitis in the hearts of his hunting dogs. Since 2014 Dirofilariasis is classified as a group of human parasitic transmissible zoonoses (Abb. TPZ). A Thelasiasis was classified in the same little Later, in 1917.

The dramatic global changes in ecosystems (16 - 20), which have been largely influenced by human activity, have been the basis of epidemiological changes in this group of infectious diseases. The high adaptability of the parasites and the increased spectrum of natural hosts and vectors, contributed to their transition from enzootic to zoonotic transmission cycles, without prolonged evolution in enzootic cycles (5, 6). Diagnostic problems occur also due to multietiological co-transmission of infectious agents via the same vector, co-infectious forms of disease, symbiotic forms of pathogens with other infectious agents (Wolbachia), resulting in consistently more severe forms and during the disease and complications(33-38). Species identification may also be difficult due to alteration of parasite structure or worms decomposition in nodules.

Among the many Dirofilaria species (spp.), D. immitis and D. Nochtiella repens are the most relevant in human and veterinary medicine, due to systemic pathological changes in the infected organism, and their increasing prevalence and incidence. D. immitis is the causative agent of canine and feline cardiopulmonary dirofilariasis (39,40), and both D. immitis (DI) and D. repens (Abb. DR) are causative agents of pulmonary and subcutaneous (40,41,42) / ocular dirofilariasis (43,44,45), irregular prolonged febrile conditions in humans and animals in worldwide. In 60% of cases, the infection proceeds asymptomatically.

DR-induced diropfiliriasis is most commonly reported Eastern Europe, southern Europe, and Asia.(42,46-48). By

1999, most of the cases reported came from the Mediterranean region, traditionally endemic to Dirofilaria spp. (Italy, France, Greece, Spain, and Serbia). Sporadic reports of minor outbreaks of subcutaneous / ocular dirofilarial infections have been reported in Germany, the Netherlands, the United Kingdom and Norway. The first case of subcutaneous human dirofilariasis in Montenegro was registered in January 2014. (49)

Only immature forms of dirofilaria (microfilariae) can cause human infections (50,51). It is not uncommon to find both types of worms in anatomical sites, which are different from those common to each species. The inclusion of vectors in the life cycle of parasites makes their transmission and distribution susceptible to global environmental and especially climate change, as evidenced by the increased infection rates in recent years, with rapid and significant changes in defined and emerging geographic regions (3,4,5). Life cycle of Dirofilaria spp. consists of the definitive host of vertebrates and vectors (Figure 1).

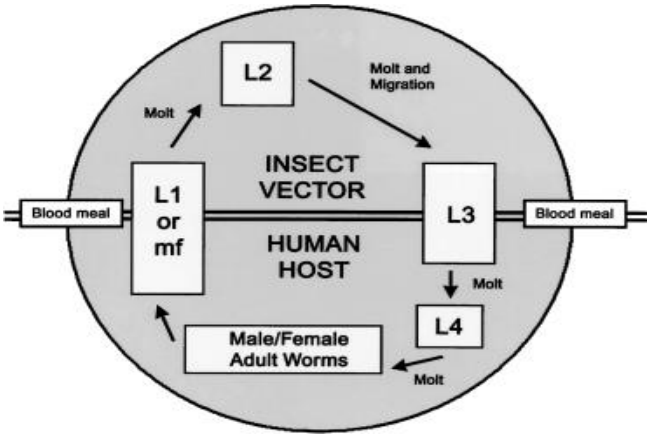


Figure 1: Life cycle of dirofilaria spp. The vectors are females of different mosquito species (genera Culex, Aedes, Anopheles, Culisera of the Culicidaea family), some species of fleas (black flea), lice and ticks are also resubmitted act as vector). DI and DR vectors become infected with microfilariae during a blood meal on infected hosts. Within 24 hours, microfilariae in the form of L-2 larvae reach the malpigia tubules of the mosquito digestive tract, and evolve to infectious L-3 larvae and then into L4. Ambient temperature is a key factor determining the time period until the development of L3 in mosquitoes.

Both Dirofilaria immitis and Dirofiliaria repens can infect many mammal species (). They are best adapted for domestic and wild canids, which are the most significant reservoirs. Humans and cats are less suitable hosts (17). In humans and cats, the development of the parasite is dramatically modified compared to the development in dogs. Adult forms of D. repens usually reside in the subcutaneous tissues of the final host (54-56), but can be found in the abdominal cavity and within the muscle fascia, where they reach sexual maturity within 6 - 9 months after infection. Microfilariae of both species reside in the bloodstream(50,51,57).

In the case of subcutaneous nodes (42,58) or ocular localization of the worm (43-45), it is usually the patient who is the first to detect the infection and seek medical treatment. In contrast, pulmonary dirofilariasis is asymptomatic in most cases, most often detected accidentally during chest X-rays (59,60). When either nodules (58) or pneumonia (60) are detected in pulmonary system or subcutaneous tumefacts are present, malignancy is not infrequently suspected, making human dirofilariasis an important differential diagnostic problem (61). The interest in dirofilariasis is due to the increasing incidence of human and animal diseases. Dirofilariae can also cause lesions in other unusual locations, including the brain, liver, eyes, peritoneal cavity, etc.(62-67) D. immitis can cause membrane glomerulonephritis due to the formation of

immune complexes, triggered by antigens from microfilariae and adult worms, with changes in the glomerular basement membrane and progression to severe nephrosis with proteinuria, renal failure and azotemia (68,69). The most common condition, which is usually registered in small dogs is vena cava syndrome (abb. VCS). This syndrome is caused by the mass of worms that go from the pulmonary arteries to the right ventricle, where they interfere with the kinetics and function of the tricuspid valvular, with the consequent increase of pressure in the right ventricle and vena cava, an increase in pressure in the systemic circulation. This complication is a common cause of death in animals due to haemolysis, haemoglobinuria, and disseminated intravascular coagulopathy (abb. DIC). Occult dirofilariasis in dogs, can result in severe respiratory syndrome due to the resulting eosinophilic inflammatory response to microfilarial antigens, with dysfunction of alveols, gas exchange disorder, hypoxia, and respiratory failure (60,70).

The first discovery of an endosymbiotic bacterium within filarias was found in D. immitis (71-76), and later in other types of filarias. Two decades later, electronic microscopy and molecular techniques have shown that this bacterium belongs to the order Rickettsiales (alpha-2-proteobacteria), of the genus Wolbachia. Wolbachiae are intracellular bacteria, observed in isolation or in clusters. They have developed a symbiotic relationship with a number of organisms, including filarias from the family Onchocercidae, of which D. immitis and D. repens are members. (Figure 2)

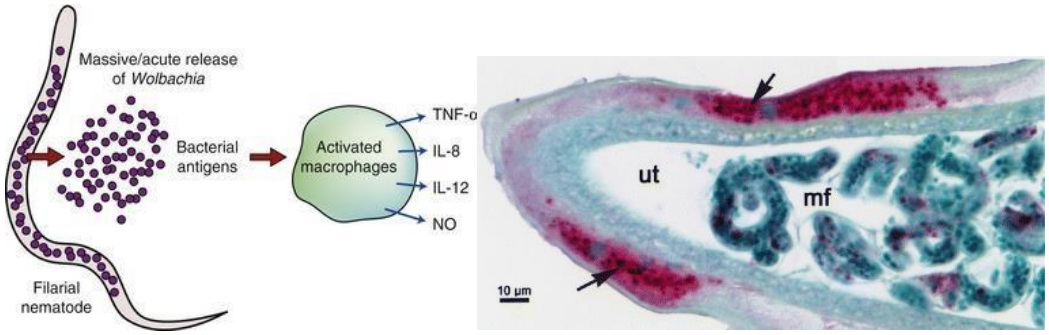


Figure 2: A In humans, soluble extracts of adult and microfilarial worms stimulate in vitro macrophage activation and production of TNF-α, IL-8, Il-12, NO, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-10 (Raman al et al., 1999; Brattig et al., 2000). Adult worm extracts exposed in vivo to doxycycline resulted in reduced IL-8 and TNF-α responses from human peripheral monocytes, as well as damage to neutrophil chemotaxis, whereas Wolbachia-free worms showed negligible inflammatory responses from human monocytes (Brattig et al., 2001)

B-Bacteria Wolbachia in the part of the adult female worm Brugia malei (200x), displays many bacteria (red) concentrated in the hypodermal lateral bands and around the uterus (ut), as well as within the microfilariae (mf) (arrows), obtained by immunohistochemical staining using rabbit antirecombinant wsp antisera (courtesy of M. Taylor, Liverpool, UK). The worms were fixed in incorporated 4% formaldehyde. in paraffin and cut into 5 preparations.

Thelaziae are spirurid nematodes of the Thelazia genus, and represent primarily veterinary parasites. They infect bovids, small ruminants, but also humans (77 – 81). Human infections are caused by 2 species: Thelazia callipaeda (Oriental eye worm) and Thelazia californiensis (California eye worm). Thelazia callipaeda was first described in 1910 in a dog in China. In 1917 Struckz from Beijing (China), reported the first case of thelaziasis in humans. Subsequently, cases of

human thelaziasis were reported in India (1948). In recent years, Romania and many other continental European countries have reported the presence of this parasite for the first time, suggesting it is rapidly expanding to new areas. Thelazia callipaeda (Spirurida, Thelaziida) infects a number of final hosts: dogs, cats, foxes, rabbits and humans. Wild and native canids are considered as the primary and final hosts for Thelazia callipaeda (77-79). T. californiensis infections in a number of mammals, wild and domestic birds, and other animals have been reported. T. gulosa is a parasite of cattle and occasionally other large ruminants.

Among the 16 known species of Thelazia, only two species of T. callipaeda and T. californiensis infect humans (79,80). The vectors for Thelasia spp. are drosophilid flies that feed on lacrimal secrets (lacrimophagous) (28). More tick species may also participate in transmission of thelazia to new hosts (Hyaloma, Haemaphysalis, Rhipicepalus, etc.). Ticks can remain infected in pasture for up to 2 years, but they do not have transovarial transmission to offspring. Theilaziae are transmitted to susceptible animals via the saliva of vector (27).

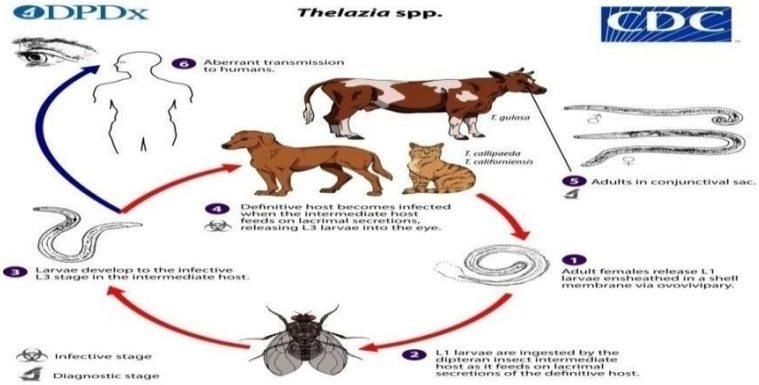


Figure 3: Thelazia californiensis is found exclusively in western part of North America. Thelazia callipaeda is responsible for most cases in India, China, Russia, Thailand, Japan, Korea, southern and northern Europe. In infected individuals or canid / bovine, first stage larvae are found in the lacrimal glands. Arthropods feeding on infected lacrimal secretions swallow larvae, which undergo 3 developmental stages in a vector for 2 - 3 weeks and develop into infectious third-stage L-3 larvae, and the vectors become L-3 donors, infectious to humans and animals. L-3 develops into adult forms within 35 days in the eye of an infected person or a sensitive animal.

In human thelaziasis main pathologic changes occur in the eye (8,81,82). This spiruroid nematode can be seen in the conjunctival sac, lacrimal gland, and tear ducts of infected mammals. []. The number of human thelaziasis (HT) cases in Asia has increased predominantly in some rural areas with low socio-economic standard. It mainly affects the elderly and children. Ocular characteristics of human thelaziasis include excessive tearing, irritation, conjunctivitis, keratitis, corneal ulcers, and ectropia. These parasites most commonly affect the anterior segment of the eye, but they can also cause serious damage to the posterior ocular segment. A case of intraocular thelaziasis has been reported with rethematogenic retinal detachment [] and intraocular inflammation involving the vitreous body, visual disturbances, and treatment requiring vitrectomy and worm extirpation.

MATERIAL AND METHODOLOGY

The first cases of human dirofilariasis in Montenegro were registered at the Clinic for Infectious Diseases in Podgorica in 2014/15 (3 cases). By the end of 2017, a total of 18 + 1 cases were registered, namely: 5 cases of pulmonary dirofilariasis, 6 cases of subcutaneous dirofilariasis, 7 cases of ocular dirofilariasis, and 1 case of ocular thelaziasis. During the same period, a private veterinary clinic "Grandov" in Bijelo Polje, Montenegro diagnosed subcutaneous dirofilariasis in dogs in

11 cases, 1 case of ocular telaziasis in cattle and 2 cases of ocular thelazias in dogs.

The methods used for the diagnosis of the disease included: anamnestic data, clinical examinations, laboratory biochemical analyses, microbiological tests (microfilaria detection tests in peripheral blood, serological (Elisa and IFA test) and PCR tests).Chest radiography and ultrasound examinations were performed if needed. Surgical extirpation of subcutaneous nodes in 4 cases in humans was supplemented by biopsy and morphological identification of worms at the Veterinary Laboratory in Podgorica. In veterinary pathology, diagnostic methods have included serological confirmation of etiologic diagnosis, indirect immunofluorescence (abb. IIF) method, PCR method, extirpation, biopsy, and morphological identification of worms.

RESULTS

The Mediterranean area is endemic to numerous parasitic diseases. Our tests, in the period from 2014 to 2017 covered vectors transmissible parasitic diseases: dirofilariasis (D. immitens and D. repens), and in 2017 thelariazis. In the same period from 2014 to 2017, at the Grandov private clinic in Bijelo Polje, Montenegro, there were conducted targeted trials, using veterinary tests. Tests confirmed subcutaneous dirofilaria was in 11 dogs, ocular thelaziasis in 1 case of cattle, and ocular thelaziasis in 2 dogs. From 2015 the number of infected animals and humans in Montenegro is on the rise.

In our studies, the diagnosis of dirofilariasis was fraught with many difficulties.

Based on epidemiological data, 78% of patients are residents of the southern part of Montenegro, the capital of Podgorica, the Skadar basin and the Montenegrin coast. One case of ocular theleziasis is a resident of the northern part of Montenegro.

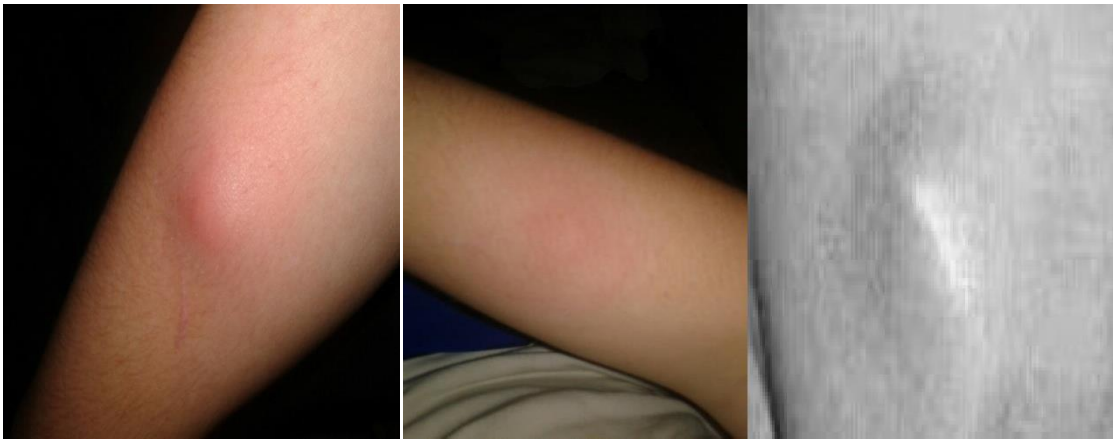
The youngest patient was 12 years old. There were 3 cases at the afe of 15 to 18 years old. The other patients were aged from 29 to 64. The distribution of respondents by gender is represented by a ratio of 68%: 42% in favour of the male gender. Over 70% of patients had pets - dogs.

The problems of clinical recognition of these TPZs are due to asymptomatic infections in 60% of those infected. In manifest infections, patients are usually the first to detect the presence of subcutaneous nodes or ocular changes, and seek help. In our investigations, 6 cases of subcutaneous and 7 cases of ocular dirofilariasis were registered. In 1 case of suspected ocular dirofilariasis, using the PCR method, we diagnosed with thelaziasis (Figure 4,5,6). Pulmonary dirofilariasis, out of 10 analysed subjects, was diagnosed with RTG findings in 5 subjects who already had symptoms suspected of subcutaneous / ocular dirofilariasis or prolonged fever with a cough (Figure 7).

Surgical extirpation, biopsy, and morphological identification of the worm were performed in 4 cases of subcutaneous dirofilariasis. In all cases, D. repens was morphologically confirmed (Figure 8). In one case, due to morphological damage to the wormby histological examination, D. repens was identified based on the morphological exclusion of Wuheriae bancrofti, Loa-loa and Onchocerce volvulus. The finding was confirmed by both serological and PCR method. Serologic examination on antibodies of Toxocara spp., Trichinella spiralis and Larva migrans were negative in all subjects. Multi pattern blood test of microfilariae was negative in all subjects. PCR is the method of choice for the diagnosis of dirofilariasis and thelaziasis, due to its high sensitivity and specificity. Positive results are obtained even when the smallest amounts of parasitic DNA are present. In our cases, using the PCR method, the etiological diagnosis of D.repens was confirmed, and in only 1 case of ocular changes was thelaziasis diagnosed. Positive immunohistochemical

methods confirmed the coexistence of Wolbachia or its molecules in 7 cases of dirofilariasis.

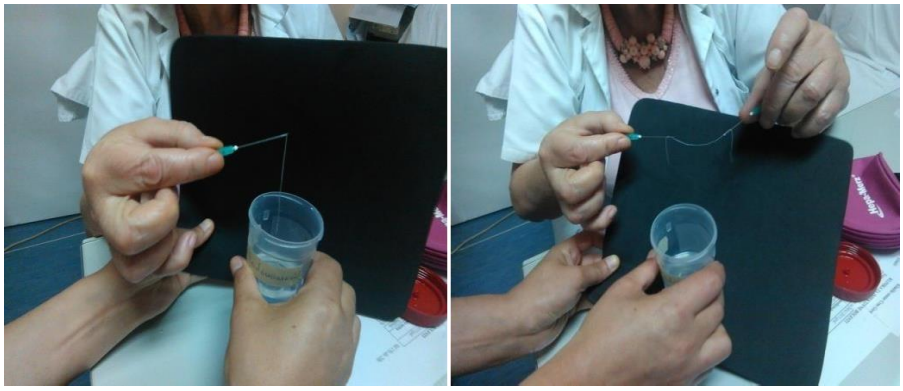
All patients were treated with oral ivemectin (150 mg / kg), doxycycline 2 x 100 mg and melarsomine pro die. The results of the treatment proved to be satisfactory.



A **B** **C**

Figure 4: **Pictures named as A and B:After Mosquito Bite, erythema and inflamed nodus in the region of antebrachi l. dex.,**

Picture named as C: Subcutaneous nodus on right breast. Needed surgical intervention. After surgical incision, thread-like worm was pulled from the wound.(A and B Original photo documentation by Prof. Dr. Bogdanka Andric 2015)

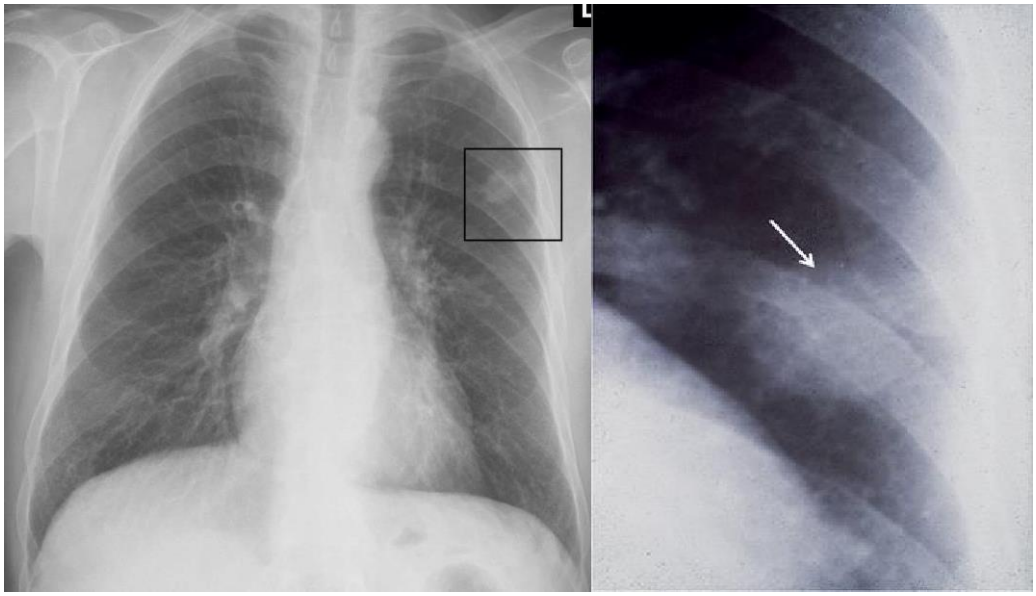


A **B**

Figure 5: A and B. Worm, longer than 10,5 cm, morphologically identified as Dirofilaria repens in Veterinary Laboratory in Podgorica (Original photo documentation of Prof. Dr. B. Andric)

A B C

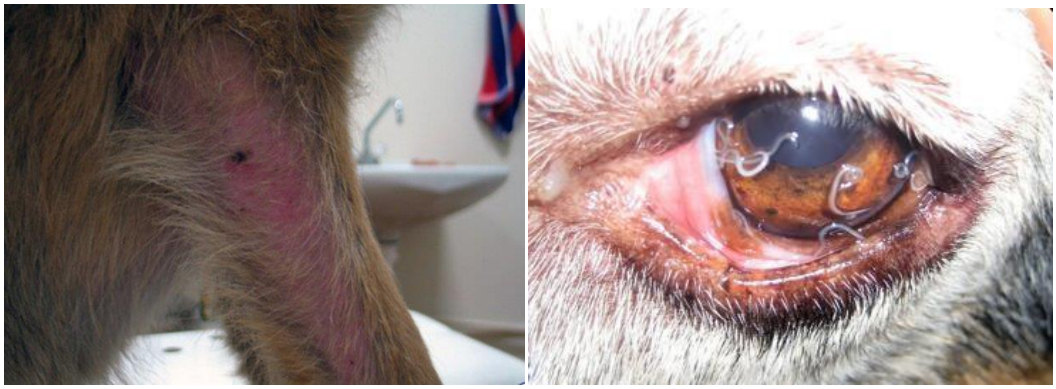
Figure 6: **Human infection with D.repens: A – Conjuctivitis and subconjuctival worm. B – Ocular worms (Original photo documentation of PhD Bogdanka Andric 2017). C-*The transmission route of oriental eye worm (Thelazia callipaeda) in Europe: a male variegated fruit fly (Phortica variegata) feeding on lacrymal secretions*. Ocular thelaziasis could be presented in different ways such as ocular irritation, inflammation and ultimately with corneal ulcerations.**



A B

Figure 7: A and B. Pulmonary form of dirofilariasis mainly proceeds as asymptomatic. When nodes are detected, it presents a significant differential diagnostic problem, primarily in relation to malignancies.

Targeted veterinary trials diagnosed subcutaneous dirofilariasis in dogs in 11 cases, in 2 cases of thelaziasis in dogs, in 1 case of thelaziasis in cattle. By serological methods (ELISA and IFA) and PCR method in the veterinary laboratory, the diagnosis was confirmed In 7 dogs, operative extirpation of subcutaneous nodes and morphological identification of D. repens were performed. In one case, the identification of an eye worm in a dog showed that it was a Thelia (Figure 8)



A **B**

Figure 8: A.Subcutaneous infiltrates in 11 dogs resulted from D.repens B. Eye worm infection (ocular thelaziasis) in a dog. Here, infection is associated with ocular discharge and blepharitis (inflamed eyelids).

DISCUSSIONS

Difficulties in the diagnosis, therapy and prognostic monitoring of TPZ (dirofilariosis, thelaziasis) can be seen through multisegmental analysis of epidemiological, clinical, immunological and microbiological characteristics.

The incidence of dirofilariasis and thelesiase spp. in a particular geographic region depends on a number of factors: drastic changes in the eco-environment, climatic conditions, which allow the rapid transition of the parasites from enzootic to zoonotic cycles (3,4,5). Transmission in the human population depends on the minimum number of dogs infected with adult microfilaria-producing worms and on the presence of one or more vector species with zooanthropophilic habits (25 – 28).

The epidemiological profile of canine dirofilaria in Europe is characterized by the coexistence of both types of D. immitis and D. repens (37). **Their spread in countries that are endemic, and a significant prevalence of autochthonous infections of dogs with DI and DR in Central and Northern European countries that have not previously had dog dirofilaria or only sporadic cases have been reported** (25 - 28). During our tests, the first case of human dirofilariasis was registered in 2014. (49) and human thelaziasis in 2017. In recent years, there has been an increase in the number of human infections with D. immitis / D. repens and Thelasiasis in our midst, both in human and veterinary medicine.

The diversity and abundance of vectors that survive infection, and the number of larvae that complete development to L3 in vectors, are factors that determine transmission efficiency and expansion of dirofilariasis in specific geographical areas (84 - 87). Studies have shown the ability of mosquito vectors to limit the number of larvae that can progress to L3, through antigen recognition and the mechanisms of humoral (HIO) and cellular (CIO) immune defense (86 -89). In the mosquito haemolymph inoculated with L3, D. immitis (90 -94), antimicrobial polypeptides have been identified that have this function. Melanization is also a limiting factor, which ends with the formation of a membrane structure on the outer zone of the cell capsule (68). The efficiency of melanization varies between species and members of the same species due to differences in enzymatic activity (phenoloxidases and other enzymes). (71,72)

Other mechanisms and structures that contribute to the destruction of larvae in vectors include buccopharyngeal armature (cibar armature), which can damage microfilariae during a blood meal. Molecules lysing the epicuticle of the worm have also been identified. Of particular importance is the coagulation of blood that traps microfilariae in the

digestive tract of mosquitoes, slowing or preventing their passage into the malpigia tubules.

The host-parasite relationship in dirofilariasis is complex mainly due to the ability of DIs and DRs to infect different hosts in which they achieve different stages of development and different pathological manifestations. The presence of Wolbachiae, an endo-symbiotic bacterium in larvae and in adult worms of both dirofilaria species, exposes infected hosts to antigens from nematodes and bacteria (61,62). The types of host immune responses elicited by the two previously mentioned antigens and the prevalence of one above another are associated with the survival or death of the parasite in the host organism and with the inflammatory response common in dirofilariasis. Seroepidemiological studies of endemic area populations worldwide have shown high rates of human infection, similar to those in canine reservoirs in the same areas (72,94 – 96).

Development from infectious larvae to adult worms and the chronic nature of most infections with DI and DR, suggest a lack of host immune response and / or the ability of the parasite to evade host control mechanisms, by reducing immunogenicity or inducing immunotolerance. By finding different isotypes of the IgM, IgG and IgE antibodies against each developmental stage of the parasite, it has become clear that in most cases the host can effectively control the infection and maintain it within the limits compatible with its own survival, destroying many of larvae. The highest level of antibodies was found in microfilariemic infections (72,73,74), which demonstrates the effectiveness of antibody-mediated mechanisms for removing microfilariae, with certain antibody classes, IgM and IgG ,mediating neutrophil adhesion to the microfilaria surface resulting in lethal cytotoxic effects on larvae. But these mechanisms are not effective against adult worms. In chronic infections, it has been observed a progressive suppression of the cellular immune response (CIO) and the preservation of the humoral immune response (HIO) along with the effect of At (75,76).

After the therapeutic treatment, mass release of antigens from dead adult worms and microfilariae is registered. Also, the release of the endosymbiotic bacterium Wolbachie is registered, which then interacts with the host tissues (75). These events are associated with an increase in host immunopathogenic responses. Antibodies of IgG classes, specific for Wolbachie dominant surface protein (WPS), have been detected in the blood and urine of dogs (), cats (), and people diagnosed with pulmonary and subcutaneous dirofilariasis (77).

Numerous reports have been published assessing differences in antibody levels and their association with the clinical status of infected hosts. **Amicrofilariomy** dogs have been described, with massive pulmonary thromboembolism that had a stronger anti-DI and anti-Wolbachia IgG response than asymptomatic **antifilariomy** dogs (77). Among dogs with glomerulonephritis, higher levels of anti WSP IgG-At were found in the urine of microfilariae dogs (77).

In humans diagnosed with pulmonary dirofilariasis, IgG or IgM responses against somatic and E / S antigens of adult worms DI were expressed, whereas IgE-At was more prevalent in asymptomatic cases. High levels of anti-WSP IgG At have also been reported in patients with pulmonary dirofilariasis, but not in asymptomatic seropositives, or in patients with subcutaneous dirofilariasis induced by DR. Based on the data presented, it could be concluded that HIO and At are associated with both parasitological status and clinical host status (72,73).

In addition to said molecular identification, later spectrometric proteomics enabled the simultaneous identification of numerous proteins. Thirty nine (39) of them were proteins from DI and 15 of them were proteins from DR, many of which were represented by several isoforms. These proteins are classified into four functional groups, including metabolic enzymes, enzymes with redox or detoxification potentials, mobility control molecules and stress response. The most

common enzymes are those involved in energy metabolism, eight of which are involved in anaerobic glycolysis in DI. Enolase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase are of particular importance. Five proteins with redox potential and four with roles in stress responses, including various heat shock proteins, have also been identified. Many of these molecules have antioxidant and detoxifying properties and are associated with the ability of parasites to neutralize reactive oxygen species released by macrophages and neutrophils. Numerous among these proteins were also found in DR, but in smaller numbers.

Several studies conducted during 1980s and 1990s identified individual molecules and their characteristics at different developmental stages of DI. In adult worms, a significant proportion of the identified molecules were acidic polypeptides from 82 kDa to 200 kDa, sensitive to various collagenases. It was later discovered that the antigenic repertoires of L2 and L3 larvae were similar, but also different from L4 larvae. A 35-kDa polypeptide has also been identified, characterized as an immunodominant surface antigen present in L3 larvae but not in larvae in subsequent stages of development. A non-immunogenic glycolipid of 6-10 kDa was also identified, as well as 35-kDa and 6-kDa molecules discharged from the surface of L3 larvae during the first days of their development in vivo and in vitro, and the released material does not replenish. Later, other proteins from D. imminis were identified, cloned, and characterized, among which the heat shock protein p27, localized to the hypodermis of larvae L3 and L4 and adult worms, was observed . Various enzymes with redox potential have been further described, including peroxy-redoxins in somatic extracts and secretory (E / S) products of adults and microfilariae as well as glutathione peroxidase in adult worms and L4, which is a precursor of neutrophil chemotactic factor. Two nuclear receptors, Di-nh-7 and Di-RXR-1, have also been reported, related to cell proliferation, differentiation and apoptosis (78). Chitin synthase and ivermectin-sensitive glutamate chloride channel subunit (79) have also been described.

Insufficiency of clinical diagnostics is due to the absence or non-specificity of symptomatology and requires differential-diagnostic considerations in relation to many other diseases with similar symptomatology. Thelazia callipaeda, Migrans larva, Toxocara canis and catis may also have similar clinical presentation.

In one of our cases, a clinical diagnosis of ocular dirofilariasis was refuted by serological and PCR evidence, which resulted in Thelasiae callipede as an etiologic agent of the disease. Pulmonary dirofilariasis, caused by both parasites, is asymptomatic in most cases and is accidentally detected. During our tests, chest RTG diagnostics were performed in 10 patients suspected of cutaneous / ocular dirofilariasis or with unclear, prolonged febrile condition. In all of 5 cases, sarcoidosis, tuberculosis, and malignancies were excluded.

Studies conducted on antibiotic treatment of host who are infected with filiaria and Wolbachiae genome sequencing have provided information on the nature of interactions between this bacterium and filariums . These studies suggested that Wolbachia participates in the structure and embryogenesis of the filarius (80,81), and the contribution of the filarius is in providing the bacterium with the amino acids required for its growth (). Wolbachia is transmitted from maternal filarias to offspring and is present in all units, at all stages of filarial development. They are particularly abundant in larvae that develop in vertebrate hosts (L3 and L4), in hypodermal structures of adult filariums of both sexes, and in the genital organs of females. These findings suggested the importance of symbiotic bacteria, as crucial for larval development in vertebrate hosts and for long-term survival of adult worms (). Wolbachia was then found in other types of filarias of the Onchocercidae family in different organs, such as the somatic gonads (epithelial layer) and the intestinal wall, suggesting that the bacterial-filarius relationship is far more complex and diverse than it was thought to be(82,83).

Symbiotic relationships between Wolbachia and various filariasis species, including DR and DI, have been seeking new options for therapeutic treatment of filariasis. Taking Wolbachia as therapeutic target (84,94,95), the objectification of this filarial blocking strategy relates to bacterial elimination and indirect elimination of the parasite (filarius). The symbiotic bacterium Wolbachia is sensitive to tetracyclines, especially in infections where L4 and L5 are present. Doxycycline administration for 2 weeks, in combination with ivemectin and melarsomine (85,86), reduced the incidence of inflammatory pulmonary lesions and thrombus. Elimination of Wolbachia induces extensive apoptosis of germ cells treated in adult worms and L3 and L4 microfilariae, suggesting that symbiotic bacteria regulate apoptosis in their host nematodes.

CONCLUSIONS

Dirofilariasis and Theileriasis are transmissible parasitic zoonoses, which have experienced great worldwide expansion over the past 10 years. This was due to the human activity and global ecological, especially climate changes, the presence of a wide range of natural reservoirs of phyla and competent vectors. The expansion of species of natural reservoirs and competent vectors allowed the parasites to be transferred from enzootic to zoonotic transmission cycles, without the need for longer evolution in enzymatic cycles resulting in the consequent expansion and disease of humans. Diagnosis and differential diagnosis of these parasitoses are not very easy, mainly due to the asymptomatic nature of the disease in 60% of cases and in manifest infections due to similarity and / or non-specificity of symptomology. Complications can be extremely tough. Diagnostic procedures are complex and involve multiple segments.

Therapy is also a significant problem. Requires surgical extirpation of subcutaneous and ocular parasitic infections, and biological identification of worms (93) . Serological analyses are also complex and burdened with an enormous number of antigens originating from dirofilaria, especially in the context of multietiological, endosymbiotic forms of the disease. Modern therapeutic treatment includes antibiotic destruction of Wolbachia, with tetracyclines to which it is susceptible, and indirect destruction of the filarius. Numerous studies are looking at the possibilities of administering new antibiotics, identifying relevant genes and enzymes involved in Wolbachia physiology, to find other target loci in the fight against dirofilaria.

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