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Review

DOG HEARTWORM DISEASE IS HERE TO STAY: THE MOST IMPORTANT ASPECTS OF CLINICAL RELEVANCE

SPASOJEVIĆ KOSIĆ Ljubica[1]*, LALOŠEVIĆ Vesna[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia

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Abstract

Heartworm disease (HWD) is a clinically important parasitic disease of the cardiovascular system in dogs, and is caused by *Dirofilaria immitis* (*D. immitis*), which resides in pulmonary arteries. Infected domestic and wild canids as hosts, and mosquitoes as intermediate hosts, are the main reservoirs of *D. immitis*. Because *D. immitis* does not reach complete development in humans, HWD is considered to be a disease with zoonotic potential. In addition to its veterinary relevance and zoonotic potential, the disease is distributed worldwide, and thus, a multidisciplinary approach is necessary to control this disease on the global level. The life cycle of *D. immitis* explains the pathogenesis and clinical signs of HWD in dogs, and determines its diagnosis, therapy and prophylactic measures. In this review, the most important aspects of HWD in canine patients are emphasized: establishment of correct diagnosis of the disease, proper diagnostic procedures for monitoring the clinical condition of an infected dog, different therapeutic protocols and how efficient they are and finally, recommendations for adequate prophylactic measures. Dogs with and without clinical signs of HWD should be tested for both microfilariae and adults of *D. immitis* at appropriate times. If positive for heartworm, the pathological changes in relevant organs, mainly lungs and heart, should be assessed in the dog. Their extent and severity determine therapeutic protocol. For dogs cured from HWD and non-infected ones, adequate preventive measures should be applied.

Key Words: clinical aspects, dogs, heartworm disease

*Corresponding author – e-mail: ljubicask@polj.uns.ac.rs

INTRODUCTION

Parasitic diseases of the dog cardiovascular system are caused by the dog heartworm *Dirofilaria immitis* (a mosquito-borne filarioid nematode) and a French heartworm *Angiostrongylus vasorum* (a snail-borne metastrongyloid nematode). Although both parasites are reported in dogs in Serbia (Spasojević Kosić et al., 2016a; 2012a; Simin et al., 2014), differences in their prevalence and transmission emphasize the greater importance of heartworm disease (HWD) caused by *D. immitis*. HWD is clinically important for dogs since it can induce serious complications such as pulmonary hypertension (PH), pulmonary thromboembolism (PTE), allergic pneumonitis, right heart failure, caval syndrome (CS), glomerulonephritis and reactive arthritis (Dunn, 2000). The adult form of *D. immitis* lives in pulmonary arteries of hosts (dogs, cats, wild carnivores), but when higher numbers of parasites occur, they can migrate towards the heart, causing CS, i.e. occlusion of the right heart and venae cavae (Jones, 2016).

For the life cycle of *D. immitis,* mosquitoes are required as intermediate hosts. Therefore, heartworm transmission is limited by climatic conditions which vary throughout the world (Simón et al., 2012). Mosquitoes ingest microfilariae (mf, larvae L1, the infective stage for mosquitoes) *via* blood meals from infected hosts. Part of the parasite's development happens within the mosquito (from larvae L1, through L2 to L3) during approximately 2 weeks (at 27°C and 80% relative humidity, but with lower temperatures maturation takes longer), and continues in a new host after the mosquito transmits infective larvae during a blood meal. The life cycle of the parasite continues from L3 (an infective stage for dogs) in the host through moulting into the L4, and then into the L5, a young worm which enters the vascular system, and finally matures in peripheral pulmonary arteries at least 6 to 7 months post-infection, when the gravid female worm is capable of releasing L1 mf (Hayasaki, 1996; Lichtenfels et al., 1985; Kotani and Powers, 1982; McGreevy et al., 1974; Taylor, 1960; Kartman, 1953).

The disease is distributed worldwide and, with the exception of the Antarctica, there is no continent where this disease has not been reported. The pooled and weighted prevalence of *D. immitis* in dogs across the world was calculated as 10.91% (Anvari et al., 2020). In Europe, heartworm has been spreading from endemic areas (southern Mediterranean) to non-endemic areas (northern and eastern areas of Europe) during the last two decades due to global climate change that favours mosquito activity, as well as travel and import of pets (Simón et al., 2017; 2012; Genchi et al., 2011).

Although the first cases of HWD in Serbia were reported as a result of a necropsy in 1989 (Milosavljević and Kulišić, 1989), it was not until the beginning of this century that interest in this disease increased. Since then, many surveys have been reported on different subjects such as prevalence, seroprevalence, diagnostic procedures, therapy and molecular characterization of *D. immitis*, as well as reports of clinical cases of HWD in naturally infected dogs (Spasojević Kosić et al., 2020; 2018; 2016a; 2016b; 2014a; 2014b; 2014c; 2012a; 2011; Potkonjak et al., 2020; Krstić et al., 2017; Pavlović et al. 2014; 2012; 2009; Savić et al., 2014; 2012; Gabrielli et al., 2012; Pajković et al.,

2010; Tasić et al 2008). *D. immitis* was reported not only in dogs, but in wild animals and mosquitoes in Serbia as well, so a number of animal species serve as reservoirs for the parasite in our country (Gavrilović et al., 2019; Kurucz et al., 2016; Gavrilović et al., 2015; Penezić et al., 2014), which highlights the necessity for both monitoring and controlling the disease's transmission by vectors.

Because of its complexity, dirofilariosis is regarded as a paradigm of an episystem. The concept of vector-borne disease episystem includes biological and epidemiological aspects, and environmental elements of the disease in defined geographic and temporal scales. It is a multidisciplinary approach (involving parasitologists, veterinarians, doctors, molecular biologists, computer scientists, mathematicians and meteorologists) that enables the progress of knowledge of animal and human dirofilariosis, and it will continue to be necessary in achieving the effective global HWD control (Simón et al., 2017).

Although having impact on humans and wildlife, HWD is primarily considered to be a veterinary problem. Even an ideal situation, i.e. complete and adequate prophylactic measures in dogs, insufficient control of this mosquito-borne disease would continue to be a problem, and HWD would, nevertheless, persist in the population of sensitive individuals, due to existence of the parasite in wild animals, global warming which prolongs annual mosquito activity, and travel of dogs worldwide. Therefore, it is essential for clinicians to know how to establish precise and reliable diagnosis of HWD, to monitor the appearance of clinical complications of this potentially fatal disease, and to successfully implement adequate therapy in diseased dogs and efficient preventive measures in healthy ones.

THE IMPORTANCE OF ESTABLISHING AN ADEQUATE DIAGNOSIS

Adequate diagnosis of HWD consists of diagnosing heartworm infection, and diagnosing and classifying HWD, which means determining the pathological consequences on relevant organs and organ systems caused by the parasite (heart, lungs, blood, immune system, renal system etc.).

The diagnosis of heartworm infection is based on identifying the mf of *D. immitis* and on finding adult heartworm antigen in the dog's blood. Techniques for detecting circulating mf of *D. immitis* include microscopic native blood examination, the modified Knott concentration test and the filter test (Bazzocchi et al., 2008, Genchi et al., 2007). The diagnosis of adult heartworm can be performed using immunoassays against antigen from the adult female heartworm reproductive tract (Ag) in blood, serum or plasma (Atkins, 2003; Goodwin, 1998). Also, adult heartworms can be directly visualized in pulmonary arteries or right heart by echocardiography (Badertscher et al., 1988). Molecular analyses (PCR) can also be used for the detection of both mf and adult heartworm. It is very important to include detection of both mf and adult heartworm in the diagnosis of heartworm infection, since the infection is diagnosed if a dog has mf of *D. immitis*, or if Ag is detected, or both. In the case of very low

heartworm burden, the level of Ag can be below the limit of Ag detection, but still, if there are *D. immitis* mf, we know that at least one female and one male exist and have produced mf. *D. immitis* mf have specific morphological characteristics, and have to be differentiated from another species of the same genus (*Dirofilaria repens*). On the other hand, occult infection with *D. immitis* is established in the case of a positive Ag test and a negative Knott test. Animals with adult heartworm may not have mf if immature adults are present in the pulmonary arteries, if the worms are sterile, if only a single sex of the parasite is present or if the host immune response has caused immunological destruction of mf (Rawlings et al., 1982). During a ten-year study on heartworm infection in pet dogs in Novi Sad, we found the majority (45.45%) of dogs in which *D. immitis* were detected harboured both Ag and mf, followed by dogs (41.82%) with *D. immitis* occult infection. The lowest percentage of dogs (only 12.72%) had *D. immitis* infection that was diagnosed based on the detection of *D. immitis* mf (unpublished data).

Table 1. Prevalence of dirofilariosis in Serbia from selected studies published during last two decades

Reference Authors and year	**Methodology**	**Number of dogs**	**Prevalence (%) (*D. immitis* or dirofilariosis)**
Tasić et al., 2008	Modified Knott test and commercial filtration test (with identification of mf), the detection of adult female *D. immitis* circulating antigen	193	7.2% mf *D. immitis* 2.2% antigen *D. immitis*
Pajković et al., 2010	Modified Knott test (without identification of mf), the detection of adult female *D. immitis* circulating antigen	71	14 % (dirofilariosis)
Pavlović et al., 2012	the detection of adult female *D. immitis* circulating antigen	387	14.99% (*D. immitis*)
Savić et al., 2012	Modified Knott test (without identification of mf), ELISA antibody test	49	18% (dirofilariosis)
Spasojević Kosić et al., 2012a	Modified Knott test (with identification of mf), the detection of adult female *D. immitis* circulating antigen	77	9.09% (*D. immitis*)
Savić et al., 2014	Knott test (without identification of mf), ELISA antibody test	152	27.6% (dirofilariosis)
Krstić et al., 2016	Modified Knott test (with identification of mf)	150	12.7% (*D. immitis*)
Spasojević Kosić et al., 2016a	Modified Knott test (with identification of mf), the detection of adult female *D. immitis* circulating antigen	190	18.95% (*D. immitis*)
Potkonjak et al., 2020	real-time PCR	59	27.1 % (*D. immitis*)

Aside from complete diagnosis of heartworm infection (for both mf and adult parasite), it is important to assess the dog's sensitivity to heartworm infection. This need can be easily understood if we recall the life cycle of *D. immitis* and the role of mosquitoes in transmission of the disease. It takes 6-7 months for the parasite to fully develop from the L3 stage (which are inoculated subcutaneously by a mosquito) into a fertile adult that resides in pulmonary arteries. Therefore, it is pointless to test a dog for heartworm infection if it is younger than 6 months old, if it has not been exposed to minimally one mosquito season or if its last potential mosquito bite is less than 6 months ago. Even worse, under such circumstances, the negative result can be false negative. For these reasons, and having in mind that annual mosquito activity in this part of Europe lasts 4 to 6 months (Simón et al., 2012), it is recommended that dogs in Serbia are tested every year in March, before the new mosquito season begins.

Finally, the diagnosis of heartworm infection is not the same as the diagnosis of HWD. HWD is dependent on the number of adult parasites, the duration of infection and the host's immune response. The higher the number of parasites, the more serious is the clinical manifestation in a dog. The parasite can live up to seven years (Newton, 1968), consistently causing pathological alterations. The initial pathological changes occur where the parasite resides in the pulmonary arteries, and are described as villous myointimal proliferation (endothelial cell swelling, widening of intracellular junctions, increased endothelial permeability, and periarterial oedema). While endothelial damage promotes thrombosis, periarterial oedema results in lung consolidation. Pulmonary infiltrates consist of neutrophils and eosinophils. Pulmonary infiltrates with eosinophils (eosinophilic bronchopneumonopathy, allergic pneumonitis) are a manifestation of immunologic hypersensitivity, which, among other known aetiological agents, can be caused by HWD. Chronic inflammatory pulmonary changes lead to fibrosis of the lung (Ware, 2011). Dead parasites induce a more intensive host reaction such as PTE, further damage of lung tissue, shock and coagulopathy (Ware 2011; Kitoh et al., 2001; Kitoh et al., 1994). Thickening of intima and media of the pulmonary arteries and obstruction of lung blood flow increase pulmonary vascular resistance and lead to PH. Furthermore, PH increases right heart afterload and causes right ventricular hypertrophy and right-sided heart failure (*cor pulmonale*). Besides changes in lung and heart, microfilarial antigens can stimulate immunological reaction and cause circulating immune complex deposition in glomeruli or synovial membranes (glomerulonephritis, reactive polyarthritis) (Dunn, 2000).

THE MONITORING OF HEARTWORM DISEASE CLINICAL SIGNS AND COMPLICATIONS

Many dogs infected with *D. immitis* have no apparent clinical signs, and the infection is accidentally discovered. For that reason, it is important to test dogs on a regular annual basis. That way, the disease can be detected at an earlier stage and, thus, is more easily cured. At the same time, the reservoir of infection is reduced. In the majority

of cases, dogs with HWD become chronically ill, with respiratory and cardiovascular clinical signs (mild to severe cough, dyspnoea, polypnoea, fatigue, shortness of breath, syncope, weight loss, right-sided heart failure). Serious acute clinical complications of HWD in dogs are PTE and CS (ESDA, 2017). Pulmonary thromboembolism is associated with heartworm death, either spontaneous or after adulticide treatment, and acutely manifested with respiratory difficulties, depression, coughing and/or haemoptysis, cyanosis, syncope, collapse and sudden death (Ware 2011; Goggs et al., 2009; Hoh and McMichael, 2009). Caval syndrome in dogs is acutely manifested with sudden collapse and weakness, anorexia, pale mucosa membranes, dyspnoea, tachypnoea, bilirubinuria and haemoglobinuria (Jones, 2016).

General clinical examination findings in patients with chronic HWD depend on the stage of disease, and commonly reveal poor body condition, polypnoea, dyspnoea, distension and pulsation of jugular vein and ascites, abnormal lung or cardiac auscultation. Auscultation of lung sounds usually detects abnormal lung sounds (crackles), while heart auscultation can reveal splitting of the second heart sound, an ejection heart murmur in the left heart base, a murmur of tricuspid regurgitation or arrhythmias (Ware, 2011).

Table 2. Classification of HWD in dogs (AHS 2020, Jones 2016, Ware, 2011)

Class 1: Asymptomatic to mild HWD	**Class 2: Moderate HWD**	**Class 3: Severe HWD**	**Class 4: Caval syndrome**
a) Clinical signs absent or occasional cough	a) Clinical signs: occasional cough, exercise intolerance, abnormal lung sounds, mild loss of body condition	a) Clinical signs: persistent cough, constant fatigue, dyspnoea, abnormal heart and lung sounds, hepatomegaly, syncope, ascites, jugular distension and pulse, death	a) Clinical signs: sudden onset of severe lethargy and weakness accompanied by dark red to black coffee coloured urine
b) Radiographic sighs absent	b) Radiographic signs present: RV enlargement, mild PA enlargement, circumscribed perivascular infiltrates and/or mixed alveolar/ interstitial infiltrates	b) Radiographic signs present: RV and RA enlargement, severe PA enlargement, circumscribed to diffuse mixed patterns of pulmonary infiltrates, signs of pulmonary embolism	b) Cardiac ultrasound: numerous short, white, parallel lines within the right atrium, ventricle and tricuspid orifice
c) Laboratory parameters normal	c) Laboratory parameters abnormal: mild anaemia, with or without mild proteinuria	c) Laboratory abnormalities: anaemia, other haematological abnormalities or proteinuria	c) Laboratory abnormalities: haemoglobinemia, haemoglobinuria, proteinuria, bilirubinuria, mf (occasionally) in urine sediment

RV – right ventricle; PA – pulmonary artery; mf – microfilariae

Classification of HWD in a patient follows after heartworm infection is diagnosed, and it is based on clinical examination, blood and urine analysis, and the dog's radiographic findings (Table 2). These routine clinical diagnostic examinations are performed by veterinarians in every day practice. Another classification of HWD disease is based on criteria that define whether the patient is at low or high risk of thromboembolic complications (Table 3).

Table 3. Staging of dogs with HWD according to ESDA (ESDA 2017)

Class 1: Low risk of thromboembolic complications (low worm burden and no parenchymal and/or pulmonary vascular lesions)	**Class 2: High risk of thromboembolic complications (high worm burden and/or parenchymal and/or pulmonary vascular lesions)**
Dogs that have all these conditions:	Dogs that have at least one of the following:
- No symptoms	- Symptoms related to the disease
- Normal thoracic radiographs	- Abnormal thoracic radiographs
- Low level of circulating antigens or a negative antigen test with circulating microfilariae	- High level of circulating antigens
- No worms visualized by echocardiography and no evidence of pulmonary hypertension	- Worms visualized by echocardiography and/or evidence of pulmonary hypertension
- No concurrent diseases	- Concurrent diseases
- Owners agree exercise restriction	- No likelihood of exercise restriction

Numerous diagnostic procedures (complete blood count, blood biochemistry tests, urine analysis, radiography, electrocardiography and echocardiography) can be useful in monitoring HWD and its most common complications (PH, PTE, right heart failure and CS). The gold standard procedures in cardiology are radiography, electrocardiography and echocardiography, methods that can fully assess cardiac morphology and function.

Thoracic radiography enables an insight into the morphology of the lung field and cardiac silhouette in a dog with HWD. Radiographic changes associated with HWD can be assessed subjectively (size and shape of the heart, pulmonary vascular and parenchymal changes, and the presence of pleural effusion) and/or objectively (sizes of heart and relevant blood vessels determined by the vertebral heart size (VHS)) (Spasojević Kosić et al., 2017; 2007; Buchanan and Bücheler, 1995). Dilation of the main pulmonary artery (MPA) and right ventricular (RV) hypertrophy, seen as a reverse D-shape of the heart on dorsoventral (DV) radiograph, are typical features of HWD (Bahr, 2018). However, the occurrence of these features is not the same in small and large dogs, and it is considered that thoracic radiography cannot reflect the severity of cardiovascular changes by HWD in small dogs (So-Young et al., 2019). The average VHS on lateral (LL) radiograph in dogs with HWD was reported to be higher than the reference value of VHS (Tudor et al., 2014). Our preliminary results of radiographic findings in dogs naturally infected with *D. immitis* showed the most common radiographic parameter of pulmonary vascular changes was enlargement of

right caudal lobar artery (RCaLA) on DV radiograph, followed by loss of margination in the pulmonary vessels and MPA enlargement on LL radiograph. The average VHS in dogs with HWD was 10.75v on LL radiograph and 11.04v on DV radiograph (Spasojević Kosić et al., 2016b). Our further study on this subject in a greater number of dogs with HWD confirmed that enlargement of RCaLA is presented in the majority of cases (96.87% of dogs), and objective measurement of heart size showed 10.26v on LL radiograph and 10.85v on DV radiograph (unpublished data).

Although not considered as a method needed for staging HWD, electrocardiography can detect arrhythmias and right heart enlargement in dogs. While being inferior to both radiography and echocardiography in detecting right ventricular remodelling in dogs with HWD, it is by far the best method to determine the clinical significance of arrhythmias heard upon cardiac auscultation. However, experimental study based on insertion of artificial worms in dogs revealed that standard electrocardiography could be useful for the diagnosis of early canine HWD, characterized by attenuation of amplitude of Q wave in lead I, R wave in lead II and S wave in lead aVR (Onyango, 2011).

Echocardiography enables the visibility of worms, their number and position. The examination is performed as two-dimensional echocardiography, and the same mode can be used for determination of right heart indices in order to assess right heart function. Several right heart indices, such as the ratio of the right to left ventricular basal diameter (RVD/LVD), left ventricular compression quantified using the eccentricity index (end diastolic LV EI) and the ratio of the MPA to aortic root diameter (MPA/AO), are of particular importance, as they are significantly higher in dogs with HWD (Tai and Huang, 2013). It seems these right heart indices are affected differently depending on dog size (So-Young et al., 2019). More advanced Doppler echocardiography is necessary to assess the right ventricular acceleration to ejection time (AT/ET), which is significantly lower in dogs with HWD (Tai and Huang, 2013). The right pulmonary artery distensibility index (RPAD Index) has been recently validated as a method capable of estimating the presence and severity of PH in heartworm-infected dogs. Among other echocardiographic parameters, MPA/AO and the RPAD Index were proven to be useful in evaluating the hypertensive status in heartworm-infected dogs. Furthermore, the RPAD Index has potential diagnostic value for the detection of PH in asymptomatic animals (Serrano-Parreño et al., 2017; Venco et al., 2014).

Recently, cardiopulmonary biomarkers have been investigated in order to assess their use as objective parameters for measuring and quantifying pathological processes in HWD. Biomarkers such as C-reactive protein (CRP) (Venco et al., 2014) and endothelin-1 (ET-1) (Uchide and Saida, 2005) can be used as indicators for PH, while D-dimer is indicator of PTE in dogs with HWD (Carretón et al., 2011). In chronically ill patients, an increase of pulmonary vascular resistance and RV afterload, together with a decrease in extracellular myocardial collagen matrix, lead to right-sided heart failure (Bowman and Atkins, 2009; McCall et al., 2008a; Wang et al.,

2005). Myocardial injury and heart failure can be measured in dogs with HWD using troponin I, myoglobin and natriuretic peptides (ANP and NT-pro BNP) (Carretón et al., 2017; 2011; Kitagawa et al., 2000; Takemura et al., 1991). Even the risk of renal damage in HWD can be identified in dogs by proving proteinuria, serum symmetric dimethylarginine and creatinine (especially in dogs with microfilariaemia, high parasite burden, chronic clinical signs and PH) (Carretón et al., 2020a; Bartges, 2012; Ludders et al., 1988). However, although cardiopulmonary biomarkers can be indicators of serious clinical complications of HWD, many other pathological conditions and even subtle pathological changes, such as ischemia, can cause elevation of their concentrations (Spasojević Kosić et al., 2012b). Therefore, in spite of their promising potential, we are still far from stepping into their complete implementation into clinical practice.

THERAPY OF HEARTWORM DISEASE

Goals of heartworm treatment are to improve the clinical condition of the animal and to eliminate all life stages of heartworm with minimal post-treatment complications. Melarsomine dihydrochloride is the only effective drug available for treating adult heartworm infections (AHS, 2020; ESCCAP, 2019). Standard treatment based on the usage of melarsomine and prednisone is started first with macrocyclic lactones and doxycycline. Alternatively, monthly oral ivermectin or topical moxidectin heartworm preventive with doxycycline at 10 mg/kg twice a day for a 4-week period might be considered as a salvage procedure. Continuous monthly administration of prophylactic doses of any macrocyclic lactone alone for up to two years' duration is possible, but this is not recommended alternative therapy for HWD (AHS, 2020). Surgical intervention is advised when multiple worms have been displaced into the right cardiac chambers, producing sudden onset of CS (AHS, 2020; ESCCAP, 2019).

Melarsomine is not registered in Serbia, so alternative therapy consisting of doxycycline and ivermectin in different application regimes is widely used (Spasojević Kosić et al. 2020; Milojković et al., 2016; Stepanović et al., 2015; Spasojević Kosić et al., 2014b; 2011). We have been studying effects of alternative therapy consisting of doxycycline and ivermectin (Spasojević Kosić et al., 2020; 2014b; 2011) in dogs naturally infected with *D. immitis* for ten years and have come to the conclusion that the majority of treated dogs have no mf after one month of the therapy, while all initially microfilariaemic dogs have no more mf after 90 days' therapy. Similar results were shown in another study (Grandi et al., 2010). Concerning antigenemia, in our study, dogs needed between 2 and 12 months' therapy duration to become negative, with the majority (29.63%) of dogs being negative after 6 months (unpublished data). The alternative therapy was effective even in cases of severe HWD, as two dogs with heart failure (stage 3 HWD) were successfully cured (Spasojević Kosić et al., 2020). More promising results come from a monthly dose of moxidectin in combination with 30 days of doxycycline. This type of alternative therapy eliminates circulating mf more quickly, within one month, and antigenemia after four to nine months of therapy

(Genchi et al., 2019). The efficiency of the therapy with only melarsomine depends on the severity of disease. The majority of dogs with mild to moderate HWD become Ag-negative by four to five months (Genchi et al., 2019; Ware, 2011).

Tetracycline treatment targets *Wolbachia*, an endosymbiont bacterium of *D. immitis*, which is crucial for the pathogenesis of filarial diseases (Bandi et al., 1999). However, depletion of *Wolbachia* is not the sole cause of worm death. Chemically modified tetracyclines without antimicrobial activity are also able to cause detrimental effects on filarial worms (Rajan, 2004). Ivermectin also causes intestinal alterations of *D. immitis,* suggesting accumulation of higher concentrations of doxycycline within the nematode, and the drug's lethal effects for *D. immitis* could be independent of *Wolbachia* depletion (Bazzochi et al., 2008). In the first report of *Wolbachia* in dogs naturally infected with *Dirofilaria* in Serbia, the prevalence was 57.14% (Spasojević Kosić et al., 2018). In a higher number of dogs infected with *D. immitis*, we found a similar prevalence (53.85%). When we compared the effectiveness of our alternative therapy in dogs infected with *D. immitis* and in dogs infected with *Wolbachia* spp., we had similar results (88% and 83%, retrospectively) (unpublished results).

While there is no question about the need to administer doxycycline as part of the treatment protocol for HWD (Kramer et al., 2005; 2003), the recommended dose is contentious from several points of view (efficacy, toleration and emergence of resistant strains of other bacteria). The recommended doses of doxycycline are 10 mg/kg either once (Menozzi et al., 2015; Bazzochi et al., 2008) or twice a day (Savadelis et al., 2018) or 5 mg/kg twice a day (Carretón et al., 2020b). According to the evaluation of antibodies against *Wolbachia* in dogs' sera (Carretón et al., 2020b), administration of the lower dose of doxycycline is sufficient to achieve a significant reduction of *Wolbachia*. The duration of doxycycline administration differed among studies (from 30 days at the beginning of the therapy to several cycles of administration with a duration of 2-3 weeks), depending on the type of the study (experimental or natural infection), and on the therapeutic protocol for HWD (the standard treatment with melarsomine or an alternative one) (Savadelis et al., 2018; Kramer et al., 2011; Grandi et al., 2010; Rossi et al., 2010; Bazzocchi et al., 2008; McCall et al., 2008b). It would be best to individually adjust therapy for each dog according to its clinical follow up (microfilariemia, antigenemia, molecular detection of *D. immitis* and *Wolbachia*). In addition, depending on the stage of HWD in a dog, therapy against *D. immitis* infection must be complemented with medications prescribed for the therapy for PH, PTE, right-sided heart failure and other possible complications of the disease, depending on the dog's clinical condition (Ames and Atkins, 2020).

EFFICIENT PROPHYLAXIS OF HEARTWORM DISEASE

Prophylaxis protects dogs from HWD and has an important role in diminishing reservoirs of *D. immitis*. Therefore, it is significant, not only on individual level, but for the general population of susceptible species (domestic and wild carnivores, humans).

For years, prophylactic measurements against HWD have been directed against mf in dogs (important for infectiveness of the dog for mosquitoes), and against metamorphosis of larvae into adults (important for interception of the parasite life cycle in the dog). Apart from this, mosquito control measurements in the surroundings where dogs are present have prevented mosquito bites. After very important research on the efficacy of prophylactic agents against *D. immitis* (McCall et al., 2017a; 2017b), a new preventive measure, i.e. the multimodal prophylactic approach, was established. This prophylactic procedure involves using a repellent (dinotefuran-permethrin-pyriproxyfen) on the dog's skin, together with a macrocyclic lactone (milbemicin oxime, moxidectin, selamectin or ivermectin), in order to block the transmission of heartworm from dog to mosquito, and from mosquito to dog. The prophylaxis should start in puppies at least at 8 weeks of age (AHS, 2020). However, the start and the duration of prophylactic treatment depend on the geographical area where the dog resides. According to European Union recommendations, this new preventive measure varies from seasonal prophylactic treatment (starting in spring before the mosquito season and lasting until late autumn) to year-round prophylactic measures (ESCCAP, 2019).

CONCLUSION

Since natural infection of dogs with *D. immitis* exists in Serbia, it is necessary to test each dog with respiratory and/or cardiovascular signs for this infection. Furthermore, each clinically healthy dog should be tested at an appropriate time (at least at 6 to 7 months of age, after being exposed to one mosquito season, and one month before the next mosquito season) with adequate diagnostic tests (for both mf and Ag to adult parasite in blood) in order to confirm or discharge heartworm infection. If a dog proves to be positive, the diagnostic process proceeds to diagnose HWD and its stage. With this information, veterinarians can recommend a therapeutic protocol, and discuss with owners the best possible treatment for their dogs. Therapy for *D. immitis* infection is specific, but is not necessarily the same as therapy for HWD, as the disease can provoke different pathological changes in various organs. Therefore, therapy for HWD should always be individually adjusted to each patient. Particular attention should be given to efficient prophylactic requirements for non-infected or cured dogs.

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Authors' contributions

Vesna Lalošević conducted parasitological and laboratory aspects of the study, and Ljubica Spasojević Kosić was responsible for the clinical aspects and procedures of the study. Both authors contributed to the writing of this manuscript.

Competing interests

The authors declare that they have no competing interests.

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BOLEST IZAZVANA SRČANIM CRVOM NASTAVLJA DA POSTOJI KOD PASA: NAJZNAČAJNIJI KLINIČKI ASPEKTI OBOLJENJA

SPASOJEVIĆ KOSIĆ Ljubica, LALOŠEVIĆ Vesna

Kratak sadržaj

Bolest izazvana srčanim crvom je klinički značajna parazitoza kardiovaskularnog sistema kod pasa, koju izaziva *Dirofilaria immitis* (*D. immitis*), koja parazitira u plućnim arterijama. Inficirani psi i divlji kanidi kao pravi domaćini, i komarci kao prelazni domaćini, predstavljaju rezervoare *D. immitis*. Budući da *D. immitis* ne dostiže potpuni razvoj kod čoveka, bolest izazvana ovim parazitom se smatra oboljenjem koje ima zoonozni potencijal. Osim što se oboljenje značajno za veterinu i ima zoonozni potencijal, činjenica da je parazit rasprostranjen po celom svetu ističe neopohodnost multidisciplinarnog pristupa u kontrolu ovog oboljenja na globalnom nivou. Pod takvim okolnostima, veterinari su u odgovorni za zdravstveno stanje pasa. Životni ciklus *D. immitis* objašnjava patogenezu i kliničke znake bolesti izazvane srčanim crvom kod pasa, ali i određuje dijagnostički proces, terapiju i profilaktičke mere. U ovom radu su istaknuti najznačajniji aspekti bolesti izazvane srčanim crvom kod pasa: uspostavljanje tačne dijagnoze bolesti, odgovarajuće dijagnostičke procedure za praćenje kliničkog

stanja inficiranih pasa, različiti terapeutski protokoli i njihova efikasnost i na kraju preporuke za sprovođenje adekvatnih profilaktičkih mera. Psi sa ili bez kliničkih simptoma bolesti izazvane srčanim crvom treba da budu testirani i na postojanje mikrofilarija i na prisustvo odraslih parazita *D. immitis*, i to u odgovarajuće vreme. Ukoliko se pokaže da su pozitivni na srčanog crva, potrebno je proceniti u kojoj meri je parazit izazvao patološke promene na određenim organima, a pre svega na plućima i srcu. Obim i ozbiljnost ovakvih patoloških promena određuju protokol lečenja. Za pse izlečene od bolesti izazvane srčanim crvom ili neinficirane pse, potrebno je primeniti adekvatnu preventivu.

Ključne reči: bolest izazvana srčanim crvom, klinički aspekti, psi