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UPDATING THE PREVALENCE OF CANINE DIROFILARIOSIS IN PET DOGS IN NOVI SAD, VOJVODINA, SERBIA*

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SUMMARY: The aim of this study is to update the prevalence of dirofilarial infections in pet dogs. From the year 2010 to the year 2014, a total of 143 blood samples were collected from privately owned pet dogs in Novi Sad. All samples were examined by wet blood smears, the modified Knott test and heartworm antigen test. Circulating microfilariae of both Dirofilaria (D.) immitis and D. repens were found in dogs. Prevalence values for D. immitis and D. repens were 16.78% (24/143) and 18.88% (27/143), respectively. We report mixed infection with both parasites for the first time. Results of this study, compared with results of previous investigations, shows increase of infection with D. immitis and increase of mixed infection with both Dirofilaria species. Further investigations are required with higher number of samples to confirm these findings.

Key words: *D. immitis, D. repens, prevalence, pet dogs.*

INTRODUCTION

Nematodes of the genus *Dirofilaria (D.)* are currently considered emerging agents of parasitic zoonoses in Europe. Two main filarial infections occur in domestic and wild carnivores in Europe: *D. immitis*, the etiological agent of canine and feline heartworm disease, and *D. repens*, the main etiological agent of subcutaneous filarial infections. Climate changes, the existance of animal reservoirs (domestic and wild canides), and global movement of dogs have caused an increase in the spreading of these mosquito-borne nematodes (Genchi et al., 2011).

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Regarding dirofilariosis from the clinical point of view, heartworm disease is the most important. It can be a serious and potentially fatal disease in both cats and dogs (Ware, 2011; Atkins, 2009, 2014). *Dirofalaria repens* is a parasite of the subcutaneous connective tissues, mainly in dogs. Infection with *D. repens* is generally asymptomatic. Main clinical manifestations of *D. repens* infection are nodular multifocal dermatitis and pruriginous papule (Scott and Vaughn, 1987; Haliwell and Gorman, 1989). Both *Dirofilaria* species have zoonotic potential which expand the interests for these nematodes into human medicine (Simon et al., 2009, Simon et al., 2014).

First cases of canine dirofilariosis in Serbia were found in dogs during the autopsy (Milosavljević and Kulišić, 1989). During the last decade studies were performed in dogs on the subject of seroprevalence, diagnostic procedures, therapy and case report (Tasić et al 2008; Pajković et al, 2010; Spasojević Kosić et al 2011; Pavlović et al., 2012; Spasojević Kosic et al., 2012, 2014; Savić et al., 2014). Although data concerning prevalence of both *Dirofilaria* infections in Serbia have been published, it is of interest for human and veterinary medicine to follow up these infections among dogs. The aim of this study is to update the prevalence of dirofilarial infections in pet dogs.

MATERIAL AND METHODS

From the year 2010 to the year 2014 pet dogs from Novi Sad were tested for dirofilaria infections. This research was done in 143 privately owned pet dogs. At the moment of testing, dogs were at least 7 months old, exposed minimally to one mosquito season and without history of treatment with macrocyclic lactones. All dogs were clinically examined and blood samples were taken from all dogs with the purpose of their parasitological examination. The parasitological examination consisted of wet blood smears, the modified Knott test and antigen testing. Techniques for detecting circulating microfilariae include microscopic examination of fresh blood smears and modified Knott test. Detection and enumeration of circulating microfilariae (mf) of both *D. immitis* and *D. repens* were carried out by the modified Knott test (Bazzochi et al., 2008). Morphological characteristics of microfilariae, such as length, width, cephalic and caudal ends, were assessed in order to differentiate microfilariae of two *Dirofilaria* species (Genchi et al., 2007) (Figure 1.). Detection of circulating *D. immitis* antigens was carried out by commercial kit SNAP Heartworm RT Test (Idexx Laboratories) according to manufacturer's instruction.

In case of *D. repens* Knott test was sole diagnostic test for subcutaneous dirofilariosis. Concerning *D. immitis* infection, detection of either microfilaria or circulating antigen, or both microfilaria and antigen detection was diagnostic for heartworm infection.

RESULTS AND DISCUSSION

In this study we used recognized methods in the diagnosing of dirofilariosis (American Heartworm Society Canine Guidelines, 2014, ESCCAP 2012); we tested sensitive population of dogs, old enough to develop the adult form of the parasite, as well as dogs with clinical manifestations that may arise as a consequence of dirofilaria infections.

Among 143 examined dogs, 61 (42.66%) dogs had clinical signs (Table 1), while the rest of the dogs were asymptomatic. The most common clinical signs in dogs were cough and skin nodules. However, neither all dogs with the clinical signs were with the diagnosis of dirofilariosis nor all dogs with the diagnosis of dirofilariosis were with the clinical signs of diseases.

Table 1. Clinical signs in 61 symptomatic dogs

Clinical signs	Number of dogs
Cough	30
Dyspnoea	6
Fatigue	2
Weakness	2
Arrhythmias	2
Syncope	2
Cachexia	3
Skin nodules	7
Lameness	3
Ascites	1
Neurological signs	3

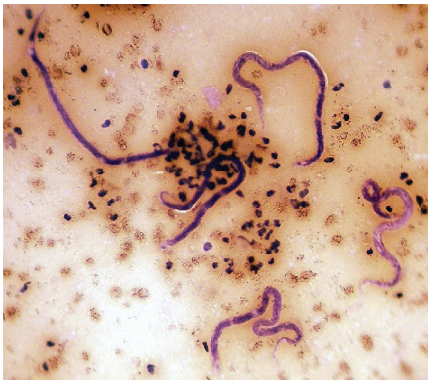

Figure 1. Microfilariae detected by modified Knott test (orig.)

Table 2. Number of infected dogs during study period

Year	Number of dogs	*D. repens*	*D. immitis*	Mixed infection
2010.	39	4	2	0
2011.	16	2	2	0
2012.	28	4	3	0
2013.	37	5	6	8
2014.	23	3	2	1
2010-2014	143	18	15	9

The number of tested dogs varied through time period. The number of dogs with *D. repens* was almost always higher in comparison with the number of dogs with *D. immitis*. Since 2013. we have observed the dogs with mixed infection, and according to our new data from this year, it is still present in 2014.

Circulating microfilariae of *D. repens* were found in 27 dogs, while circulating microfilariae of *D. immitis* were found in 17 dogs. The number of microfilariae vary from the minimum of 10 mf/ml to the maximum of 1750 mf/ml in case of *D. repens* infection. In dogs infected with *D. immitis*, minimum number of microfilariae was 40 mf/ml and maximum was 26900 mf/ml. Counting the number of microfilariae in blood is particularly important in assessing the efficacy of therapy (Grandi et al., 2010, Spasojević Kosić et al., 2014). In 22 dogs antigenemia related to *D. immitis* was detected, and in 7 of them no circulating microfilariae of *D. immitis* were found. In these dogs we perform Baermann test due to potential cross-reactions with *A. vasorum* in commercially available test kits for the detection of *D. immitis* antigen (Schnyder and Deplezes, 2012). The larvae of *A. vasorum* were not detected in these dogs. Opposite to these dogs with no circulating microfilariae, in 2 dogs with *D. immitis* infection no antigen was detected. Overall prevalence of *D. repens* in examined dogs was 18.88% (27/143), while the prevalence of *D. immitis* was 16.78% (24/143). Since mixed infection with both parasites was detected in 9 dogs (6.29%), the prevalence of heartworm as a single infection was 10.49% (15/143), while the prevalence of subcutaneous dirofilariosis as a single infection was 12.59% (18/143).

In order to compare the prevalence reported in this study with the prevalence of dirofilariosis in the previous studies it is necessary to take into consideration the methods used for the diagnosing of *D. immitis* and *D repens* infection. By using both Knott test and antigen detection, studies of dirofilariosis in Novi Sad at first showed an infection only with *D. repens*, with the prevalence of 28.9%. At that time *D. immitis* was not diagnosed (Tasić et al., 2008). Among pet dogs in Novi Sad, prevalence of 5% (3/60) was determined by using only heartworm antigen testing (Pavlović et al., 2012). In the meantime, two studies were published on the subject of the prevalence of dirofilariosis. The first one reported the prevalence of dirofilariosis in military dogs of 14% (Pajković et al., 2010), but the authors did not differentiate microfilariae. The second one reported prevalence of

7% in the year 2004 and 11% in 2009 in pet dogs in Novi Sad (Savić et al., 2012), but in this study antibodies against *Dirofilaria* were detected. Compare to the study of Tasić et al. (2008), and our study from 2012 (Spasojević Kosić et al., 2012), which have provided full diagnostic protocol for dirofilarial infections, this study shows increase of infection with *D. immitis* and we report mixed infection in dogs with both parasites for the first time. Infection with *D. repens* in this, as well as in our previous study (Spasojević Kosić et al., 2012), is still lower than in first report of Tasić et al. (Tasić et al., 2008).

Due to the clinical importance of heartworm disease in veterinary medicine and zoonotic potential of *Dirofilaria* infection it is necessary to continue the study of prevalence among dogs and report data on periodic occasion.

CONCLUSION

This study reveales a 10.49% prevalence of *D. immitis* as single infection, 12.59% prevalence of *D. repens* as single infection, and 6.29% prevalence of mixed infections with both *Dirofilaria* sšecies in pet dogs in Novi Sad.

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AŽURIRANJE PREVALENCE DIROFILARIOZE KOD PASA KUĆNIH LJUBIMACA U NOVOM SADU, VOJVODINA, SRBIJA

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Izvod

Cilj ovog rada je da prikaže nove podatke o prevalenci infekcije dirofilarijama kod pasa kućnih ljubimaca. Od 2010. do 2014. godine pregledano je 143 uzoraka krvi prikupljenih od pasa iz Novog Sada. Uzorci krvi od svakog psa su pregledani nativnim krvnim razmazom, modifikovanim Knotovim testom i dokazivanjem postojanja antigena odraslog parazita. U krvi pasa su dijagnostikovane mikrofilarije i *D. immitis* i *D. repens*. Prevalenca infekcije *D. immitis* iznosila je 16,78% (24/143), dok je za *D. repens* prevalenca bila 18,88% (27/143). Ovo istraživanje prvi put otkriva mešanu infekciju sa oba parazita kod pasa. Rezultati ovog istraživanja, u odnosu na prethodna, pokazuju porast broja infekcija pasa sa *D. immitis* i povećanje broja mešanih infekcija sa oba parazita. Dalja ispitivanja, na većem broju pasa, su potrebna kako bi se potvrdile procene ovog istraživanja.

Ključne reči: *D. immitis*, *D. repens*, prevalenca, psi kućni ljubimci.

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