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DIROFILARIOSIS AND ANGIOSTRONGILOSIS IN PET AND HUNTING DOGS IN NOVI SAD, VOJVODINA, SERBIA

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Abstract

The aim of this study is to update the data on the prevalence of *Dirofilaria immitis* (*D. immitis*) and *Dirofilaria repens* (*D. repens*) infections in pet dogs, to report a preliminary result of the prevalence of *Angiostrongylus vasorum* (*A. vasorum*) in hunting dogs, and to assess the presence of concurrent infection with *D. immitis* and *A. vasorum* in hunting and pet dogs in Novi Sad. The methods used to estimate the prevalence of dirofilaria infections were modified Knott test and detection of antigen of *D. immitis*. The prevalence of *A. vasorum* was determined using Baermann fecal technique and detection of *A. vasorum* antigen. Concurrent infection with *D. immitis* and *A. vasorum* was assessed only by detection of antigens of each parasite. Overall prevalence values for *D. immitis* and *D. repens* were 18.95% (24/143) and 16.32% (27/143), respectively. The prevalence of *A. vasorum* in hunting dogs was 1.96% (1/51). Concurrent infection with *D. immitis* and *A. vasorum* did not exist in examined hunting and pet dogs. Further studies with larger number of examined dogs and samples from other region of the country are needed to determine the prevalence of these parasites.

Key words: *Dirofilaria spp*., *A. vasorum*, prevalence, dogs.

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DIROFILARIOZA I ANGIOSTRONGILOZA KOD PASA KUĆNIH LJUBIMACA I LOVAČKIH PASA U NOVOM SADU, VOJVODINA, SRBIJA

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Kratak sadržaj

Cilj ovog istraživanja je da pruži nove podatke o prevalenciji *D. immitis* i *D. repens* kod pasa kućnih ljubimaca u Novom Sadu, da objavi preliminarne rezultate prevalencije *A. vasorum* kod lovačkih pasa u Novom Sadu, i da proceni da li postoji istovremena infekcija sa oba parazita kod lovačkih pasa i pasa kućnih ljubimaca u Novom Sadu. Za procenu prevalencije infekcije dirofilarijama korišćeni su modifikovani Knotov test i detekcija antigena *D. immitis*. Prevalencija *A. vasorum* je ispitivana kod lovačkih pasa na osnovu Bermanovog testa i detekcijom antigena parazita. Postojanje istovremene infekcije sa *D. immitis* i *A. vasorum* kod lovačkih i pasa kućnih ljubimaca je proveravano samo na osnovu detektovanja antigena oba parazita. Ukupna prevalencija infekcije *D. immitis* iznosila je 18,95% (24/143), dok je za *D. repens* prevalencija bila 16,32% (27/143). Prevalencija *A. vasorum* kod lovačkih pasa je bila 1,96% (1/51). Nije utvrđeno postojanje istovremene infekcije sa *D. immitis* i *A. vasorum* u ispitivanoj populaciji lovačkih i pasa kućnih ljubimaca. Dalja ispitivanja sa većim brojem pasa i uzoraka od pasa iz različitih delova zemlje su potrebna kako bi se odredila prevalencija za ove parazite.

Ključne reči: *Dirofilaria spp.*, *A. vasorum*, prevalencija, psi.

INTRODUCTION

Heartworm diseases caused by *Dirofilaria immitis (D. immitis)* and angiostrongylosis caused by French heartworm *Angiostrongylus vasorum (A. vasorum)* are canine parasitic diseases affecting mainly the respiratory and cardiovascular system. Both parasites are lungworms having the same primary site of residence - pulmonary arteries, where they can induce severe pathologic alterations. On the other hand, intermediate hosts of these parasites are

different, thus defining their specific seasonal characteristics. Mosquitoes as intermediate hosts for *Dirofilaria spp*. determine transmission period of the diseases. In case of *A. vasorum,* a wide range of terrestrial and aquatic gastropods act as obligatory intermediate hosts. Mild and wet climate is suitable for rapid multiplication of these intervertebrate hosts. Both parasites mostly cause chronic diseases with severe clinical complications such as pulmonary thromboembolism, right-sided heart failure and caval syndrome associated with *D. immitis* infections, whereas verminous pneumonia, right-sided heart failure and bleeding tendencies are associated with *A. vasorum*. In addition, the diseases are different in their zoonotic potential. *D. immitis*, capable to form pulmonary nodules in humans, is considered to have a zoonotic potential, while *A. vasorum* is not a zoonotic agent (Ware, 2011; Morgan and Show, 2010). While canine cardiopulmonary dirofilariosis and pulmonary angiostrongylosis are diseases of obvious clinical importance, *D. repens* infection, also known as subcutaneous dirofilariosis, is less clinically important in dogs (Scott and Vaughn, 1987). Due to the importance of *D. repens* in humans, it is upon veterinary profession to deal with this agent as well.

Previous study on the prevalence of dirofilariosis in pet dogs in Novi Sad has shown the increase of *D. immitis* infection and decrease of infection with *D. repens* (Spasojević Kosić et al., 2012, 2014) as compared with first reports on the prevalence of *D. repens* infection (Tasić et al., 2008) and mixed infection with both parasites in dogs (Spasojević Kosić et al., 2014). Such significant prevalence rates make the diseases highly important from both epizootical and clinical point of view. Clinical importance of heartworm disease in dogs and zoonotic potential of *Dirofilaria* spp. prompted us to monitor the prevalence of both parasites among dogs and report data periodically.

Fecal examination technique or sera analysis have been used for studying the prevalence for *A. vasorum* in dogs also in some surrounding countries including Greece, Hungary, Bulgaria (Papazahariadou et al., 2007; Schnyder et al., 2015; Pantchev et al., 2015). Couple of years ago, the first case of *A. vasorum* was reported in Posavac hound in Serbia. Moreover, Serbia is abundant with terrestrial and aquatic gastropods proved to be either natural or experimental host for *A. vasorum*, while climate in Vojvodina offers suitable conditions for their survival (Simin et al., 2014). From the epizootical point of view, this finding is important, yet not sufficient; thus, further studies are needed to estimate the prevalence of canine pulmonary angiostrongylosis among dogs in Serbia. In regions where this disease is endemic, the true prevalence of the disease is probably underestimated, because most diagnoses of angiostrongylosis are made when infection results in clinical signs (Morgan et al. 2005; Koch and

Willesen, 2009). To our knowledge, this is the first report on the prevalence of *A. vasorum* in dogs in Serbia. Due to the fact that both heartworm diseases and French heartworm can manifest as subclinical conditions (Savić et al., 2012; Simin et al., 2014) or with respiratory signs such as cough and dyspnea, in this study, we investigated the existence of the concurrent infection in some asymptomatic hunting dogs and in pet dogs with clinical signs. Hence, parasitic infestations of respiratory and cardiovascular system should be considered by a clinician when a differential diagnosis list is made for patients with respiratory and/or cardiovascular system signs.

MATERIAL AND METHODS

In the period from 2010 to 2016, pet dogs from Novi Sad were tested for dirofilaria infections. The research included 190 privately owned pet dogs. At the moment of testing, the dogs were at least 7 months old, exposed minimally to one mosquito season (in Serbia it is from April to October), and without history of treatment with macrocyclic lactones. All animals were clinically examined and blood samples were taken from all dogs to the purpose of parasitological examination. The parasitological examination consisted of wet blood smears, modified Knott test and antigen testing. Techniques for detecting circulating microfilariae included 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 using 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). Detection of circulating *D. immitis* antigens was carried out using commercial kit (SNAP Heartworm RT Test, IDEXX Veterinary Diagnostics) according to manufacturer's instruction.

In order to detect infestation with *A. vasorum* in hunting dogs, Baermann fecal examination method and antigen detection (Angio Detect Test, IDEXX Laboratories) were used. For the detection of the *A. vasorum* larvae, modified Baermann test was performed (Zajac and Conboy, 2006). A total of 51 hunting dogs were examined for *A. vasorum* infestation by both Baermann fecal examination and antigen detection. Fecal examinations by Baerman test in dogs were done first. The sera of these dogs were frozen and later tested for *A. vasorum* antigen.

The number of hunting dogs examined for concurrent infections with heartworm and French heartworm was 37, and the analyses were done by anti-

gen detections for both parasites (SNAP Heartworm RT Test, Idexx Veterinary Diagnostics and Angio Detect Test, Idexx Laboratories). Twelve pet dogs with respiratory clinical signs (mainly cough and dyspnea) were also evaluated for the infestation with both *D. immitis* and *A.vasorum* using antigen detection for each parasite.

RESULTS AND DISCUSSION

In this study as well as in our previous studies (Spasojević Kosić et al., 2012, 2014), we have been applying well established and recognized methods in the diagnosing of dirofilariosis (American Heartworm Society Canine Guidelines, 2014, ESCCAP 2012). Our main criteria for testing a dog included it's classification into the susceptible population with respect to age, exposure to mosquitoes and lack of prophylactic treatment. The number of tested dogs varied throughout the time period, and we observed increase in number of dogs with clinical signs among the susceptible population. Clinical signs observed in dogs included cough, dyspnea, fatigue, cachexia, weakness, syncope, skin nodules, lameness, ascites, neurological signs, with cough and skin nodules being the most common ones. The number of dogs infested with *D. repens* was either higher or equal to the number of dogs infested with *D. immitis*. The exceptions in view of numbers of infested dogs were recorded in 2013, 2015 and 2016, when we observed more dogs infested with *D. immitis* and dogs infested with both *Dirofilaria spp*. We started with diagnosing concurrent prevalence of infection with both *Dirofilaria spp*. among pet dogs in 2013, and for the entire study period the infection was confirmed in 11 dogs (table 1).

Table 1. Prevalence of infection with *Dirofilaria spp*. as single or mixed infection in pet dogs during the study period

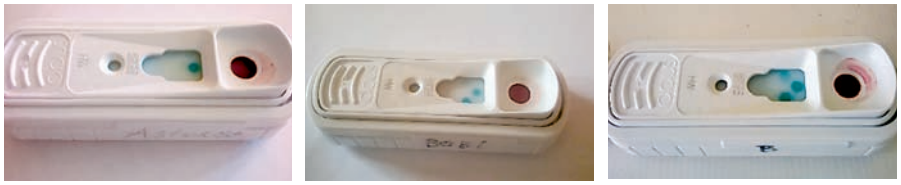
Year	Number of dogs	Prevalence *D. repens*	Prevalence *D. immitis*	Prevalence Mixed infection
2010	39	10 .26% (4/39)	5 .13% (2/39)	0
2011	16	12 .5% (2/16)	12 .5% (2/16)	0
2012	26	15 .38% (4/26)	11 .54% (3/26)	0
2013	39	12 .82% (5/39)	15 .38% (6/39)	20 .51% (8/39)
2014	26	15 .38% (4/26)	7 .69% (2/26)	3 .84% (1/26)
2015	24	4 .17% (1/24)	12 .5% (3/24)	0
2016	20	0	35% (7/20)	10% (2/20)
2010 -2016	190	10 .53% (20/190)	13 .16% (25/190)	5 .79% (11/190)

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. Having in mind this fact, we can compare our new results with our previously published results, and with the study of Tasić et al. (2008). In this study, we observed an overall prevalence for *D. repens* of 16.32% (31/190), which is lower than that from our previous study (18.88%, 27/143 dogs) (Spasojević Kosić et al., 2014) and lower than that from the first report of *D. repens* in dogs in Novi Sad (Tasić et al., 2008). The prevalence of *D. immitis* in dogs is 18.95% (36/190), which is higher than that reported in our previous study (16.78%, 24/143 dogs). The prevalence of *D. immitis* is particularly increased in this year, which could be explained by the fact that the majority of dogs examined in this year were those with respiratory clinical signs. For the period 2009-2013, the prevalence of dirofilariosis in Vojvodina was reported by Savić et al. (Savić et al., 2014.); however, in this study, the differentiation of microfilariae has not been done and no conclusion can be made on the prevalence of *D. immitis* and *D. repens*. In the study of Savić et al. (2015), the prevalence of dirofilariosis, being 15.29%, was established for the period of 2 years of study, and in 92.3% of positive samples, *D. immitis* were determined by PCR.

In order to diagnose *A. vasorum*, Baermann fecal examination technique was used because the test is useful when larvae are being shed. In hunting dogs examined by this test, *A. vasorum* larvae have been detected in one dog (Simin et al., 2014) making the prevalence for *A. vasorum* of 1.96%. Prevalence of 1.1% was found in Greece based on fecal examination of 281 samples (Papazahariadou et al., 2007), while the prevalence determined in 1247 dogs from Hungary ranged from 1.36% to 2.73% (depending on sera analyses) (Schnyder et al., 2015). In 167 sera of dogs from Bulgaria, no positive findings were recorded (Pantchev et al., 2015). Overall estimated prevalence in this region of Europe in not so high, but the number of examined dogs was higher as compared with our study population. Having in mind that some dogs might not be shedding larvae at the moment of examination, we wanted to increase a detection of infestation with *A. vasorum* by using the detection of the parasite's antigen. However, the antigen of *A. vasorum* was confirmed in none of these animals, not even in the dog in which the larvae of the parasite were detected. The possible explanation for this result could be the formation of antigen-antibody complexes, which inhibit detection of antigen in a sample as it has been previously shown with *D. immitis* detection (Matsumura et al., 1986; Brunner et al., 1988) and with *A. vasorum* detection by rapid device (Schnyder et al., 2014).

In hunting dogs examined for antigen of both parasites, only low level of

heartworm antigen was detected in two dogs, indicating the prevalence of *D. immitis* in examined hunting dogs of 5.40% (2/37). Concurrent infection with *D. immitis* and *A. vasorum* was established in neither hunting nor pet dogs. In Portugal, where the aforementioned study was conducted, concurrent infection with *D. immitis* and *A. vasorum* was found in one dog (Alho et al., 2014).



A B C

Fig 1. SNAP Heartworm RT Test: (A) negative (one dot) and positive findings: (B) two dots (low level) and (C) three dots (high antigen level)

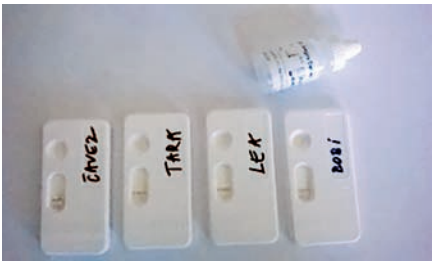


Fig 2. Angio Detect test – showing negative findings (one line) in several pet dogs

Further studies are needed to determine the actual prevalence of *A. vasorum* in dogs in Serbia. Studies like these, aimed at emphasizing the need for specific diagnosis and prevention in dogs, could be useful for clinical practice. With regard to proven heartworm disease and *A. vasorum* finding in Serbia, use of specific diagnostic procedures for detection of these two parasites in dogs with signs indicating respiratory disease is essential. This approach would provide reliable results of clinical studies on dogs.

CONCLUSION

This study revealed the prevalence rates of 13.16% and 10.53% for single infections with *D. immitis* and *D. repens,* respectively, whereas the prevalence for mixed infections with both *Dirofilaria* species in pet dogs in Novi Sad was

5.79%. Prevalence of *D. immitis* in hunting dogs was 5.40%. According to the results of Baermann fecal examination method, the prevalence of *A. vasorum* in hunting dogs from Novi Sad was 1.96%. Concurrent infection with *D. immitis* and *A. vasorum* was established in neither hunting nor pet dogs in Novi Sad, Vojvodina, Serbia.

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