

The prevalence and distribution of *Dirofilaria repens* in dogs in the Mazovian Province of Central-Eastern Poland

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Abstract

The aim of this study was to determine the prevalence and distribution of canine dirofilariasis from different districts of the Mazovian Province in central-eastern Poland. 462 dogs aged from 1.5–14 years were examined for dirofilariasis. Blood samples were examined for the presence of microfilariae using the Knott method, as well as the method of Kingston and Morton, after centrifugation in haematocrit microtubes in order to determine the intensity of infection as the number of microfilariae in 60 microliters of blood. The species of microfilariae found were determined after staining on the basis of the morphological characters. Samples were also examined using Canine Heartworm Antigen Test Kit SNAP HTWM (IDEXX, USA) that allows the detection of circulating antigens of females of *Dirofilaria immitis*. The positive samples were examined using multiplex PCR assay for species confirmation. Microfilariae belonging to the species *D. repens* were found in the blood samples of dogs originating from the city of Warsaw and from 18 districts of Mazovian Province. The mean prevalence of that species observed in the province was 25.8%. The range of intensity counted with the number of microfilariae found in 60 microliters of blood amounted to between 1 – 150; median intensity 9 microfilariae. The highest prevalence, reaching 52.9% and the highest median intensity of infection reaching 28 microfilariae, was found in Radom district. The lowest prevalence, amounting to 4.2%, was registered in Grójec district; however, the lowest median intensity reaching 6 microfilariae was noted in Żyrardów district. The infected dogs had not been outside of Poland, which means that this is an autochthonous infection. Microfilariae and antigens of *D. immitis* were not found in any examined blood samples. Results of the presented study show that autochthonous dirofilariasis caused by nematodes *D. repens* commonly occurs in the area of the Mazovian Province.

Key words

Dirofilaria repens, dogs, autochthonous infection, Mazovian Province

INTRODUCTION

Subcutaneous dirofilariasis is caused by nematodes *Dirofilaria repens* Railliet et Henry, 1911. This parasitosis is widely dispersed in southern Europe among dogs, cats and wild carnivores. It also occurs in Asia and Africa. Adult nematodes *D. repens* is located in the nodules, in subcutaneous or intramuscular connective tissue of dogs. The females release microfilariae which migrate to the blood and are ingested by the female mosquitoes during feeding. The mosquitoes from genera *Anopheles*, *Aedes* and *Culex* are the intermediate hosts of the mentioned parasites. *D. repens* invasive larvae are transmitted by more than 60 mosquito species. Development to invasive III stage larvae takes place in the mosquito, and the final host is infected when the mosquito takes a further blood meal. In the dog, the III stage larvae migrate to the subcutaneous tissue and undergo two moults over the next few months. The prepatent period is 6–9 months [1, 2].

During the course of infection of this parasite there are observed: nodular multifocal dermatitis, presence of itching exanthemas in the form of papules, and also alopecia, erythema, hyperpigmentations of skin and its hyperkeratosis. Occasionally, purulent inflammatory changes occur in the skin. Local pathological changes in skin are associated with the presence of matured nematodes under or in the microfilariae of the skin. Sometimes, the infection of the parasite has an asymptomatic course. It is possible that it is also caused by microfilariae circulating in the blood, causing generalized cardio-hepato-renal insufficiency [3, 4, 5, 6]. The objective of the presented study was to determine the prevalence and distribution of canine dirofilariasis from different districts of the Mazovian Province.

MATERIALS AND METHOD

During 2011–2012, dogs from the Mazovian Province were investigated for dirofilariasis. 462 dogs were examined, aged from 1.5–14 years. The examined dogs did not show any pathological symptoms of infection. Blood samples, taken from the cephalic vein to tubes with the addition of anticoagulant EDTA, were examined for the presence

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of microfilariae using the Knott method [7], as well as the method of Kingston and Morton [8], after centrifugation in haematocrit microtubes in order to determine of intensity of infection as the number of microfilariae in 60 microliters of blood. The species of the microfilariae found were determined after staining on the basis of morphological characters.

Samples were also examined using Canine Heartworm Antigen Test Kit SNAP HTWM (IDEXX, USA) that allows detection of circulating antigens of the females of *Dirofilaria immitis*.

Adult *D. repens* nematodes, microfilariaemic blood samples, as well as negative blood samples were examined using the multiplex PCR assay, according to Gioia et al. [9], in order to confirm species-specificity of morphological identification of *D. repens* infection. Genomic DNA was extracted using commercial kit DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Multiplex PCR reaction for the amplification of the small subunit ribosomal RNA gene of the mitochondrion (12S rRNA) of *D. repens* and *D. immitis* was performed in a final volume of 25 µl in a TECHNE TC-512 Thermal Cycler (TECHNE, Staffordshire, UK), using two specific sets of primers in the same mixture reaction [9]. The thermal profile used was 92 °C for 1 min; 40 cycles of 92 °C for 30s, 49 °C for 45s, 72 °C for 1 min, and the final elongation step at 72 °C for 10 min. Amplification products were visualized on 1.5 % Gel View agarose gel (Novazym, Poznań Poland).

RESULTS

Microfilariae belonging to the species *D. repens* were found in blood samples of dogs originating from the city of Warsaw and from 18 districts of the Mazovian Province. The mean prevalence of that species observed in the province was 25.8%. The range of intensity counted with the number of microfilariae found in 60 microliters of blood amounted to between 1 and 150; median intensity 9 microfilariae. The highest prevalence, reaching 52.9%, and the highest median intensity of infection reaching 28 microfilariae, was found in Radom district. In four districts, the prevalence in dogs amounted to 27.2% – 36.7%. In five districts, the highest intensity was found, exceeding 100 microfilariae in 60 microliters of blood. The lowest prevalence, registered in Grójec district, was 4.2%, and the median intensity – seven microfilariae. In several districts, blood was taken randomly from single dogs which proved to be infected. This attested to the frequent occurrence of this parasite (Fig. 1). The infected dogs had not been outside Poland, which means that this is an autochthonous infection. Detailed data concerning the infection of dogs with dirofilariosis in the Mazovian Province is presented in Table 1.

Microfilariae of *D. immitis* were not found in examined blood samples. By using the Canine Heartworm Antigen Test Kit SNAP HTWM, circulating antigens of that parasite were also not found. The multiplex PCR results confirmed infection of *D. repens* in all cases of microfilaraemic blood samples. The positive controls showed the expected amplification products of approximately 500 bp for the genus *Dirofilaria* and of 327 bp for *D. repens* (Fig. 2). No cases of *D. immitis* or mixed infections were revealed.

Table 1. Infection of dogs by nematodes *Dirofilaria repens* in the Mazovian Province

Locality	Examined	Infected	Prevalence %	Intensity range	Median intensity
Warsaw	173	51	29.8	1–150	9
Piaseczno district	18	6	33.3	1–75	18.5
Żyrardów district	30	11	36.7	1–130	6
Pruszków district	60	13	21.7	2–50	15
Nowy Dwór/Mazovian district	11	3	27.2	3–75	8
Grójec district	71	3	4.2	1–7	7
Plock district	28	5	17.8	2–52	7
Radom district	17	9	52.9	1–120	28
Legionowo district	22	1	4.5	15	-
Siedlce district	5	2	-	6–52	-
Warsaw Zachodni district	6	1	-	3	-
Wołomin district	8	5	-	1–5	3
Ostrołęka district	3	3	-	3–30	26
Sierpc district	2	1	-	102	-
Otwock district	2	1	-	33	-
Białobrzeg district	1	1	-	108	-
Wyszaków district	1	1	-	2	-
Żuromin district	1	1	-	2	-
Mińsk Mazowiecki district	3	1	-	2	-
TOTAL	462	119	25.8	1–150	9



Figure 1. Studied area (grey colour – *D. repens* positive districts)

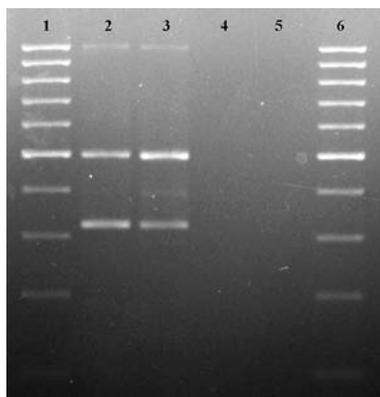


Figure 2. Results of Multiplex PCR amplification of 12S rDNA. Lane 1, O'Gene Ruler 100 bp DNA Ladder, 100–1000 bp (Fermentas); Lane 2, *D. repens* positive control; Lane 3, *D. repens* positive blood sample; Lane 4, *Dirofilaria* spp. negative blood sample; Lane 5, no DNA control; Lane 6, O'Gene Ruler 100 bp DNA Ladder, 100–1,000 bp (Fermentas)

DISCUSSION

Until recently, there has been an opinion that the northern border of the occurrence of this parasitosis reached Cherbourg in France [10]. However, in 2005, dirofilariosis were found in native dogs in Slovakia [11], in 2006 in the Czech Republic [12], and in 2009 in the Netherlands, Austria, Germany and Poland [13, 14, 15, 16]. In Poland, dirofilariosis was first registered in three focuses in central Poland: Warsaw, Pruszków and Żyrardów. 64 dogs were examined, among which 24 were infected. The prevalence observed, at that time reaching between 21.4% – 60%, was caused by the fact that the dogs examined were held in three kennels, in open boxes in small areas where they were susceptible to mosquitoes' bites [16]. In 2010, cases of microfilaremia were registered again in Poland, caused by eight dogs and in two of them the infection was confirmed by the PCR method. However, the authors did not publish where the infected dogs came from [17]. In the subsequent year, microfilaremia was confirmed by the PCR examination in another ten dogs. The infected animals originated within a radius of about ten kilometers from the centre of Pruszków [18]. Matured nematodes *D. repens* were relatively rarely found in dogs in Poland. So far, they have been found in the scrotum, subcutaneous connective tissue, nodule on the eyelid and inside a cyst in testicular parenchyma [19, 20, 21, 22]. Subsequent examinations which found microfilariae *D. repens* in 119 dogs in Warsaw and in 18 districts of Mazovian Province, substantially widen the already discovered extent of this parasitosis. Taking into consideration the fact that the parasite was registered in the centre as well as in the peripheral districts, it can be assumed that dirofilariosis occurs throughout the entire area of the Mazovian Province.

The presented results and published data suggest that autochthonous dirofilariosis of dogs caused by *D. repens* often appears in central Poland. The detection of this parasitosis in recent years in neighbouring countries means that its range is increasing. This could be caused by the change of climate and high temperatures in summer. These changes can influence the increase in the occurrence of certain mosquito species, and allow the development of invasive *Dirofilaria* larvae in them.

Dirofilariosis as a zoonosis is a danger also for human. On the territory of the European Union, over 270 cases of dirofilariosis have already been diagnosed in human. Most were evoked by nematodes *D. repens*, and only 10 cases by *D. immitis*. Until 1995, 410 cases of dirofilariosis in men had been described in 30 countries worldwide. The next 270 cases were registered in 25 countries in 1995–2000. Nematodes were located in subcutaneous connective tissue in different areas of the body, in lungs, omentum, epididymis and spermatic chord, palpebra and under the conjunctiva, and also in lymphatic nodules. The diameter of tumours with parasites varied between 10 – 25 mm. Subcutaneous and pulmonary tumours evoked by *D. repens* were sometimes false and recognized as those of neoplastic origin. Dirofilariosis in humans has been the most often diagnosed during postoperative histological examination [23, 24, 25]. Between 2007 – 2012, 19 cases of dirofilariosis caused by *D. repens* were reported in humans in Poland. Three were confirmed to be autochthonous. In sixteen cases, single nematodes were localised in nodules located in different parts of the body, and in three cases – subconjunctivally [26].

CONCLUSIONS

The results of the presented study show that autochthonous dirofilariosis caused by nematodes *D. repens* commonly occurs in the area of the Mazovian Province. It can be assumed that the number of cases of this parasitosis in humans can also increase. Examinations of dogs for the detection of dirofilariosis on the area of the entire country are necessary in order to determine the epidemiological situation of this parasitosis.

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