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Prevalence of *Dirofilaria repens* in dogs in central-eastern Poland and histopathological changes caused by this infection

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Abstract

The aim of the study was to determine the prevalence of canine dirofilariosis in four provinces of central-eastern Poland and to describe pathological changes occurring during the course of this parasitosis. A total of 188 dogs aged from one and a half up to 16 years were examined for dirofilariosis. Blood samples were analysed for the presence of microfilariae using Knott method, as well as the method of Kingston and Morton. Pathomorphological examination of two dogs was also performed. Histological sections were stained with H&E, AB-PAS, van Gieson, and von Kossa methods. Microfilariae of *Dirofilaria repens* were found in blood samples of dogs that originated from the Mazowieckie, Lubelskie, and Podlaskie provinces. The mean prevalence of that species was 12.7%. Autopsy revealed the adult nematodes in the subcutaneous connective tissue. Microscopic examinations indicated the presence of microfilariae in the liver, kidneys, lungs, heart muscle, and intestines. Necrotic foci, thrombosis, and infiltrations of different intensity with eosinophils in internal organs were also observed. Significant lesions in different internal organs together with the presence of numerous microfilariae suggest their important role in the disease process.

Key words: dogs, *Dirofilaria repens*, dirofilariosis, histopathological analysis, Poland.

Introduction

Until recently, dirofilariosis, caused by nematode *Dirofilaria repens*, had been regarded as parasitosis common in Southern Europe, Asia, Australia, Africa, and North and South America (20, 18). Since 2005, cases of this parasitosis have been observed in dogs as well as in humans in Slovakia, Czech Republic, Germany, Russia and Poland (3, 4, 5, 17, 21, 23, 24, 29).

Dogs, cats, wild carnivores, and accidentally also humans are definitive hosts of *Dirofilaria repens* (1, 17, 19, 21, 29). Adult nematodes localise in the subcutaneous and intramuscular connective tissue of the definitive host. Females of the nematodes, in the place of their localisation in their final host, bare numerous first stage larvae called microfilariae. Microfilariae circulate in blood, and they occur in the skin capillaries in the evening and at night, which is coincident with the increased activity of mosquitoes. Over 60 species of mosquitoes belonging to genera *Anopheles*, *Aedes,* and *Culex* are intermediate hosts and vectors of these nematodes. During feeding of mosquitoes on animals infected with microfilariae, larvae penetrate from the gut of insects into the Malphigi tubes, where they grow and moult twice. After reaching the invasive stage, larvae migrate to the mosquito's head and penetrate into the mouth organs. The period of larval development to the invasive stage depends on the species of the mosquito and the temperature of the environment, and ranges from 10 to 21 d. The development to the stadium of invasive larvae in mosquitoes (intermedial host) requires daily temperature not lower than 14ºC. During the further uptake of blood by the mosquito, invasive larvae penetrate to the definitive host's skin and begin moving to the places of permanent location. Then larvae moult twice and between six to nine months they reach sexual maturity. Mature nematodes live from two to five years in a host (4, 5, 28).

In the place of localisation of the adult individuals, nodules, focal dermatitis, papules, alopecia, erythema, skin pigmentation, or keratosis are usually observed (25, 26). In humans, *D. repens* was detected in the subcutaneous tissue, eye, lung, abdominal cavity, and breast (8, 16, 17, 21, 29).

The aim of the study was to determine the prevalence of the canine dirofilariosis in four provinces of central-eastern Poland and to describe pathological changes, which occur during the course of this parasitosis.

Material and Methods

Between 2011 and 2012, 188 dogs originating from Mazowieckie, Lubelskie, Podlaskie, and Łódzkie provinces were tested for dirofilariosis. The age of the dogs ranged from 1.5 to 16 years. The dogs did not reveal any pathological symptoms of infection. Blood samples, collected from the cephalic vein into EDTA-coated tubes, were examined for the presence of microfilariae using Knott method (13), as well as Kingston and Morton's method (12). After centrifugation in haematocrit microtubes, the intensity of infection, presented as a number of microfilariae in 60 μL of blood, was determined. The species of the microfilariae was determined on the basis of their morphometrical characteristics after staining with 0.1% methylene blue solution. Samples were also examined using Canine Heartworm Antigen Test Kit (SNAP HTWM, IDEXX, USA), which allows detecting circulating antigens of *Dirofilaria immitis* females.

The necropsies of two dogs were done. Dog no. 1 from Lubelskie province, male, mixed breed (mongrel dog), aged eight years was treated symptomatically because of increased levels of alanine aminotransferase and bilirubin in serum as well as hypoalbuminaemia for five weeks and due to poor clinical condition was subjected to euthanasia. Dog no. 2 from Mazowieckie province, male, Cane Corso, aged 11 years was not treated, and had a reduced appetite and lost weight. The dog died suddenly. Both dogs have never been outside Poland. Samples of the skin with subcutaneous tissue containing nematodes were collected. Nematode species was determined by the morphological characteristics. Samples of the liver, kidneys, lungs, heart muscle, and intestines collected for histopathological examination were fixed in 10% buffered formalin, and paraffin sections stained with haema-toxylin-eosin and with van Gieson, AB-PAS, and von Kossa methods were prepared.

Results

Blood examination. Detailed data concerning the dogs' infection with dirofilariosis is presented in Table 1. *D. repens* microfilariae were found in blood samples of dogs, which originated from the Mazowieckie, Lubelskie, and Podlaskie provinces. The mean prevalence of the infection was 12.7%. The range of intensity amounted between 1 and 102 microfilariae, and the mean intensity was 16 microfilariae. The highest prevalence, reaching 21%, and the highest mean intensity of infection, reaching 19 microfilariae, were found in Mazowieckie Province. Circulating antigens of that parasite were not found.

Table 1. Infection of dogs with *Dirofilaria repens* microfilariae

Province	Number of dogs	Prevalence (%)	Intensity*	
			Range	Mean
Mazowieckie	86	21	1-102	19
Lubelskie	20	15	1-20	8
Podlaskie	25	12	2-7	4
Łódzkie	57	0	0	0
together	188	12.7	1-102	16

*Number of microfilariae in 60 μL of blood

Anatomopathological and histopathological examinations. Anatomopathological examinations of two dogs did not reveal any lesions in the skin. In the subcutaneous tissue, nematodes of *D. repens* and a considerable congestion of surrounding vessels were observed (Fig. 1). In addition, post-mortem examination showed right cardiac ventricular dilatation and hypertrophy of the left cardiac ventricular wall of the heart, endocarditis, pulmonary congestion and oedema, and nephritis in both dogs. In addition, dog no. 1 indicated visible features of liver cirrhosis, and in dog no. 2 a considerable congestion of the liver was observed.

Histopathological examination revealed the presence of *D. repens* microfilariae in the samples from parenchymal organs and intestines. Apart from the presence of relatively numerous microfilariae in the kidneys, membranoproliferative glomerulonephritis with crescents - focal glomerular sclerosis, chronic interstitial inflammation, deposition of calcium in walls of vessels, renal tubules, glomeruli, and renal interstitial tissue were noted. Small focal haemorrhages and thrombus in the lumen of the vessels were present. In addition, besides deposition of calcium, also fibromatous necrosis was observed in the walls of blood vessels (Figs 2, 3).

Focal atrophy, degeneration, necrosis, and fragmentation of muscle fibres were found in cardiac muscle. Focal inflammatory infiltrations, mainly with eosinophils, and single microfilariae between the cardiac muscle fibres were also observed (Fig. 4).

In the liver of dog no. 1, characters of cirrhosis were found (proliferation of intralobular connective tissue, reorganisation of hepatic lobules, occurrence of pseudocanalicules). In both dogs, focal atrophy and necrosis of hepatocytes, focal infiltration of inflammatory cells with the presence of eosinophils, focal cholestasis and haemostasis were observed. Thrombosis and wall fibrinous necrosis were noted in blood vessels. Microfilariae were found in vascular sinusoids, walls of the bile ducts, interlobular connective tissue, and portal spaces (Figs 5, 6).



Fig. 1. Adult organisms of *Dirofilaria repens* in subcutaneous tissue

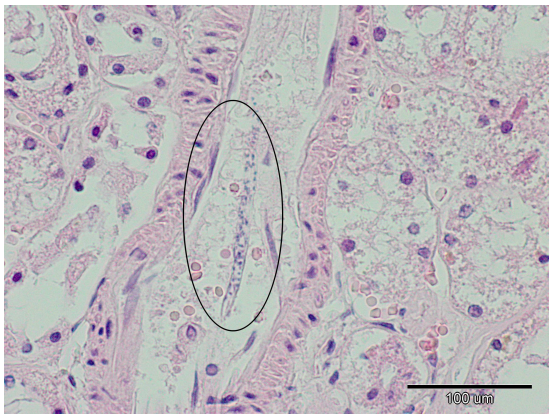


Fig. 2. *Dirofilaria repens* microfilaria in the lumen of kidney blood vessels

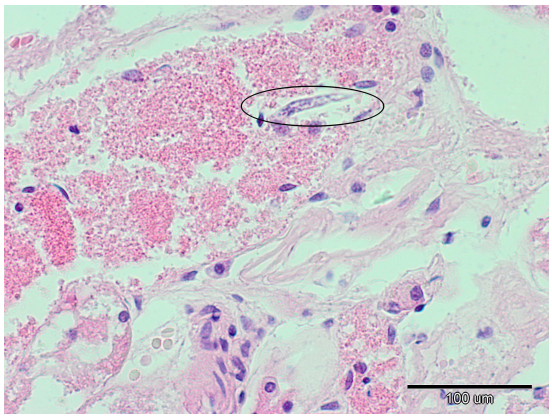


Fig. 3. *Dirofilaria repens* microfilaria in a focal haemorrhage in the kidney

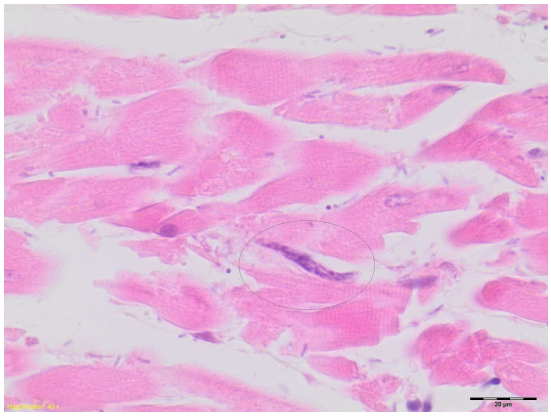


Fig. 4. Microfilaria in heart muscle

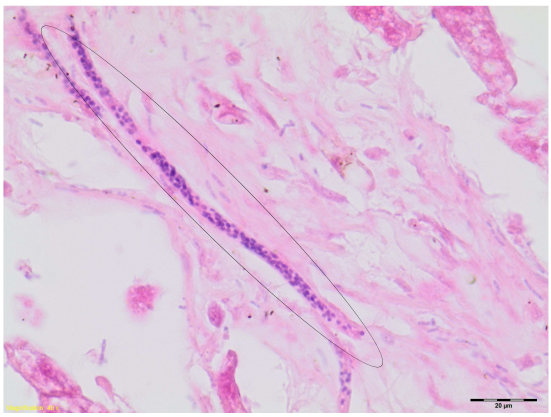


Fig. 5. *Dirofilaria repens* microfilaria in the interlobular connective tissue of the liver

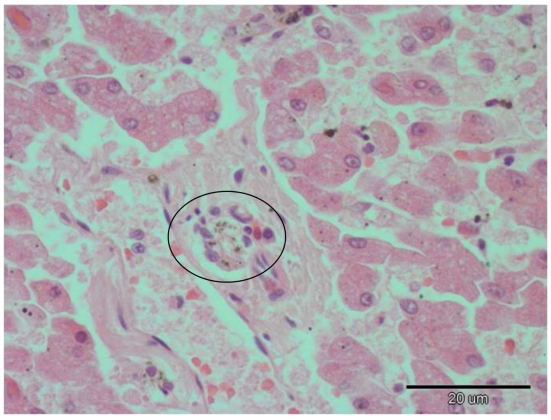


Fig. 6. *Dirofilaria repens* microfilaria in a vascular sinusoid of the liver

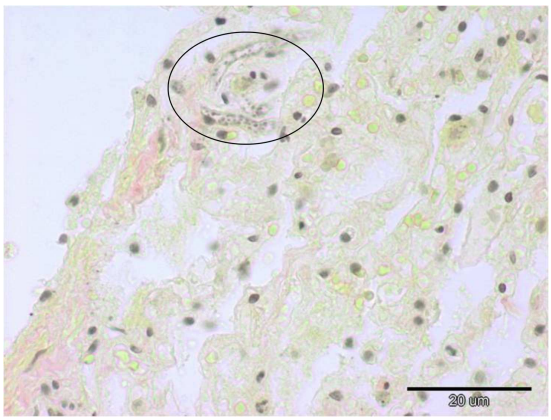


Fig. 7. *Dirofilaria repens* microfilariae and focal atelectasis of the lungs

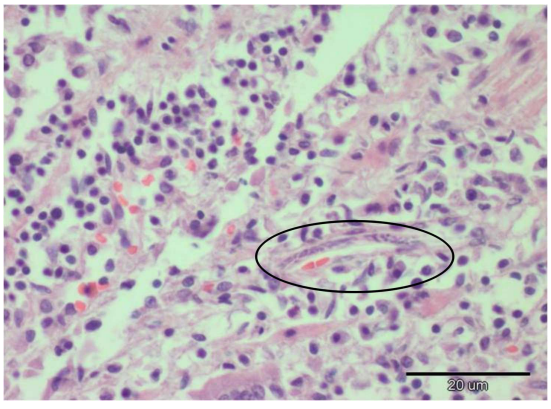


Fig. 8. *Dirofilaria repens* microfilaria and infiltration of inflammatory cells in the intestine

The microscopic examination of the lungs showed oedema, focal congestion, atelectasis, and focal points of emphysema and damage to the pulmonary alveolus walls with the presence of relatively numerous microfilariae. Thrombosis, thickening, and fibrinous necrosis of blood vessel walls were also found (Fig. 7).

In the spleen, focal haemorrhages, siderocytes, focal atrophy due to blood stasis, single microfilariae, and thrombosis, thickening, and fibrinous necrosis in blood vessel walls were noted. Microscopic examination of the stomach and intestines revealed congestion, inflammatory cell infiltration, mainly with eosinophils, and microfilariae in the mucosa (Fig. 8).

Discussion

The results of parasitological examinations of blood collected from the dogs from Mazowieckie, Lubelskie, and Podlaskie provinces indicate *D. repens* infection. It is likely that it is resulted from biting the dogs by infected mosquitoes (intermediate hosts of this nematode) on Polish territory. This indicates the presence of a new zoonotic parasitosis in Poland.

In 2008, Żarnowska-Prymek *et al.* (29), for the first time in Poland, described five cases of *D. repens* infection in men. The cases were recognised between March 2007 and April 2008. All patients lived in Warsaw or in the surrounding area. The medical history revealed that at certain times they were in countries where *D. repens* had occurred before, but in two cases the authors were not convinced if the infection really began outside of Polish borders. The results of examinations of dogs described in this paper, and reports by Demiaszkiewicz *et al.* (4, 5) Sapierzyński *et al.* (22), and Masny *et al.* (15) indicate that the infection probably took place in Poland. Twelve new cases of human dirofilariosis were recognised between April 2009 and December 2011 in Mazowieckie Province (3).

Results of our study also indicate that dogs, in which no cutaneous changes characteristic for dirofilariosis were observed, could be infected with *D. repens*. Sometimes, as in the case of the dogs described in the study, a bad general state of patient's health is observed without cutaneus lesions.

The clinical examination may indicate kidney and liver injury and cardio-respiratory system disorders. Symptomatic treatment brings a moderate effect or no significant improvement. Parasitological examination of blood can, in such cases, reveal the presence of *D. repens* microfilariae, which allows undertaking causal treatment (2, 9, 11). In numerous papers, lesions associated with the presence of adult *D. repens* are described (6, 14, 25, 26). Less frequently changes associated with the presence of *D. repens* microfilariae in internal organs are reported (7, 11, 28).

In this study, histopathological examination of the internal organs of dogs infected with *D. repens* demonstrated the presence of relatively numerous microfilariae in blood, interstitial tissue, and parenchyma of the organs. Significant pathological changes observed in the kidneys, heart muscle, lungs, and blood vessels of the examined dogs are similar to those described by Džaja *et al.* (7), Vasylik (28), and Kamalu (11). These authors suggest that a damaging role is played mainly by *D. repens* microfilariae, which was confirmed by our anatomopathological examinations. The significance of damaging role of microfilariae in the course of dirofilariosis has been confirmed by cases of infection with *D. repens* in humans, where no reproduction of this parasite is found. Changes in these cases are limited to a place of settlement of the parasite and surgical removal of the lesions eliminates health problem. Human is an accidental host, in which only single individuals of *D. repens* (male or female) are usually observed. Furthermore, it is believed that this parasite does not reach sexual maturity in humans; however, cases were reported where the presence of microfilariae was detected (8, 21, 27). Poppert *et al.* (21) as well as Vasylyk (28) indicate not only the mechanical tissue damage associated with the migration of microfilariae through blood vessels, but also the cellular and humoral reaction of the immune system to the presence of microfilarial antigens and toxins.

In light of the studies by Demiaszkiewicz *et al.* (4, 5, 6), Masny *et al.* (15), and Sapierzyński *et al.* (22) on the prevalence of *D. repens* in dogs, it can be assumed that this zoonosis is not only an "imported" disease in Poland. Parasitological examination of blood for the presence of *D. repens* and *D. immitis* microfilariae should be included in routine diagnosis of dogs and cats in our country. It appears that the test for the presence of dirofilariosis in humans, particularly in the cases of suspicion of *D. repens* infection, should also be carried out.

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