

Research note

The first report of autochthonous dirofilariosis in dogs in the Czech Republic

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Summary

In the Czech Republic, canine dirofilarial infection (*Dirofilaria immitis* and *D. repens*) is usually diagnosed in dogs coming from endemic areas and as such has been considered an imported infection. Here, 77 dogs that had never travelled abroad from the Břeclav area, close to Slovak border, were tested for *Dirofilaria* spp. infection. The presence of microfilaria in peripheral blood was detected by Knott test. Microfilariae were further examined by acid phosphatase staining and molecular methods (PCR). The presence of adult female *D. immitis* circulating antigens in blood was assessed by a commercial kit (PetChek, IDEXX Laboratories, Portland, USA). Microfilariae were detected in 7 (9 %) out of the 77 animals by the Knott test. The result of the acid phosphatase staining and PCR for all seven samples agreed with *Dirofilaria repens* species. Other five dogs of the 77 sera (6.5 %) sampled were serologically positive for circulating *D. immitis* antigens. No *D. immitis* microfilariae were found in these five dogs. *D. repens* positive dogs were negative on the ELISA for *D. immitis*. This is the first report of autochthonous cases of heartworm disease and subcutaneous dirofilariosis in dogs in the Czech Republic.

Key words: *Dirofilaria repens*; *Dirofilaria immitis*; dog; autochthonous infection

Introduction

D. immitis is the causative agent of the canine and feline heartworm disease, a severe/very severe infection in dogs living in stable endemic areas, characterized by high abundance of animal reservoirs and of mosquito vectors. The adult worms are localized in the pulmonary arteries and in the right atrium and ventricle, and they cause chronic, progressive cardiovascular disease. *D. repens* is the agent of

the subcutaneous filariosis in dogs and cats. Its pathogenicity is often underestimated, though the presence of adult worms in the subcutaneous connective tissue may cause moderate to severe dermatological symptoms (Szell *et al.*, 1999; Baneth *et al.*, 2002). The parasite can also be incidentally found in the deep perimuscular connective tissue during surgery or necropsy.

Adult females of both the species produce circulating microfilariae. *D. immitis* and *D. repens* infective larvae are transmitted by more than 60 mosquito species, including those commonly found in the Czech Republic (Rettich, 2000). Global warming, the increasing abundance of mosquitoes, movement of dogs between endemic and non-endemic countries and the abundance of reservoirs in endemic areas have increased the risk of spreading of filarial infections in formerly *Dirofilaria*-free areas (Genchi *et al.*, 2005). The endemic distribution of *D. immitis* has recently been reported from tropical and subtropical areas, and in the temperate zones (McCall *et al.*, 2004; Bucklar *et al.*, 1998; Genchi *et al.*, 2005). *D. repens* infection in dogs are quite common in Africa, southern Europe and Asia (Baneth *et al.*, 2002), and this species is prevalent mainly in eastern European countries such as Hungary (Szell *et al.*, 1999) and Ukraine (Vasylyk, 2004). Recently, the first autochthonous infections caused by both *Dirofilaria* species have been confirmed in Slovakia (Svobodova *et al.*, 2005). Both species can cause potentially serious risks for public health (Orihel & Eberhardt, 1998) and *D. repens* has recently indicated as an emergent zoonotic infection (Logar *et al.*, 2001; Pampiglione *et al.*, 2001).

In the Czech Republic, canine infections caused by *D. immitis* and *D. repens* have been diagnosed sporadically and all cases were found in dogs imported from abroad (Svobodova & Mišoňova, 2005). The present study was aimed at surveying the possible presence of autochthonous *Diro-*

filaria infections in dogs in an area of the Czech Republic where the climatic conditions are favourable for the life cycle of the parasite.

Material and Methods

The study was carried out from November 2005 to February 2006 on 77 dogs, 51 males and 26 females, aged 1-12 years. Most were hunting dogs that spend the majority of their time outdoors, from border area between southern Moravia and Slovakia, not far from the confluence of the Morava and Dyje rivers. The area has high mosquito abundance, and a mean temperature from June to September above 18°C (<http://www.meteo-web.cz>), which is the threshold below which the development of dirofilarial larvae does not proceed in the mosquito host (Fortin & Slocombe, 1981). The sampled dogs had no history of travelling abroad and were free of clinical symptoms. Samples of

test was evaluated spectrophotometrically using a 650 nm filter.

Results

Seven of the 77 dogs tested were microfilaremic (9 %). Five were male aging 6-11 years and 2 were female, both aged 2 years. Acid phosphatase staining identified *D. repens* species in all the samples. The active site of acid phosphatase, which was highlighted by a red-brown colour, was restricted to anal pore. PCR definitely confirmed *D. repens* diagnosis in these samples. Numerous adult *D. repens* were found in the subcutaneous tissue during necropsy of one *D. repens* microfilaremic dog euthanized following the owner's request.

Five of the 77 sera (6.5 %) sampled were positive on ELISA for *D. immitis* circulating antigens. Four dogs were male aged 3 – 9 years and one was a 3-year-old female. No

Table 1. Results

	Knott test (mf)	<i>D. repens</i>		<i>D. immitis</i>		
		Acid phosphatase staining (mf)	PCR	Acid phosphatase staining (mf)	PCR	ELISA (<i>D. immitis</i> antigen) PetChek®
Positive (%)	7 (9)	7 (100)	7 (100)	0 (0)	0 (0)	5 (6.5)
Negative (%)	70 (91)	0 (0)	0 (0)	7 (100)	7 (100)	72 (93.5)
Total	77	7	7	7	7	77

whole blood, blood serum and 2 fresh blood smears were obtained from each dog.

Microfilariae

The Knott test (Knott, 1939) was used to detect microfilariae (mf) that were then identified on the basis of their morphology (Orihel, 1961). Species identification was confirmed by histochemical staining of acid phosphatase with naphthol AS-TR-phosphate as a substrate, and pararosaniline as a chromogen (Chalifoux & Hunt, 1971). Stained blood smears were examined by microscopy at x 200 magnification.

PCR

DNA was extracted from 100 µl of canine blood samples positive for circulating mf using a commercial kit (QIA amp DNA blood; QIAGEN GmbH, Germany). PCR reactions for the amplification of the mitochondrial *coxI* gene were performed in a final volume of 10 µl using general filarial primers and thermal profile described in Casiraghi *et al.* (2004). PCR products were gel-purified and sequenced directly using ABI technology. The obtained sequences were compared to those deposited in gene banks.

Adult *D. immitis* female antigen

The detection of circulating adult female heartworm antigens in blood serum was performed using the PetChek kit (IDEXX Laboratories, Portland, USA). The ELISA-based

D. immitis microfilariae were found in these dogs. *D. repens* positive dogs were negative on the ELISA for *D. immitis*.

Discussion

Both canine heartworm disease and subcutaneous dirofilariosis are classified as emerging infectious diseases, particularly in eastern European countries (Szell *et al.*, 1999; Vasylyk, 2004; Genchi *et al.*, 2005; Simon *et al.* 2005; Svobodova *et al.*, 2005). The emergence of both diseases is likely associated with the increasing movement of dogs and with global changes in climate. Previous reports of *Dirofilaria* infection from the Czech Republic were confirmed as imported cases (Svobodova & Mišoňova, 2005). The climate in the Czech Republic and the numerous mosquito vectors present enable the development and autochthonous transmission of the infectious stages of the parasites (Svobodova & Mišoňova, 2005). The dogs included in this study were intentionally sampled from the area in Southern Moravia characterized by high annual mean temperatures and high mosquito abundance. Moreover, the area is close to the Slovak Republic where autochthonous cases of both *D. immitis* and *D. repens* infections have recently been found in dogs (Svobodova *et al.*, 2005).

D. repens microfilariae were found in 7 dogs and the species identification was confirmed by histochemistry and PCR. No serological methods are currently available for

diagnosis of *D. repens* infection and *in vivo* diagnosis is based on the detection and the morphological identification of circulating microfilariae. Furthermore, in one microfilaremic dog our findings were confirmed by necroscopy. *Post mortem* examinations showed a large number of *D. repens* adult worms in the subcutaneous tissues.

D. immitis-ELISA was positive in 5 dogs, though no microfilariae were found. The ELISA for detecting circulating *D. immitis* antigens is very sensitive and specific (Nelson *et al.*, 2005). Different factors can affect the presence of microfilariae in the blood, including the age of worms (female worm fecundity decreases with age), host immunity and worm abundance. It is possible, that in an area where the parasite has been recently introduced, such as the Czech Republic, the infection could be due to a low/very low parasite burden. Furthermore, several dogs were over 5 years of age. It has previously been demonstrated that microfilaremia may be absent in older dogs (Genchi *et al.*, 1988).

Our results show that dirofilariosis must now be among the autochthonous emerging infections in the Czech Republic, though so far its prevalence appears low, particularly for *D. immitis*. In our study, both *D. repens* and *D. immitis* infections were asymptomatic in all examined dogs. However, considering environmental factors such as temperature (Genchi *et al.*, 2005; Svobodova & Mišoňova, 2005) and mosquito abundance, the risk of rapid spread and increasing frequency of infection is likely high and dogs with clinical symptoms may be expected to occur.

The pathogenicity of *D. immitis* is well known, and heartworm infection is among the most severe diseases for which prophylaxis is recommended (McCall *et al.*, 2004). On the other hand, the clinical importance of *D. repens* tends to be underestimated, even though dermatological symptoms may be severe (Szell *et al.*, 1999; Baneth *et al.*, 2002). Furthermore, both *Dirofilaria* species are able to infect humans. In Europe, most zoonotic infections are due to *D. repens* (Pampiglione *et al.*, 1995). The findings of autochthonous canine infections in the Czech Republic confirms the spread of *Dirofilaria* infection into new territories and suggests the opportunity of preventive treatment during the mosquito season against *D. repens* also (Marconcini *et al.*, 1993; Pollono *et al.*, 1998; Genchi *et al.*, 2002; Rossi *et al.*, 2004). Chemoprophylaxis is the only measure that will minimize the risk of the infection spreading in both the mosquito vectors and dogs and decrease the risk of human infection. Furthermore, close cooperation with human practitioners is necessary to assess the potential risk of the zoonotic infections.

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