

First cases of canine dirofilariosis in Slovakia: a case report

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ABSTRACT: Dogs from two locations in West Slovakia suspected to have dirofilariosis were tested with the help of microfilariae detection, histochemical identification of microfilaria species and *Dirofilaria immitis* specific antigen detection in the blood serum. Six out of the group of 15 dogs from Bratislava region were found positive with *Dirofilaria repens* microfilariae. In the second location situated farther to the south, near Komarno, 6 out of 7 dogs were found positive with *D. repens* microfilariae and 2 with *D. immitis*. Serological detection of *D. immitis* antigen was positive in 3 cases among the dogs from this region, 2 of which were found positive with *D. immitis* microfilariae simultaneously with *D. repens*. The infection was asymptomatic in all of the dogs included in the trial.

Keywords: *Dirofilaria repens*; *Dirofilaria immitis*; dog; autochthonous canine dirofilariosis

The incidence of canine filarial infections in Central Europe in the past period was regarded as rare and associated with import only. The most notorious filariae included primarily *Dirofilaria immitis*, inhabiting pulmonary arteries, the right ventricle and auricle, and often causing serious canine cardiac diseases. Other less notorious species found in the subcutis such as *Dirofilaria repens* or *Dipetalonema reconditum* were considered nonpathogenic (Kassai, 1999; Georgi and Georgi, 2000). Females of all these species produce microfilariae circulating in blood. *D. immitis* and *D. repens* are transmitted by over 60 common mosquito species. The intermediate hosts of *D. reconditum* are fleas and lice.

Extensive movement of dogs across countries and continents has contributed to the spreading of filariae to new areas with vectors and favourable climatic conditions (Schrey and Tautvetter, 1998). Endemic distribution of *D. immitis* has recently been reported in most countries throughout the tropical and subtropical zones, and also the temperate zone (McCall et al., 2004). *D. repens* was originally abundant in the Mediterranean, South Asia and Africa (Lock, 1998). Endemic distribution

was however documented in Hungary (Szell et al., 1999) and in Ukraine (Vasylyk, 2004). *D. reconditum* is also distributed across North America, Europe, Africa (Quinn et al., 1997) and Australia (Boreham and Atwell, 1985). Although subcutaneous dirofilariosis is usually asymptomatic, dermatological complaints may occur (Kleiter et al., 2001). The aim of this paper is to report the first incidence of autochthonous canine dirofilariosis in Slovakia.

MATERIAL AND METHODS

The finding of microfilariae in the blood of two dogs on the occasion of haematological testing in May 2005 became an impetus for us to take and analyse blood samples of dogs from the same locations upon agreement with general veterinary practitioners. Both regions are situated in West Slovakia, about 140 km from each other. Group 1 consisted of 15 dogs living in the location to the northwest of Bratislava by the Morava River close to the Czech-Slovak border while another 7 dogs (Group 2) came from a region farther to the south

by the Vah River in the Komarno region close to the border with Hungary. All experimental dogs were free of clinical symptoms of the disease and lived in Slovakia permanently. Full unagglutinated blood, 2 blood smears and blood serum were taken from each dog.

Microfilariae

Microfilariae were detected using the method developed by Knott (1939) and with the help of Giemsa staining for one of the two blood smears.

In the animals found positive with microfilariae the other blood smear was used for species determination based on the histochemical method taking advantage of the somatic distribution of the activity of acid phosphatase with naphthol AS-TR-phosphate as a substrate and pararosaniline as a chromogen (Chalifoux and Hunt, 1971; Schrey and Trautvetter, 1998).

Detection of adult *D. immitis* antigen

Detection of the antigen of sexually mature females in canine blood serum was performed using the PetChek[®] kit (IDEXX laboratories, Portland, ME, USA). The ELISA-based test was evaluated spectrophotometrically, using a 650 nm filter. It included positive and negative controls and the results were subjected to PC evaluation.

RESULTS

In Group 1 microfilariae were detected in 6 out of the 15 animals, both by the Knott test and in stained blood smears. The result of the histochemical reaction for all samples agreed with *D. repens* species. *D. immitis* antigen was detected in no blood serum sample.

In Group 2 microfilariae were detected in 6 out of the 7 dogs both by the Knott test and in stained blood smears. The histochemical reaction identified two dirofilaria species, namely *D. repens* and *D. immitis*. Microfilariae of *D. repens* were detected in all 6 dogs and microfilariae of *D. immitis* in 2 dogs simultaneously with *D. repens*. Serological testing detected *D. immitis* antigen in 3 dogs. In two of them microfilariae of *D. immitis* were detected simultaneously while in the third dog microfilariae

of *D. repens* only were identified by histochemical staining.

DISCUSSION

Dissemination of emerging infectious diseases is a worldwide phenomenon associated with the movement of people and animals as well as climatic change. The first cases of dirofilariosis were described as imported e.g. in Austria (Kleiter et al., 2001), Great Britain (Ridyard, 2005), Czech Republic (Svobodova and Misonova, 2005), resulting in autochthonous infections where conditions are favourable.

The two foci of dirofilariosis in Slovakia testify to the observation. Both locations are known for mosquito outbreaks and relatively high temperatures, which provided conditions for rapid dissemination of *D. repens* and slower dissemination of *D. immitis*. The canine blood samples contained a great amount of microfilariae, which enabled the species determination. Microfilariae were also identified in all blood smears where the amount of detected microfilariae is significantly lower than in Knott test method (Courtney and Zeng, 2001). Serological testing confirmed the *D. immitis* antigen in 3 dogs in the location near Komarno by the Hungarian border only. In the positive animals we identified both dirofilaria species by the histochemical method, with microfilariae of *D. repens* prevailing over *D. immitis* significantly. In the third *D. immitis* serologically positive dog only microfilariae of *D. repens* were detected, indicating that the intensity of *D. immitis* infection was probably lower. Based on the results we can assume higher prevalence and intensity of *D. repens* infection in Slovakia which unlike *D. immitis* infection we also detected near Bratislava. On the other hand, the identification of 3 *D. immitis* positive dogs out of the 7 dogs living near Komarno suggests with a relatively significant likelihood a high incidence of heartworm disease in the given locality from where it may spread to other areas in summer months.

D. repens as well as *D. immitis* findings were asymptomatic in all the dogs tested but patients with clinical symptoms of the disease may be expected to occur as the intensity of the infection grows. The pathogenicity of *D. immitis* is well known, qualifying the heartworm disease as one of the serious veterinary diseases where prophylaxis is recommended (McCall et al., 2004). On the

other hand, *D. repens* pathogenicity is often underestimated. Nevertheless the disease caused by this filaria may be characterized as an opportunistic infection with potentially serious dermatological symptoms. The symptoms most often include erythema, papulae, focal or multifocal alopecia, hyperpigmentation, lichenification, crusts and nodular changes as well as secondary pruritus (Tarello, 2002). Thromboembolic changes may also be found in different organs (Vasylyk, 2004) and both species can cause zoonoses (Orihel et al., 1997; Pampiglione et al., 1999).

The detection of the first cases of dirofilariosis in Slovakia implies that filariae have colonized another area. Their incidence and dissemination can be restricted by the application of prophylactic measures in dogs throughout the period of mosquito activity. Measures to prevent mosquito outbreaks protect the health of people and animals.

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