

Climate changes implicated for *Dirofilaria* dissemination in Slovakia¹

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ABSTRACT. Dirofilariosis is a parasitic disease caused by helminths of the genus *Dirofilaria*. Climatic changes are considered to be main risk factors for dirofilariosis spreading. In the Slovak Republic, canine subcutaneous dirofilariosis was recorded for the first time in 2005. In 2007 the first coordinated research project started to detect possible endemic infections and to determine their magnitude. A total of 984 dogs were examined for the presence of microfilariae within 2007–2008. Modified Knott test and PCR were used for microfilariae detection and for *Dirofilaria* species identification. Dirofilariosis was diagnosed in 196 dogs which represents an overall prevalence of 19.9%. The majority of infected dogs came from southern regions of Slovakia. In the regions of Trnava and Nitra 45.2% and 31.4% of the dogs surveyed were infected, respectively. The highest prevalence of dirofilariosis was detected in shepherd and watch dogs (45.7%), and hunting dogs (40.5%). In the group of police dogs, 20.5% animals were infected. *Dirofilaria repens* was detected in all infected dogs. In seven animals co-infection with *Dirofilaria immitis* was present.

Key words: vector-borne diseases, dirofilariosis, dogs, Slovakia

Introduction

Dirofilariosis is a parasitic disease caused by helminths of the genus *Dirofilaria*. In Europe the most important species are *Dirofilaria repens* and *Dirofilaria immitis*. *D. repens* is a parasite of subcutaneous tissues, *D. immitis* is a causative agent of pulmonary and heart form of dirofilariosis. Life cycle of *Dirofilaria* spp. involves carnivores (dogs, cats, foxes, etc.) as definitive hosts and several mosquito species are intermediate hosts (or vectors) of the parasite. Viviparous females living in subcutaneous tissues or pulmonary arteries, release microfilariae into the blood circulation. Microfilariae are ingested by mosquito through the blood meal and moult into second and third larval stages. Larval development in mosquito takes 8–20 days depending on the temperature [1]. Infective L3 larvae are transmitted to a new definitive host during a next blood meal. In subcutaneous tissues L3 larvae moult into L4 and L5 stages. L5 larvae migrate to the pulmonary arte-

ries (*D. immitis*) or stay in subcutis (*D. repens*). Development of L3 infective larvae to adult worms in definitive host takes 6–9 months. Humans became accidental hosts while bitten by an infected mosquito.

Climatic changes, in particular global warming, extensive floods resulting in increased abundance of mosquitoes, along with an increased dog mobility are considered to be main risk factors for *Dirofilaria* spreading. At present, subcutaneous dirofilariosis caused by *D. repens* is considered to be the fastest spreading arthropod-borne disease in Europe [2,3].

In the Slovak Republic, canine subcutaneous dirofilariosis was recorded for the first time in 2005 [4] while the first consistent and coordinated research started in February 2007. The aim of this study was to perform the first detailed epidemiological survey of canine dirofilariosis in Slovakia, to detect possible endemic infections and to determine their real magnitude.

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Materials and methods

Within 2007–2008 a total of 984 dogs of different age, gender, breed and use were examined for the presence of microfilariae in their blood stream. The blood sample collection was performed in cooperation with several veterinary practices. Blood samples from police and military dogs were collected within cooperation with the Slovak Ministry of Interior and the Slovak Ministry of Defense. All the veterinarians were asked to choose dogs randomly and to fill the questionnaire covering all basic information on dog (age, gender, breed, utilization, possible visits abroad, locality of residence, clinical signs).

Modified Knott test was used for microfilariae detection. Blood and 2% formalin mixture was centrifuged (1500 rpm, 5 min), supernatant was decanted and sediment was stained with 0.5% methylene blue. Stained sediment was examined under a microscope at 150× magnification [5].

All samples positive by Knott test were analyzed by PCR for species determination. DNA of microfilariae for PCR analysis was extracted from 200 µl of citrate-treated blood using DNeasy Blood and Tissue Kit (Qiagen). Amplification of the fragments of cytochrome oxidase subunit 1 (CO1) gene was performed using specific *D. repens* and *D. immitis* pairs of primers according to Rishniw et al. [6].

Results

Microfilariae were detected in 196 of 984 dogs which represents an overall prevalence of 19.9%. Significant differences in the level of infection occurrence were found between individual regions. The majority of infected dogs came from southern regions of Slovakia. In the regions of Trnava and Nitra 45.2% and 31.4% of the dogs surveyed were

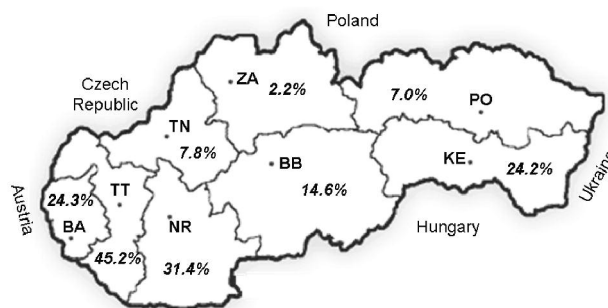


Fig. 1. Prevalence of canine dirofilariosis in the regions of Slovakia (BA–Bratislava, TT–Trnava, NR–Nitra, BB–Banská Bystrica, TN–Trenčín, ZA–Žilina, PO–Prešov, KE–Košice)

infected, respectively. More than 24.0% dogs were positive in Bratislava and Košice regions. The lowest prevalence of canine dirofilariosis was detected in northern part of Slovakia (Žilina 2.2%, Prešov 7.0% and Trenčín region 7.8%) (Table 1, Fig. 1). The number of microfilariae in 1 ml of blood varied between one up to several hundred specimens.

The highest prevalence of dirofilariosis was detected in shepherd and watch dogs (45.7%), and hunting dogs (40.5%). In the group of police dogs, 129 (20.5%) individuals out of 629 examined were infected. Low prevalence of dirofilariosis was found among companion dogs (7.4%) (Fig. 2).

Dirofilaria repens was detected in all infected dogs using PCR. In seven animals co-infection with *Dirofilaria immitis* was present. No single infection caused by *D. immitis* was found.

Autochthonous source of infection has been confirmed almost in all infected dogs.

Discussion and conclusions

The results of this survey demonstrate that cani-

Table 1. Canine dirofilariosis in Slovakia: number of dogs examined and infected in individual regions

Region	No. of dogs examined	No. of dogs infected	Prevalence (%)
Bratislava	140	34	24.3
Trnava	73	33	45.2
Nitra	185	58	31.4
Banská Bystrica	96	14	14.6
Trenčín	102	8	7.8
Žilina	92	2	2.2
Prešov	143	10	7.0
Košice	153	37	24.2
Slovak Republic	984	196	19.9

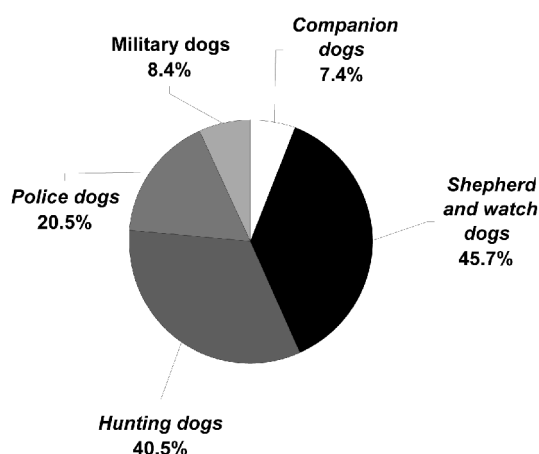


Fig. 2. *Dirofilaria* infections in dogs of different utilization

ne subcutaneous dirofilariosis is widespread among dogs in southern part of Slovakia. Two endemic localities were identified in the territory of Slovakia: Danubian Lowland and Záhorie region in southwestern part of the country and Eastern Slovak Lowland in southeastern Slovakia. Aforementioned regions are characterized as the warmest parts of Slovakia with high mean annual air temperatures and periodic floods in the river system.

High prevalence of infection in working dogs (shepherd-, watch-, hunting- and police dogs) represents an inconsiderable veterinary problem, whereas the disease (in particular pulmonary and heart forms caused by *Dirofilaria immitis*) and its pharmacotherapy may cause the thrombosis and heart failure especially in animals exposed to physical exercise.

Despite the fact that all infections were considered to be autochthonous, the exact determination of the locality of the origin of individual infection cases is very difficult. The reason is the extensive mobility of dogs. For example, police dogs spend several weeks per year in a training centre located in Záhorie that is considered to be endemic area of subcutaneous dirofilariosis. It could be assumed that it is a main reason of high number of infected police dogs in northern regions of Slovakia with climatically less favorable conditions as well as of great differences between prevalence in police and military dogs.

In relation to zoonotic character of this parasitic disease, the risk of human infection is also of importance in particular in endemic localities. In Slovakia two autochthonous cases of human subcutaneous dirofilariosis have been reported in 2007 and in 2008. Both cases were reported from western Slovakia, in the regions of Bratislava and Trnava [7,8].

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