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Different epidemiological pattern of canine dirofilariosis in two neighboring countries in Central Europe—the Czech Republic and Slovakia

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Abstract

The known data resulting from individual surveys of canine dirofilariosis point to the great differences in the epidemiological situation among countries where *Dirofilaria* parasites emerged approximately at the same time. In this regard, the Czech Republic and Slovakia, neighboring countries situated in Central Europe, could serve as an illustrative example of such a situation. The present study aimed to assess the prevalence of canine dirofilariosis in both countries and to discuss the reasons for potential differences shown. Between October and December 2019, 429 dogs from the Czech Republic and 644 from Slovakia were examined for canine dirofilariosis using the Knott test for microfilariae detection and conventional PCR for the species determination. The results' analyses showed notable differences. While in the Czech Republic autochthonous *Dirofilaria repens* cases are reported sporadically and *Dirofilaria immitis* infections have been confirmed only as imported so far, in Slovakia, both *Dirofilaria* species seem to have become endemic. Concretely, in the Czech Republic, microfilariae were detected in the peripheral blood of 8 dogs (1.86%): in seven, *D. repens* was confirmed, and in one dog, mixed infection with *D. repens* and *D. immitis* was diagnosed. Seven infected animals came from the eastern part of the country neighboring Slovakia. In Slovakia, microfilariae were detected in 68 (10.56%) dogs examined. DNA analysis confirmed *D. repens* mono-infection in 38 (5.90%) dogs, single *D. immitis* infection in 21 (3.26%) animals, and both *Dirofilaria* species were detected in 9 (1.40%) samples. Although we are unable to determine the cause of the differences, our study confirmed that the long-registered low number of canine dirofilariosis cases in the Czech Republic is not due to insufficient investigation (monitoring), but due to a low prevalence of the parasite in this area.

Keywords Canine dirofilariosis · *Dirofilaria immitis* · *Dirofilaria repens* · Czech Republic · Slovakia

Introduction

In Europe, *Dirofilaria immitis* and *Dirofilaria repens*, mosquito-transmitted canine filarioid nematodes with zoonotic potential, were found to be hyper-endemic in Mediterranean territories (Italy, Spain, Greece) in the past. During the last decades, the parasites were reported for the first time also in previously non-affected countries of Central and Eastern Europe (Hungary, Slovakia, Czech Republic, Austria, Poland, Germany, etc.), in some cases with significantly high prevalence in local dog population (Capelli et al. 2018).

The known data resulting from individual surveys and case reports point to the great differences in the epidemiological situation among the countries where *Dirofilaria* parasites emerged approximately at the same time. In this regard, the Czech Republic and Slovakia could serve as an illustrative example of such a situation. In both countries, the first autochthonous cases of canine dirofilariosis were recorded after the year 2000, and the presence of two species, *D. repens* and *D. immitis*, was confirmed (Svobodová et al. 2005, 2006). Since then, intensive research has been carried out in these regions, the awareness of veterinary practitioners has been

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markedly increased, and diagnostic and prophylactic approaches have been improved and put into practice. Nowadays, 15 years after the first findings, the epidemiological situation of canine dirofilariosis appears to be very different in the two countries.

For that reason, the aims of this study were (i) to assess the prevalence of canine dirofilariosis in both neighboring countries, the Czech Republic and Slovakia, over the same period and (ii) to discuss the reasons for potential differences shown.

Material and methods

Study areas

The presented survey was carried out concurrently in two neighboring countries. The Czech Republic (49° 45′ N 15° 30′ E; 78,866 km^2; population 10,693,939) and Slovakia (48° 40′ N 19° 41′ E; 49,035 km^2; population 5,457,926) are landlocked Central European countries laid between temperate and continental climate zones with relatively warm summers and cold, humid winters. The eastern part of the Czech Republic borders Slovakia, and both countries are bordered by Poland to the north and Austria to the south. The Czech Republic shares borders with Germany to the west; Slovakia neighbors Ukraine to the east and Hungary to the south.

The Czech Republic comprises three historic regions: Bohemia covering the largest western part of the republic, Moravia situated in the east of the country, and Czech Silesia located on the north-eastern borders with Poland. Currently, the Czech Republic is subdivided into 14 regions including the capital city Praha (Prague).

Present Slovakia is divided into eight self-governing regions: Bratislava (capital city), Trnava, Nitra, Trenčín (Western Slovakia), Žilina, Banská Bystrica (Central Slovakia), Prešov, and Košice (eastern Slovakia).

According to the last annual report of the European Pet Food Industry Federation, dogs are the most popular pets in both countries under a study with a stable population of 2.0 million in the Czech Republic and 900,000 in Slovakia (FEDIAF 2019).

Sample collection

Within 3 months, between October and December 2019, a comparative epidemiological survey of canine dirofilariosis was performed simultaneously in the territory of the Czech Republic and Slovakia. The monitoring was carried out under the auspices of the Bayer Animal Health Czech Republic & Slovakia Company; in Slovakia, also the Institute of Parasitology, Slovak Academy of Sciences (IP SAS) and Veterinary and Food Institute in Bratislava were engaged in sampling.

Veterinarians participating in the research were asked to encompass the dogs into the survey at random, but preferably animals older than 1 year and kept outdoors or with frequent outdoor activities. Individual data related to age, breed, sex, locality of residence, health status, and travel history were collected in a questionnaire form. Also, veterinarians and owners were asked to notice any antiparasitic preparation they had administered to the dogs during the last 12 months.

About 2.5 ml of peripheral blood was collected into a sodium citrate tube from each dog and stored at 4–12 °C for 1 week maximum. Subsequently, all samples were delivered to the Institute of Parasitology, Slovak Academy of Sciences, Slovakia.

In the Czech Republic, blood samples from 429 dogs (213 females, 216 males) were collected from 13 veterinary practices situated in six regions of Bohemia territory (124 samples) and two Moravian regions (305 samples). Out of all 429 examined dogs, 296 were kept indoor, 87 outdoor, 23 animals were raised in combined conditions, and no data were obtained from 23 dogs' owners.

In Slovakia, a total of 644 blood samples were investigated: 359 from Western Slovakia (4 regions), 35 from two regions of Central Slovakia, and 250 dogs originated in two regions of eastern Slovakia. A total of 278 of the animals were females, 262 males, and in 104 cases no data regarding the dogs' ages was available (particularly with shelter dogs). The majority of the dogs (330) were kept indoor, 129 outdoor, 114 only outdoor, 129 animals were bred in combined conditions, and no data was available for 71 examined individuals.

Concerning the life cycle and the biology of *Dirofilaria* spp., all dogs integrated into the study were on purpose older than 1 year.

Diagnostics

All samples delivered to the IP SAS in Košice, Slovakia, were examined for the presence of microfilariae using the Knott test (Knott 1939). Shortly, whole blood (1 ml) and 2% formalin (9 ml) mixture was centrifuge at 1500 rpm for 5 min, the supernatant was decanted, and sediment was stained with 1.0% methylene blue and examined under a microscope at × 400 magnification.

From all positive samples, DNA was extracted from 200 μl of blood with the DNeasy Blood and Tissue Kit (Qiagen, Germany) and subsequently tested using conventional PCR approach which amplifies a 203-bp fragment of the cytochrome c oxidase subunit 1 (COI) gene of *D. immitis* and a 209-bp portion of *D. repens* COI gene according to Rishniw et al. (2006) in a total volume of 25 μl of EliZyme HS Robust MIX reaction mixture (Elisabeth Pharmacon, Brno, Czech Republic). Nuclease-free distilled water and previously

positive samples, confirmed by sequencing, were used as negative and positive controls. All PCR reactions consisted of a denaturing step at 94 °C for 2 min and 35 cycles of denaturing (30 s at 94 °C), annealing (30 s at 56 °C), and extension (30 s at 72 °C) with a final extension at 72 °C for 7 min. The PCR products were electrophoresed on a 1.5% agarose gel stained with GoodView Nucleic Acid Stain (SBS Genetech Co., Ltd., Beijing, China) and visualized under the UV light.

Results

The Czech Republic

Out of 429 dogs examined, microfilariae were detected in peripheral blood of 8 individuals that represent an overall prevalence of 1.86%. In seven infected dogs, *D. repens* was confirmed as a causative agent; in one dog, mixed infection with both *Dirofilaria* species, *D. repens* and *D. immitis*, was diagnosed.

The dog with diagnosed mixed infection came from town Příbram situated in the Central Bohemian region. This infection cannot be considered unambiguously an autochthonous forasmuch as the dog was brought to the Czech Republic from Hungary. All seven dogs infected with *D. repens* originated in the Moravia part of the Czech Republic bordering Slovakia (Fig. 1): six of them lived in the South Moravian region (municipalities Břeclav—3 animals, Brno, Vyškov, and Hodonín); one infected dog came from Zlín region (the town of Uherské Hradiště). Three of these dogs had a positive travel history to Austria, Slovenia, and Germany, respectively; one of them originated in Ukraine. None of the infected dogs showed signs of any health concerns except one with diagnosed epilepsia.

Treatment or control of ecto- and/or endoparasites was carried out in 133 out of 429 dogs (31.0%). Most frequently, vets and dogs' owners (91 out of 429) mentioned the application of ectoparasiticides (fluralaner, flumetrin, fipronyl, and sarolaner) against ticks and fleas, administered orally, topically, or in a form of dogs' collar. Only in 7 dogs (1.6%) remedies with active substances against mosquitoes (permethrin, deltamethrin) were used, and prevention against heartworm and lungworm on the base of milbemycin oxime or macrocyclic lactones was applied in 24 (5.59%) animals. Broad-spectrum dewormers (fenbendazol, praziquantel, pyrantel, and others) were used in the last 12 months in 11 (2.56%) examined dogs.

Slovakia

In Slovakia, microfilariae were detected in the blood of 68 dogs examined (49 from Western Slovakia and 19 from the eastern part of the country) (Fig. 1), which represent an overall prevalence of 10.56%. DNA analysis confirmed *D. repens*

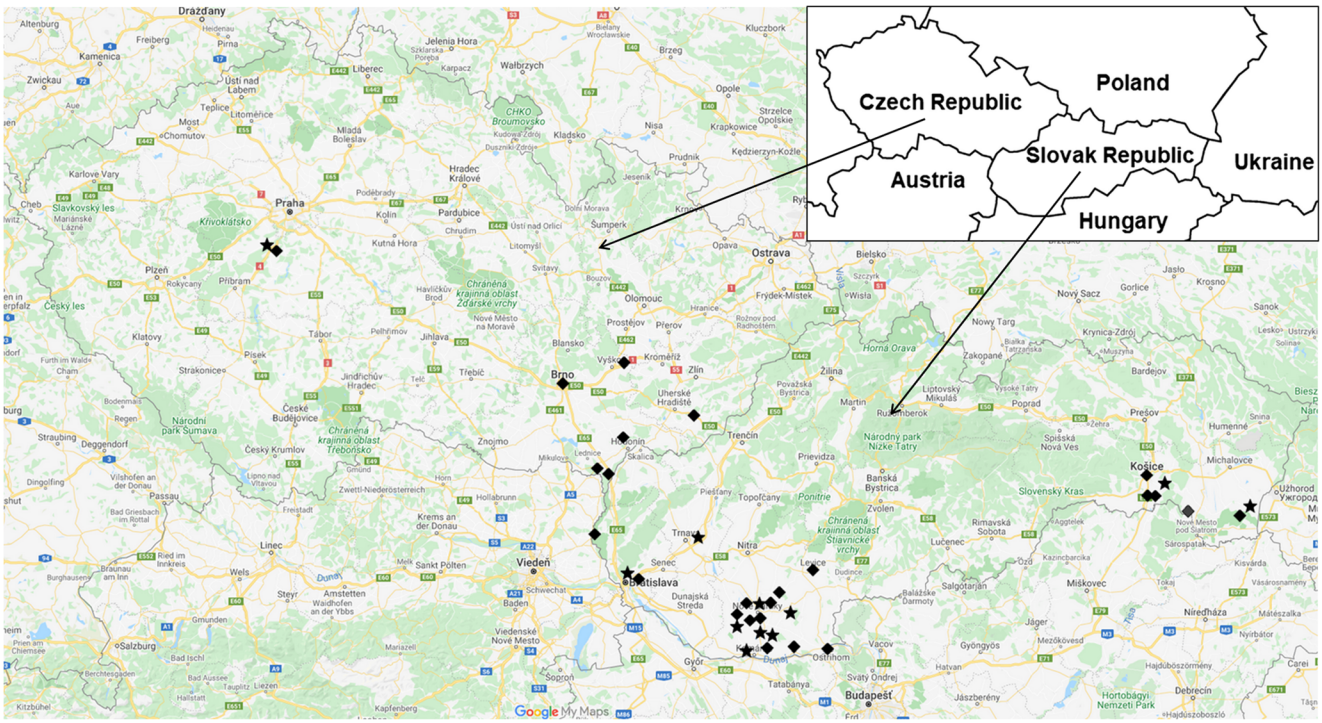

Fig. 1 *Dirofilaria repens* (rhombus marks) and *Dirofilaria immitis* (star marks) diagnosed in dogs from the Czech Republic and Slovakia within the present study

mono-infection in 38 (5.90%) dogs, single *D. immitis* infection in 21 (3.26%) animals, and both *Dirofilaria* species were detected in 9 (1.40%) of the investigated blood samples.

Out of 359 dogs originated in the western part of Slovakia, 49 (13.65%) were found microfilaraemic; in 27 individuals, *D. repens* was subsequently detected, 15 animals were infected with *D. immitis*, and in 7, co-infection with the two species was diagnosed. Examination of 250 dogs from eastern Slovakia revealed microfilariae in 19 (7.60%) samples. DNA analysis confirmed *D. repens* in 11 animals, *D. immitis* in 6, and both *Dirofilaria* species were detected in 2 dogs. None of the 35 dogs originated in the central part of the country was confirmed *Dirofilaria*-positive in this study. In four dogs from Western Slovakia infected with *D. repens*, also *Babesia canis* was diagnosed, and one dog suffered from renal failure. In one dog with confirmed *D. immitis*, a heart murmur was recorded by a veterinary surgeon, and one dog died of heart failure following severe clinical signs of heartworm disease. This fatal case of heartworm infection was published separately by Miterpáková et al. (2020).

In 220 (34.16%) out of 644 dogs involved in the study, the veterinarians or owners declared application of certain antiparasitic medicaments over the last year. Most of the dogs (81) were treated by acaricides effective against ticks and fleas. In 32 (4.97%) animals, anti-mosquito remedies on permethrin and deltamethrin base were used, and in 58 dogs (9.00%), milbemycin oxime or macrocyclic lactones as prevention against *Dirofilaria* spp. and lungworms were administered. Together, 49 dogs were dewormed using broad-spectrum anthelmintics during the last 12 months.

Discussion

The results' analyses showed notable differences in the number of *Dirofilaria*-infected dogs and also in the geographical distribution of the parasites in both studied countries.

In the Czech Republic, autochthonous cases of canine subcutaneous dirofilariosis were unambiguously detected in only two regions of Moravia—the eastern part of the country neighboring Slovakia. This lowland area of the South Moravian region bounded with rivers Morava and Dyje represents a stable natural focus of *D. repens* circulation ever since the parasite was observed here for the first time in dogs in 2005 (Svobodová et al. 2006). Later, within the xenomonitoring carried out between 2009 and 2011, *D. repens* was confirmed in *Aedes vexans* mosquitoes from this area (Rudolf et al. 2014). Additionally, at least three cases of human dirofilariosis were diagnosed in patients living in the South Moravian region who never traveled abroad (Matějů et al. 2016). These previous findings, along with the results of this here-presented research, have confirmed this rather small area to be endemic for *D. repens*.

There is a completely different situation in neighboring Slovakia. While in the Czech Republic the occurrence of canine dirofilariosis is limited practically to one coherent territory, in Slovakia, the epidemiological pattern concerning *Dirofilaria* infections has been dynamically developed. The very first systematic research in the Slovak territory in 2007 revealed an occurrence of *D. repens*–endemic areas with high prevalence rates recorded in working, in particular, police dogs (Miterpáková et al. 2008, 2010). Consecutive full-area decennial research discovered that canine dirofilariosis had expanded to the whole Slovak territory with mean prevalence rates ranging between 2.0 and 25.0% in individual regions (Miterpáková et al. 2016). The first screening of mosquito vectors performed in 2013 and also the following one carried out between 2015 and 2017 confirmed several mosquito species being involved into both *D. repens* and *D. immitis* life cycles in Slovakia (Bocková et al. 2015; Čabanová et al. 2018). Human subcutaneous dirofilariosis in Slovakia was diagnosed for the first time in 2007, and since then, new cases are reported every year (Miterpáková et al. 2017).

Discrepancies have been recorded not only in prevalence rates reported from the Czech Republic and Slovakia (1.86% and 10.56% in this study) but also in the abundance of both *Dirofilaria* species. While in the Czech Republic only *D. repens* autochthonous cases in dogs were confirmed up to now, in Slovakia, besides *D. repens*, which is considered the predominant species, also an increasing number of heartworm infections in dogs from the southern part of the country have been recorded during the last 5 years (Miterpáková et al. 2018, 2020, and unpublished data).

Herein-observed different epidemiological patterns of canine dirofilariosis might be caused by several factors. In the view of the common social and political background of the two countries, and following the authors' personal experiences with the veterinary medicine scope, it might be said that the level of prevention measurements and awareness of veterinary practitioners in both countries is coequal. Also, according to the evaluation results of the questionnaires, the proportion of dogs treated with any of the antiparasitic products was approximately the same in both countries (31.0% and 34.16% in the Czech Republic and Slovakia, respectively). Targeted prevention against *Dirofilaria* parasites was applied in 9.0% of Slovak dogs and only in 5.6% of dogs from the Czech Republic.

On the other hand, as a spread of dirofilariae depends largely on the vectors' occurrence and abundance, climatic and environmental conditions might be of great importance. For instance, the latest study from Croatia revealed a continuous increase of human dirofilariosis in continental region presumably related to changes in Croatian mosquito fauna and expansion of the host range of the

competent vector species, *Aedes albopictus* and *Aedes japonicas* (Pupić-Bakrač et al. 2020). In any case, research focused on heartworm carriers is still in its infancy in both Slovakia and the Czech Republic, and is mostly regionally oriented; therefore, it is too early to draw unambiguous conclusions.

Besides the presence of mosquito species capable of transmitting the parasites, the number of infected and microfilaremic dogs, as well as circumstances of dog keeping, might be the limiting factors for the establishment of dirofilariosis in a certain territory (Simón et al. 2012; Fuehrer et al. 2016). However, even this does not explain the significant differences in prevalence, as in both, Slovakia and the Czech Republic, more than half of the examined dogs (330 in Slovakia, 296 in the Czech Republic) were kept exclusively indoors. Additionally, the Czech Republic and Slovakia are adjacent to the countries with a very different epidemiological situation of canine (and human) dirofilariosis, which may also result in the observed discrepancy. Concretely, in Germany and Austria, neighboring the Czech Republic from the west and the south, autochthonous cases of canine dirofilariosis occur very rarely and the majority of the infections are imported with dogs from abroad (Vrhovec et al. 2017; Schäfer et al. 2019; Sonnberger et al. 2020). In Poland, neighboring both countries from the north, *D. repens* was recorded in several regions with the mean prevalence rates varied from 1.2 to more than 25.0% and with a recognized endemic area in the central part of the country (Demiaszkiewicz et al. 2014; Bajer et al. 2016).

Quite a different situation has been noticed in the countries bordering Slovakia, Hungary to the south and Ukraine to the east. In Hungary, the first autochthonous cases of *D. repens* in dogs were reported at the end of the twentieth century, but according to retrospective analyses, it could be assumed that the country had become endemic earlier (Farkas et al. 2019). While the *D. repens* prevalence in Hungary varies steadily between 14.0 and more than 19.0%, and several endemic localities of the species have been identified, *D. immitis*, for the first time detected in 2007, has increased its prevalence continually over the past 12 years (Széll et al. 2020). Only a few publications focused on canine dirofilariosis in Ukraine are accessible. Most of *Dirofilaria* surveys were performed in the Kyiv area with *D. repens* prevalence varied between 18.0 and 30.0% (Vasylyk 2004; Hamel et al. 2013; Soloviova 2017). The high number of human cases of dirofilariosis in Ukraine (1465 reported between 1997 and 2012) also points to the highly endemic status of *D. repens* in this European region (Sałamatin et al. 2013). Nevertheless, no data is available on the parasite distribution in the western part of Ukraine bordering with Slovakia.

Conclusion

The present study revealed significant differences in the occurrence of canine dirofilariosis in the Czech Republic and Slovakia—two neighboring countries of Central Europe. While in the Czech Republic autochthonous *D. repens* cases are reported sporadically and *D. immitis* infections have been confirmed only as imported so far, in Slovakia, both the *Dirofilaria* species seem to have become endemic. Based on our study, we can confirm that the long-term registered low number of canine dirofilariosis cases in the Czech Republic is not caused by insufficient investigation (monitoring), but by a really low prevalence rate in this area.

By taking a closer look at the epidemiological situation in other Central European countries, the odds are that non-endemic countries share a border with hyper-endemic regions for both *Dirofilaria* species. Considering the frequent absence of clinical signs and persistent low recognition of the infection among veterinary practitioners, we can presume that many microfilaremic dogs remain undetected, which increases the risk of *Dirofilaria* expansion to non-endemic territories. Hence, routine screening for the parasites together with increasing awareness of veterinary practitioners is highly eligible measurements to prevent the further spread of this zoonotic infection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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