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Canine dirofilariosis under specific environmental conditions of the Eastern Slovak Lowland

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Abstract

The aim of the present study was to collect data from Eastern Slovak Lowland, southern Slovakia, to assess risk of the spread of canine dirofilariosis. Climate and environmental conditions in the Eastern Slovak Lowland are ideally suitable for the occurrence of vector-borne diseases. In the past, an endemic locality of dangerous mosquito transmitted malaria was found in this area. Today, another zoonotic parasitic disease threatens – dirofilariosis. The results of the first detailed study revealed a 34.44% prevalence in dogs harbouring dirofilariae. *D. repens* was diagnosed in all infected specimen, with 2 individuals being co-infected also with *D. immitis*.

Key words

dirofilaria, dirofilariosis, dog, environmental conditions, Slovakia, Eastern Slovak Lowland

INTRODUCTION

In the past few decades, a new phenomenon has come to professional and lay public awareness, represented foremost by global warming, redundant rainfall, strong storms and flooding. These climate changes have considerable impact on the epidemiological patterns of many infectious diseases. The demographic characteristics of suitable host, vector and pathogen reservoir species undergo changes. The increase in average temperatures in many areas of the world for prolonged periods, has created conditions favourable for parasite survival in a vector organism or environment, and multiplied the number of parasite generations developing during one season. Indeed, not only climate changes are leading to the establishment of new host-parasite-environment systems. Uncontrolled anthropogenic interferences with the environment, urbanisation, global trade, travelling intensification, increase in exotic pets breeding, etc., have significantly contributed to the spread of infections.

Recent climate changes, above all, are responsible for the rapid spread of vector-borne infections, including viral diseases (e.g. Dengue fever, West Nile fever) and parasite infections (malaria, leishmaniosis, human and animal filarioses). In Europe, subcutaneous dirofilariosis is presently considered as the arthropod-borne disease with the fastest spread propensity [1].

Dirofilariosis is caused by parasitic helminths from the genus *Dirofilaria*, with *D. repens* responsible for the subcutaneous form of the disease and *D. immitis,* which affects primary the respiratory system. The parasite life cycle involves carnivores as definitive hosts, and different mosquito species as both vectors and intermediate hosts. Female dirofilariae living in subcutaneous tissue or pulmonary blood-vessels produce larvae – microfilariae which circulate in peripheral blood. During their blood meal, mosquito ingests microfilariae that start developing into the infective L3 stage. Development in the vector organism is dependent on the environmental temperature and lasts from 8 up to more than 20 days [2]. During the next blood meal, infective L3 larvae are transmitted to a new, definite host, where they moult twice and transform into L4 and L5 stages. L5 larvae are pre-adult worms that settle in subcutaneous tissues (*D. repens*), or migrate into the pulmonary arteries (*D. immitis*). After sexual maturity, the female and male worms copulate and produce microfilariae. Parasite development in the definitive host organism takes 6-9 months. Humans can also be involved in the *Dirofilaria* life cycle as a aberrant host [3].

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MATERIAL AND METHODS

Studied area. The Eastern Slovak Lowland, covering an area of 2,500 km^2, is located in the eastern part of Slovakia (Fig. 1). Bordering with Ukraine and Hungary, 3 districts are situated there: Trebišov, Michalovce, Sobrance. geographical co-ordinates: 49°11´-48°24´, North Latitude, 21°51´-22°12´ East Longitude, at an altitude of 95-200 m above sea level. The region belongs to the mild climatic zone and generally has a warm and fairly dry climate; average annual air temperature: about + 9-10 °C, with 50-70 summer days; the warmest month is July with n average temperature +19.7 °C. Annual rainfall: 600-750 mm, with profound rainstorms often occurring and causing intensive floods. The area is characterised by a fan-shaped network of streams, with an important river, the Bodrog, and its tributaries Ondava, Laborec, Latorica, Uh and Topľa. A big water reservoir, Zemplínska Šírava (33 km²), and a system of protective dikes and drainage canals have been built to prevent frequent floods. Lowland meadows, grasslands, pastures and fields with swamps and swamp forests prevail in the landscape, with protected habitats in nature preserves. The above-mentioned conditions are associated with recurring mosquito calamities.

Samples collection. During 2007–2010, dogs originating from the Eastern Slovak Lowland area were investigated for dirofilariosis. 151 dog samples were collected by co-operating veterinarians and dog breeders. Peripheral blood was taken into tubes with the addition of anticoagulans and stored at +4 °C until examination.

The dogs were subdivided into groups according to age (< 3 years, 3-6 years, 6-9 years, and older than 9), gender (male, female), canine utilisation (watchdog, police and military, hunting, company and shepherd dogs), keeping manner (outdoor, indoor), breed size (small, medium, large and giant) and hair length (short-, long- and wire-haired). All dogs were older than 1 year, without previous chemoprophylaxis or treatment by microfilaricide preparations.

Figure 1. Studied area – Eastern Slovak Lowland (charcoal grey)

Methods used. Microfilariae were in the total blood detected by means of a modified Knott test [4]. One ml of blood was mixed with 9 ml of 2 % buffered formalin and centrifuged at 1,500 rpm for 5 min. The supernatant was descanted and the sediment tinctured by 1 % methylene blue stain. The tained sediment was examined under a microscope at 150 × magnification. All positive samples had undergone the PCR analyses for species identification.

Microfilarial DNA for PCR analyses was extracted from positive blood samples using commercial isolation set DNease 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.* [5].

Statistical analysis. The Chi-square test was carried out to compare the prevalence of dirofilariosis according to district, sex, age, utilization, type of coat, breed categories and housing. The Odds Ratios (OR) and the 95% confidence intervals (95% CI) were calculated. A value of $p < 0.05$ was considered significant. All the statistical analysis was performed using Open Epi 2.3.1 software.

RESULTS

A total of 151 dogs from the Eastern Slovak Lowland and adjacent areas were examined. The age of dogs varied from 12 months to 13 years. Microfilariae were detected in 52 individuals (34.44%) with the highest prevalence in Trebišov district (54.38%) (OR = 4.145, CI = 2.033-8.448, p = 0.019). The percentage of infected dogs was lower in the districts of Michalovce (25.42%) (OR = 0.507, CI = 0.247-1.040, p = 0.019) and Sobrance (17.14%) (OR = 0.315, CI = 0.121-0.818, p = 0.019) (Tab. 1). Not a single dog showed any clinical symptoms incidental with infection.

Table 1. Risk factors of canine dirofilariosis

Variable	N	Pos.	Prevalence (%)	OR (odds ratio)	95% CI (confidence interval)	p-value
District						
Michalovce	59	15	25.42	0.507	0.247-1.040	
Trebišov	57	31	54.38	4.145	2.033-8.448	0.019
Sobrance	35	6	17.14	0.315	0.121-0.818	
Sex						
Female	40	14	35.00	1.034	0.484-2.209	0.95
Male	111	38	34.23	0.967	0.453-2.065	
Age (years)						
< 3	60	10	16.66	0.233	0.106-0.516	
3-6	44	16	36.36	1.127	0.541-2.347	0.03
6-9	29	17	58.62	3.521	1.525-8.129	
>9	18	9	50.00	2.093	0.776-5.648	
Utilization						
Pet dogs	22	4	18.18	0.375	0.120-1.173	
Hunting dogs	10	5	50.00	2.000	0.552-7.251	
Guard dogs	25	11	44.00	1.629	0.680-3.900	0.35
Police dogs	91	29	31.87	0.753	0.380-1.488	
Shepherd dogs	3	3	-	-	-	
Type of coat						
Shorthaired	57	19	33.33	0.924	0.461-1.851	
Longhaired	92	32	34.78	1.040	0.522-2.071	0.94
Wirehaired	2	1	-	-	-	
Breed						
Small	6	1	16.67	0.369	0.042-3.241	
Medium	8	1	12.50	0.258	0.031-2.153	
Large	109	41	37.61	1.699	0.772-3.742	0.63
Gigant	28	9	32.14	0.881	0.367-2.115	
Housing						
Indoor	22	4	18.18	0.375	0.120-1.173	0.05
Outdoor	129	48	37.21	2.667	0.852-8.343	

Number of dogs examined (N) and positive for dirofilariosis (Pos.).

Mutual relations between infection and individual factors potentially facilitating it were analysed within the study (Tab. 1). The results show that canine utilisation is a considerable risk factor for dirofilariosis incidence. The prevalence was significantly higher in working dogs (37.21%) (OR = 2.667, CI = 0.852-8.343, p = 0.05) in comparison with companion dogs (18.18%) (OR = 0.375, CI = 0.120-1.173, p = 0.05). The animals most infected were in the group of hunting dogs (50.0%) (OR = 2.000, CI = 0.552-7.251, p = 0.35), followed by watchdogs (44.0%) (OR = 1.629, CI = 0.680-3.900, p = 0.35), and police and military dog groups (31.87%) (OR = 0.753, CI = 0.380-1.488, p = 0.35).

Dogs older than 3 years harboured microfilariae more frequently in comparison with specimen less than 3 years of age. The prevalence rose to 36.36% (OR = 1.127, CI = 0.541-2.347, p = 0.03) in the category 3-9 years, and reached maximum in old dogs aged 6-9 years (58.62%) (OR = 3.521, CI = 1.525-8.129, p = 0.03), whereas in dogs over 9 years of age it was only slightly lower (50.0%) (OR = 2.093, CI = 0.776-5.648, p = 0.03).

In the comparison, between a group of small and medium breeds (OR = 0.2919, CI = 0.04281-1.215, p = 0.05) on one side, with a group of giant and large breeds (OR = 3.448, CI = 0.7417-16.03, p = 0.05) on the other, the difference was significant.

Microfilariae were more often found in the blood of female dogs (35.0%) (OR = 1.034, CI = 0.484-2.209, p = 0.95) than in males (34.23%) (OR = 0.967, CI = 0.453-2.065, p = 0.95); the difference, however, was not statistically significant.

From the sampling, 57 dogs were included in the short-haired group (prevalence 33.33%) (OR = 0.924, CI = 0.461-1.851, p = 0.94), 92 individuals had long hair (prevalence 34.78%) (OR = 1.040, CI = 0.522-2.071, p = 0.94), and 2 specimens were wire-haired (one was infected). The length of hair did not significantly influence prevalence.

In all infected dogs, *D. repens* was detected as the infection agent (rate 96.15%). Two dogs bred in the Veľké Slemence village on the Slovak-Ukrainian border also harboured *D. immitis* in mixed infection with *D. repens*. No *D. immitis* mono-infection was detected.

DISCUSSION

Environmental conditions in the area of the Eastern Slovak Lowland (continental warm summer, number of swamps and wetlands, frequent floods followed by repetitive mosquito calamities) are most suitable for the occurrence and spread of mosquito transmitted diseases. For a half of the 20th century the Eastern Slovak Lowland was an endemic region of malaria and involved recurring (tertian) malaria (caused by *Plasmodium vivax* and *P. ovale*) and dangerous tropical malaria (caused by *P. falciparum*). According to Dziuban, during World War II, 10-30% of the human population of Eastern Slovakia were infected [6, 7]. The last autochthonous case in Slovakia was registered in 1959, and in 1963 the country was proclaimed malaria-free [8].

Mosquito transmitted diseases, including malaria, also re-emerge nowadays in Central Europe. Subcutaneous dirofilariosis is the arthropod-borne disease with the fastest spread propensity in Europe [1].

D. repens in dogs was first recorded in Slovakia in 2005 [9]. In 2007, the first targeted epizootological study was performed that revealed nearly 35% of dogs living in south-western regions with a warm climate were infected with *D. repens* [10]. All cases were confirmed as autochthonous. Subsequent research revealed a relatively high occurrence in police dogs, with an average prevalence of 20% [11].

Since 2005, new records of canine dirofilariosis have gradually been reported from adjacent countries. The first infected dog in the Czech Republic was recorded in 2006 [12]. Monitoring performed during 2005-2006 in Hungary revealed a prevalence of 14% in dogs, and 2 infected cats [13]. The first *D. immitis* autochthonous infection in Hungary was presented by Jacsó et al. in 2009 [14]. In Austria, subcutaneous dirofilariosis was diagnosed in 2009 when microfilariae were detected in the blood of 7 dogs living in regions neighbouring Hungary and Slovakia [15]. In 2009, Demiaszkiewitz also found *D. repens* infected canines in central Poland, with the average prevalence reaching 37.5% [16]. Parasite investigation in Ukraine was focused foremost on the Kiev area. In 2000-2002, Vasylyk reported *D. repens* incidence in 30% of stray dogs and in 22% of domestic dogs [17]. No data are available on parasite occurrence in western part of Ukraine bordering with Slovakia and Hungary. Although dirofilariosis research did not map all regions of Central Europe, the results to date confirm its occurrence in the entire territory, with a more or less restricted range of endemic localities in all countries.

The Eastern Slovak Lowland is situated in the south-eastern part of Slovakia, and local climatic and ecological conditions create a suitable environment for parasite development in vectors. Woodland meadows, swamps, flood plain forests and inundation areas by Latorica River and other rivers, are essential for vector survival. Substantial rainfall and abundant floods are causative for mosquito calamities in this region. According to the monitoring of mosquito species composition carried out in 2007-2008, 23 out of 49 known species in Slovakia are located in the territory of the Eastern Slovak Lowland [18]. The most important are blood-sucking biters with calamity occurrence, i. e. *Aedes vexans*, *Ae. cinereus*, *Ae. rossicus*, *Ae. sticticus*, and *Ae. cantans*.

Canine utilisation and age appear to be important factors in parasite transmission. Dogs used as working animals: hunting, watchdog or police and military dogs, were infected more frequently compared with companion animals. This can be incidental with outdoor keeping of working dogs, as well as with a longer period of time spent in the open-air and more frequent exposure to mosquitoes.

Districts situated in the region of the Eastern Slovak Lowland are mostly rural with small towns and villages, where dogs are bred in large numbers and kept backyards. It is generally known that the attitude of the owners to veterinary care and prevention in the country is less keen compared to urban breeders, who consider dogs to be a member of the family. *D. immitis* infection, especially in working dogs, may negatively bias their serviceability and cause rejection from active duty.

Age appears to be the next important factor in dirofilariosis transmission. The presented results are in compliance with several studies, and point out that older dogs are infected more frequently than younger animals of less than 3 years of age. This is probably due to a longer exposure time to mosquitoes that increases the opportunity for acquisition of infection. The slight decrease of infection in dogs over 9 years of age may be explained by the *Dirofilaria* life span that ranges from 5-7 years, and also by the fact that the first autochthonous dirofilariosis case in Slovakia was reported in 2005.

A high dirofilariosis prevalence was recorded in the large and giant dog breeds. This might be connected with the more spacious body surface, and probably also with a higher body weight, followed by lower activity compared with small and medium-sized dogs, which encourages mosquito-sucking and increases the risk of infection.

The results of our research show that *D. repens* is the predominant species circulating in the Eastern Slovak Lowland area. *D. immitis*, responsible for the pulmonal (resp. heart) form of disease, was detected in only 2 dogs, both in co-infection with *D. repens*. The rare *D. immitis* occurrence can be explained by the findings of Genchi *et al.* [1] who analysed the mutual relationships between the 2 *Dirofilaria* species, and affirmed that *D. immitis* spread is suppressed in areas with high *D. repens* prevalence. In fact, the prevalence of *D. immitis* might indeed be much higher, taking into account that in the case of mixed infection, the number of *D. immitis* microfilariae can be very low and thus difficult to detect. The sensitivity of commercial serological kits for *D. immitis* diagnostic is also limited, and according to available data they are able to detect the infection only in the case of a minimum of 2 adult females present in the organism [19]. Our study provides the evidence that the microfilariemia in dogs is not necessarily accompanied by the clinical signs of *D. repens* or *D. immitis* infection. Such dogs often become the source of parasite for other canids and humans, and in interaction with a long patent period and high number of sucking-mosquitoes they represent a great risk of infection.

With regard to the zoonotic character of dirofilariosis, the risk of human infection in the Eastern Slovak Lowland is enhanced by reason that a large part of the area is constituted by the Latorica protected natural reserve, intensively exploited for tourism. To date, 2 autochthonous human cases of subcutaneous dirofilariosis have been recorded in Slovakia, both of them in the western part of the country [20, 21].

CONCLUSION

Restriction of the spread of dirofilariosis by using preventive measures throughout the whole period of mosquito activity, is a momentous task. The education of lay and professional communities and preventive measures focused on anti-mosquito protection and preclusion of the occurrence of mosquito calamities are major instruments in combating the parasite. Furthermore, regarding the zoonotic pattern of dirofilariosis, the monitoring and risk analysis in different environmental conditions are valuable contributions to avert the infection risk for both humans and animals.

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