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Original papers

Endoparasites of the grey wolf (*Canis lupus*) in protected areas of Slovakia

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ABSTRACT. Although the grey wolf was on the brink of extinction in Central Europe in the last century, it never became extinct in Slovakia and nowadays its population is considered stable. The wolf population in Slovakia is estimated to be around 400 individuals with seasonal variations, and due to these small numbers, studies on the parasite fauna of wolf are scarce. Of the 35 parasitic species recorded worldwide in grey wolves in temperate and mountain zones of the Palearctic region, 15 were detected in Slovakia. In our study, 256 grey wolf faeces samples taken from three protected areas in Slovakia were examined using the modified flotation method with a zinc sulphate solution. In total, 169 samples (66%) displayed propagative stages belonging to ten parasitic taxa (*Isospora* spp., *Alaria alata*, *Taenia* spp., *Strongyloides stercoralis*, Ancylostomatidae, Trichuridae, *Toxocara canis*, *Toxascaris leonina*, *Spirocerca lupi*, *Angiostrongylus vasorum*). The Trichuridae was the most prevalent group, with a prevalence of 17.7–60.3%. The parasitic species *Isospora* spp. (3.5%) and *A. vasorum* (0.8%) are reported for the first time in wolves in Slovakia. Considering the zoonotic potential of some parasites, and the increasing co-existence of human and wildlife in protected areas, the present study provides important findings for further epidemiology research in the grey wolf population.

Key words: grey wolf, endoparasites, *Angiostrongylus vasorum*, coprological study

Introduction

The grey wolf (*Canis lupus*) is one of the largest predators in Europe. This carnivore has a Holarctic distribution and inhabits Eurasia and Northern America. The human impact on its population associated with persecution and habitat change has resulted in its current distribution range in Europe being fragmented. The grey wolf population has expanded across Slovakia and the rest of Europe over the last 35 years, and has been the focus of numerous studies and conservation efforts [1].

The wolf is a European protected species of importance and its population in the European Union is growing thanks to the habitat directive initiative and its conservation measures. Nowadays, the population of wolves numbers around 12,000 individuals divided between 10 populations present in 28 countries [2,3]. In Slovakia, the wolf was never absent but due to persecution, the population has been on the brink of extinction several times, especially at the end of the 19th Century. The last significant decline in the wolf population was recorded in the 1960s [4,5]. Despite their low number, the wolves have remained in the north-eastern part of Slovakia. Today the species is protected by law and managed through a hunting quota determined annually by the Ministry of Agriculture and Rural Development of the Slovak Republic. Hunting is prohibited in all national parks and Sites of Community Importance. Nowadays, the wolf population in Slovakia is considered stable by the International Union for Conservation of Nature, and based on an official report elaborated from the

Slovak minister to the European Commission, an annual average of 400 individuals roam the territory distributed across approximately 60% of the country [5]. The most natural wolf habitat in Slovakia is composed of a mosaic of hills connected with forests, meadows and agricultural landscapes with low human density. However, wolves are widespread in most of the central Slovak mountains, where the habitat is composed of continuous forests, agricultural land at the lower boundary of the forest, grassy uplands rising to about 2,000 m above sea level, and widespread deciduous forests which have recently colonized the south-western and southern foothills of the Carpathians [6].

In Central Europe, humans and wildlife co-exist closely in a shared environment, and hence parasites and pathogens can easily be transmitted between them [7]. Due to the low numbers of wolves present in the last century, data concerning their parasitology is rare. According to Craig and Craig [8], 35 agents of parasitic infections were recorded in the temperate and mountain zones of the Palearctic region; however, the most prevalent in this host are believed to be Taeniid cestodes (*Taenia hydatigena*, *Taenia krabbei*, *Taenia multiceps*), the nematodes of the genus *Trichinella*, the *Alaria alata* fluke, and the *Uncinaria stenocephala* hookworm [7–9].

In Slovakia only few parasitological surveys in wolves have been performed so far. The very first study on parasite diversity in Slovakian wolves was carried out in 1961 by Baruš [10], who examined three individuals by necropsy. Two wolves were obtained from the central part of the country: one, from Banská Štiavnica, was infected with only one parasitic species, *T. hydatigena*, while the second, from Poľana, harboured a significantly broader parasite spectrum, comprising *Toxocara canis* and *Toxascaris leonina* roundworms, *U. stenocephala* and *T. hydatigena*. In the third wolf, from eastern Slovakia (Prešov), *Dipylidium caninum* tapeworm was found.

A necroscopy study by Mituch identified a total of four cestodes (*T. hydatigena*, *Taenia pisiformis*, *D. caninum*, *Mesocestoides lineatus*) and six nematode species (*Trichinella spiralis*, *T. canis*, *T. leonina*, *Spirocerca lupi*, *Capillaria plica* and *U. stenocephala*) in 24 wolf carcasses from eastern Slovakia [11]. The parasites were found in 21 wolves with *T. spiralis* being the most prevalent species (54.1%), followed by *T. hydatigena* (41.6%), *T. pisiformis* (16.6%), and *T. canis* (12.5%).

In 1972, the new parasitic species *Taenia crassiceps*, *Hydatigera taeniaeformis* (syn. *Taenia taeniaeformis*) and the *Ancylostoma caninum* hookworm were recognized in wolves from Slovakia [12]. The investigation of wolf parasites continued in 1990s in the Tatra National Park area, where parasitic infection was detected in seven of eight examined wolves; three cestodes (*T. hydatigena*, *T. crassiceps*, *M. lineatus*) and two nematode species (*T. canis* and *Trichinella* spp.) were found in this region [13].

Recent parasitological studies have focused predominantly on the monitoring of echinococcosis and trichinellosis: the most important zoonotic infections in wolves. Martinek et al. [14] used copro-PCR for *Echinococcus multilocularis* detection in 23 wolves from the Muránska Planina National Park and Bukovské Vrchy mountains situated in central Slovakia. *E. multilocularis* DNA was detected in two wolf samples from the Muran Plateau and one sample from Bukovské Vrchy. Interestingly, trichinellosis monitoring in the Tatra National Park found 27% of the examined wolves to be infected with *T. britovi* [15]. In summary, 15 parasite taxa have so far been identified in wolves from Slovakia. As the data on helminth fauna from the recent period is lacking, the aim of our study was to determine the parasite composition in wolves from protected areas of Slovakia under current environmental conditions.

Materials and Methods

Study area and sampling. The parasitic fauna of grey wolves in Slovakia was monitored. Sampling was performed with the use of invasive and non-invasive methods in two national parks and one protected area. The first sampling locality, the Tatra National Park (Tatra NP), is the oldest national park of Slovakia, having been established in 1949. The Tatra NP is situated in the northern part of the country on the border with Poland. It covers a total of 73,800 hectares and its buffer zone accounts for another 30,703 hectares. The park is a mosaic of different and rare landscapes and vegetation types, according to geology, elevation, aspect and slope. Forest cover is about 70% of the total area, with the remaining 30% being meadows, rocks and alpine zones [16]. The climate of the Tatra Mountains ranges from cool to very cool and moist. Various animal species live in the park, including the brown bear, wolf, lynx, marmot and chamois.

Located on the border of central and eastern

Slovakia, Muránska Planina National Park (Muránska Planina NP), with its wild mountain karstic landscape, is characterized by an area with low human intervention. It was declared a national park in 1997. Regarding its composition, 85.4% of its 20,318 hectares is forest, while only 10% is agricultural land. The national park territory does not include larger settlements and is distant from main roads and communication links [17].

The Pol'ana Protected Landscape Area (Poľana PLA) is located in Central Slovakia, it covers an area of 20,360 hectares, of which more than 83.5% is represented by forest land and over 14.7% by agricultural land; in addition the area and surroundings are characterised by an old volcano. Due to the varied relief and the geographical location of the area, both thermophilic and mountainous species of plants and animals can be found. Pol'ana PLA was declared as a protected hunting area in 1965, with specific regulations to safeguard conservation of the genetic fund of the animals hunted [18,19].

From October 2015 to November 2016, 256 wolf faecal samples were gathered in two national parks and one protected landscape area of Slovakia. These were divided into two different collections (Fig. 1).

From the Tatra NP, 79 samples were collected from animals killed during the hunting season or by car accidents. These samples were collected by zoologists from the Museum and Research Station of the TANAP.

From Muránska Planina NP and Pol'ana PLA, 104 and 73 faecal samples from grey wolves were gathered. Samples were mainly collected in two annual transect surveys during two different periods of the year.

All collected scats were frozen at –20°C and delivered to the Institute of Parasitology, Slovak Academy of Sciences in Košice for faecal examination. The faeces were examined using a modified flotation method with zinc sulphate solution of specific gravity 1.2. Propagative stages of the parasites (eggs, oocysts or larvae) were identified based on morphological features. In the event that the parasite could not be distinguished to a species, the superior taxon (genus, family or order) was indicated [20].

In two samples, larvae with the characteristic morphological features of *Angiostrongylus vasorum* were found by the modified flotation method described above. The copro-PCR assay was performed to determine the species of the nematode larvae. DNA isolation was performed using a QIAamp DNA Stool Mini Kit (Qiagen®, Germany) according to the instructions given by the manufacturer but with the following modification: 200 mg of faeces were added to a 2.0 ml tube with one 5 mm stainless bead and 1.5 ml of lysis buffer. The samples were homogenized in Qiagen TissueLyser (Qiagen®, Germany) for 20 s at 30 Hz.

PCR targeting a fragment of internal transcribed spacer 2 (ITS2) was performed using a set of

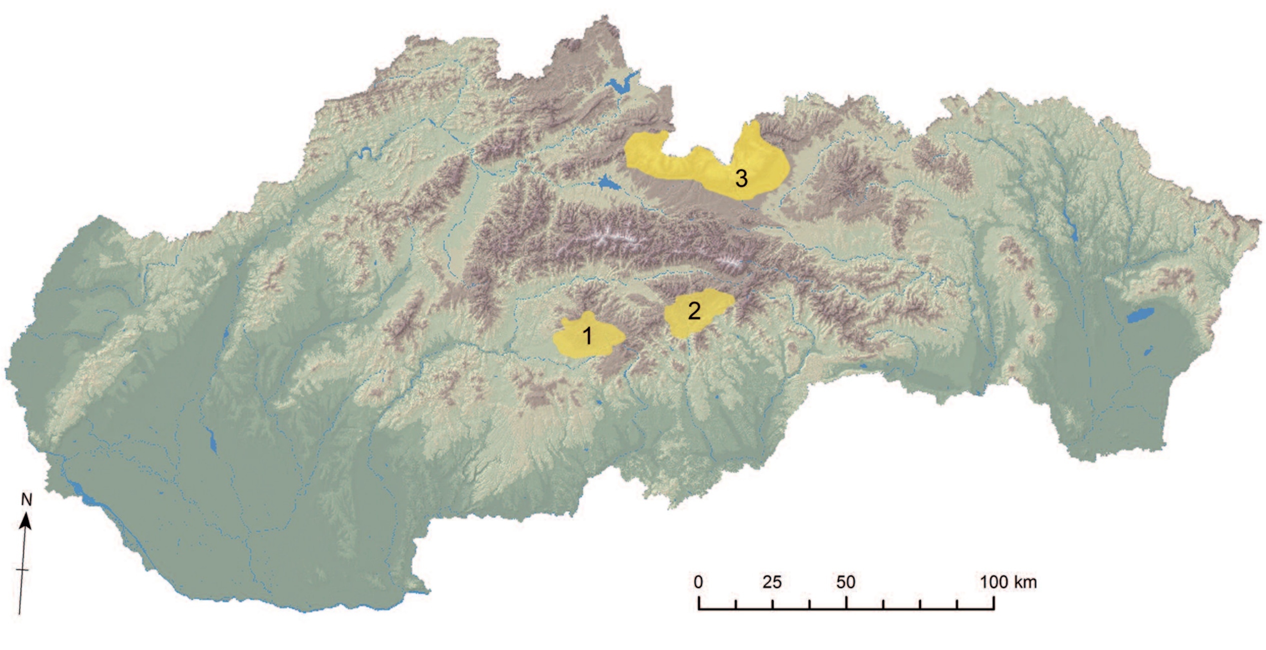

Fig. 1. Map of studied localities. 1: Pol'ana Protected Landscape Area; 2: Muránska Planina National Park; 3: the Tatra National Park.

specific primers (AV4/AV5) for *A. vasorum* [21]. Sterile water was used as a negative control and *A. vasorum* DNA from a naturally infected dog was used as a positive control. PCR reactions consisted of polymerase activation step at 94°C for two minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by final extension at 72°C for seven minutes. The PCR products were visualized using 1.5% agarose gel electrophoresis.

Results

Propagative stages of parasites were found in 169 out of 256 (66%) wolf faecal samples from three protected areas. Ten parasitic taxa were identified, including the protozoan *Isospora* spp., one trematode (*A. alata*), one cestode (*Taenia* spp.) and seven nematode taxa (Table 1).

Oocysts of the genus *Isospora* were detected in 3.5% of the samples examined: two samples from Tatra NP and seven samples from the Muránska Planina NP. No *Isospora*-infected specimens were found in PLA Pol'ana. *A. alata*, the only trematode found in the present study, was diagnosed in four faeces samples: three of which were collected in PLA Pol'ana. Taeniid eggs were diagnosed in only one wolf scat from PLA Pol'ana.

Several nematode groups were observed. The most common group of nematodes was the Trichuridae, which were found in 113 wolf samples. These species were more prevalent in the PLA Pol'ana area (60.3%) than Tatra NP (17.7%). The second most numerous parasitic group found in faecal samples was the Ancylostomatidae, with the propagative stages detected in samples from all studied areas with prevalence ranging from 6.8 to 13.9%. *T. canis* was present in eight wolf faeces, while the related species *T. leonina* was diagnosed in two. The eggs and larvae of *Strongyloides stercoralis* were detected in two wolf scats from the Tatra NP and Muránska Planina NP. *S. lupi* was also found in two specimens from the two locations. Metastrongylid larvae, identified as *Angiostrongylus vasorum* morphology and PCR analysis, were also found in two faecal samples from Tatra NP and Pol'ana PLA. ITS2 fragments of the tested amplicons were the same in size as positive control (218 bp). Acarida (*Demodex* spp. and *Sarcoptes* spp.) were also present in several samples.

Pol'ana PLA and Muránska Planina NP were found to harbour higher densities of parasites (79.5% and 74.0% positivity, respectively) than the Tatra NP (41.8% positivity).

Table 1. Endoparasites detected in faeces of wolves from three protected areas in Slovakia

Parasites taxa	No. of positive samples/Prevalence (%)			
	Tatra NP (n=79)	Muránska Planina NP (n=104)	Pol'ana PLA (n=73)	Total (n=256)
Protozoa				
Isospora spp.	2/2.5	7/6.7	0/0	9/3.5
Trematoda				
Alaria alata	0/0	1/1.0	3/4.1	4/1.6
Cestoda				
Taenia spp.	0/0	0/0	1/1.4	1/0.4
Nematoda				
Strongyloides stercoralis	1/1.3	1/1.0	0/0	2/0.8
Ancylostomatidae	11/13.9	10/9.6	5/6.8	26/10.2
Trichuridae	14/17.7	55/52.9	44/60.3	113/44.1
Toxocara canis	3/3.8	1/1.0	4/5.5	8/3.1
Toxascaris leonina	0/0	2/1.9	0/0	2/0.8
Spirocerca lupi	1/1.3	0/0	1/1.4	2/0.8
Angiostrongylus vasorum	1/1.3	0/0	1	2/0.8
Total	33/41.8	77/74.0	58/79.5	169/66.0

n – number of examined samples, Tatra NP – Tatra National Park, Pol'ana PLA – Pol'ana Protected Landscape Area, Muránska Planina NP – Muránska Planina National Park

Discussion

Our findings reveal the presence of eight parasite taxa of 15 known to infect wolves and two new parasitic agents in wolves from the three studied areas in Slovakia. Several differences with previous studies were observed with regard to parasite fauna composition. Previous studies have found the most prevalent to be *T. hydatigena* (41.6–34%) tapeworms [11–13], while in our study cestode infection was detected in only 0.4% of the wolf population. In addition, while the nematodes of the Trichuridae were found to be the most frequent agents of infection in the present study (44.1%), Mituch found *C. plica,* a member of the Trichuridae, in only 4.1% of investigated samples. Interestingly, the hookworms of the Ancylostomatidae were recorded in the same prevalence during our study as well as was published in 1963 by Mituch (8.3%) [11]. Trichuridae eggs are generally considered to be extremely resistant [22]; however, the very cold climate in the Tatra Mountains could have reduced occurrence of these nematodes in the area (17.7%). Similar results were obtained in a study from Poland, where Trichuridae (34.5%) and Ancylostomatidae (13.8%) species were most prevalent [23].

Following environmental changes, new invasive hosts and parasites have been identified in the last decades [24]. In our study, *A. vasorum*, an etiological agent of emerging canine disease, was detected in two grey wolves from Slovakia. This important canine infection was detected for very first time in Slovakia in 2012. Extensive monitoring in domestic and wild carnivores identified the parasite in 5.11% of examined red foxes and 6.22% of domestic dogs [25–27]. As they roam over extensive territories (150–300 km^2), wolves may transmit pathogens over long distances from their foci, distributing the infection to unaffected areas [23,28].

These findings demonstrate the great ability of *A. vasorum* to spread quickly throughout the environment, and show that wolves may serve as an additional parasite reservoir among wildlife. As *A. vasorum* infection causes significant pathology in dogs [29] and foxes [30], we can assume that it has also an detrimental effect on the health of wolves.

Parasite diversity strongly depends on prey composition and wolf biome [8]. A study in Latvia found the trematode *A. alata* to be widely prevalent (85.3%) in the wolf population; an analysis of the diet found the main prey to be cervids and wild boar. A study of the parasite fauna of wild boars in Latvia identified the presence of *A. alata* in their muscle tissue, confirming that a relationship exists between prey infection and predator parasite diversity [9]. In Slovakia, *A. alata* was found only in four wolf samples from Pol’ana PLA and Muránska Planina NP. While no data is available on the occurrence of *A. alata* in intermediate hosts, particularly wild boars, in Slovakia, the prevalence in wild boars in neighbouring Austria and the Czech Republic was found to be 6.6 and 6.8%, respectively [31,32].

Generally, little is known about protozoan infections in wolves. Our findings are the first confirmation of the presence of *Isospora* spp. oocysts in faeces of wolves from Muránska Planina NP (6.7%) and Tatra NP (2.5%); however, no such infection was found in wolves from Pol’ana PLA. No previous data on protozoan infections is available in wolves in Slovakia .

In the present study the prevalence of parasites was much higher in the Pol’ana PLA (79.5%) and Muránska Planina NP (74%) protected areas than in the large Tatra NP national park (23.9%). Both protected areas are situated in central Slovakia and create a suitable habitat for wolves, being composed predominantly of deciduous forests with an abundance of roe deer prey [17]. As the Tatra NP is found in a colder, mountainous area, this difference may be due to the influence of climate conditions on the survival of the propagative stages of the parasites.

In conclusion, our research revealed the presence of ten parasite taxa in grey wolves from Slovakia. Two of them, *Isospora* spp. and *A. vasorum*, are reported for the first time in this host and country. Taking into account the zoonotic potential of some parasites, and the interference of wildlife and human leisure activities in protected areas, the risk of parasite transmission is of great importance. Further research is needed to piece together the missing knowledge about the role of the wolf in the epidemiology of zoonotic agents.

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