Respiratory and cardiopulmonary nematode species of foxes and jackals in Serbia

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Summary

As part of routine monitoring of foxes (*Vulpes vulpes*) and jackals (*Canis aureus*) on the territory of Vojvodina province (northern Serbia), an analysis of respiratory and cardiopulmonary parasitic nematodes was conducted. Both host species harbored *Eucoleus aerophilus*, *E. boehmi* and *Crenosoma vulpis*, whereas *Angiostrongylus vasorum* was found only in foxes. A high prevalence of infection (72.6 %) was noted for *E. aerophilus* in foxes. The remaining parasite species occurred less frequently in both host species. In all species where it could be quantified, a high degree of parasite aggregation within host individuals was noted. Single species infections were most common, whereas two and three species infections occurred less frequently in both host species. The distribution of abundance of *E. aerophilus* was affected by host sex, with abundances higher in male foxes. Sampling site and year influenced abundance variation in *E. boehmi*.

Keywords: respiratory nematodes; cardiopulmonary nematodes; red fox; jackal; Serbia

Introduction

Red foxes (*Vulpes vulpes*) are principal reservoirs of numerous parasites, owing to their wide ranging geographic distribution (Duscher *et al*., 2014), frequent urbanization (Contesse *et al*., 2004; Morgan *et al*., 2008; Millán *et al*., 2014) and population increase following oral vaccination programs throughout Europe (Goszczinski *et al*., 2008; Borecka *et al*., 2009). Among these parasites are respiratory nematodes such as *Eucoleus aerophilus* (Creplin, 1839), *E. boehmi* (Supperer, 1953) and *Crenosoma vulpis* (Dujardin, 1844) and the cardiopulmonary nematode *Angiostrongylus vasorum* (Baillet, 1866) (Traversa *et al*., 2010; Otranto *et al*., 2015; Latrofa *et al*., 2015). Another canine species that plays a significant part in dissemination of pathogens is the golden jackal (*Canis aureus*), whose populations are also on the rise, particularly on the Balkan Peninsula (Šálek *et al*., 2015).

Eucoleus aerophilus (Enoplida, Capillaridae) lives submerged in the epithelium of the trachea, principal bronchi and the bronchi of the caudal lobes (Traversa *et al*., 2009), rarely localizing in the bronchiolar epithelium of infected animals (Nevárez *et al*., 2005). Its life cycle is described as either direct or indirect with earthworms as paratenic hosts (Di Cesare *et al*., 2012, 2014; Veronesi *et al*., 2013). While its primary hosts are various carnivorous mammals, it has also been known to infect humans. Twelve cases of human *E. aerophilus* infection have been reported worldwide, including one in Serbia, where a woman admitted with clinical signs pointing to bronchial carcinoma was found to be infected with the parasite (Lalošević *et al*., 2008).

The congeneric species *E. boehmi* has been increasingly reported in many European regions since the turn of the century (Sréter *et al*., 2003; Lalošević *et al*., 2013; Veronesi *et al*., 2014). This parasite inhabits the nasal cavity and frontal sinuses of the host,

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and its life cycle is poorly known. The hosts exhibit clinical manifestations such as sneezing and increased nasal secretion, or subclinical signs of respiratory obstruction. More recently, the parasite has been considered a main cause of intracranial disease, such as meningococcal encephalitis in dogs (Clark *et al*., 2013). However, in spite of its pathogenicity and occurrence in several European countries (Veronesi *et al*., 2014; Otranto *et al*., 2015; Hodžić *et al*., 2016a), *E. boehmi* remains underestimated and is often overlooked. Present knowledge of its range in domestic and wild animals in Europe is scarce, mostly available from studies that focus on other cardiopulmonary parasites (Conboy, 2009; Hodžić *et al*., 2016a).

Adults of *Crenosoma vulpis* (Strongylida, Crenosomatidae) can be found in small bronchi and bronchioles of all pulmonary lobes in wild and domestic canids (Bihr & Conboy, 1999). Its intermediate hosts are mollusks, and the distribution of *C. vulpis* larvae is directly influenced by climatic factors such as temperature and humidity, as well as occurrence of suitable hosts. The nematode, much like *E. aerophilus*, causes damage to the pulmonary parenchyma and chronic respiratory bronchitis. Where infections are particularly intense, bronchopneumonia may cause death of the host (Holmes & Kelly, 1973; Bowman *et al*., 2002; Taylor *et al*., 2007).

Angiostrongylus vasorum (Strongylida, Angiostrongylidae) is a highly pathogenic metastrongylid nematode that parasitizes on different carnivore species. L1 stages are localized in the lungs, with adults found in the pulmonary artery and right side of the heart. Gastropods act as intermediate hosts and L3 carriers (Woolsey *et al*., 2017). Frogs and birds may also figure into its life cycle as intermediate or paratenic hosts (Bolt *et al*., 1993; Mozzer & Lima, 2015). Dogs infected with this nematode display subclinical, sometimes even fatal cardiopulmonary and neurological symptoms (Barutzki & Schaper, 2009; Moeremans *et al*., 2011). Its endemic hotspots are in Western Europe (France, southern Great Britain, Ireland). In Central and Eastern Europe, *A. vasorum* was reported from dogs and foxes in Croatia (Rajković-Janje *et al*., 2002), Greece (Papazahariadou *et al*., 2007), Hungary (Majoros *et al*., 2010), Poland (Schnyder *et al*., 2013), Romania (Deak *et al*., 2017) and Slovakia (Hurníková *et al*., 2013), with a tendency of spreading further southeast. In Vojvodina, the northern province of Serbia, there has been only one documented case of canine *A. vasorum* infection (Simin *et al*., 2014).

Previous studies of respiratory and cardiopulmonary nematodes have shown that these parasites have high pathogenic potential. Thus, it is necessary to develop efficient measures of parasite control and diagnosis, and to monitor their transmission pathways in domestic and wild animals. The aim of this study was to bring attention to the presence of these parasites in wild carnivores – foxes and jackals – of the Vojvodina region in Serbia, and to emphasize the epidemiological and epizootiological significance of carnivores as hosts of respiratory nematodes that cause diseases harmful not only to wild and domestic animals, but also to man.

Materials and Methods

Sample collection and processing

Adult foxes and jackals were collected from 2009 to 2016 as part of routine rabies diagnostic procedure in the Pasteur Institute of Novi Sad, National Reference Laboratory for rabies. The animals were collected from various regions of Vojvodina, obtained through cooperation with local hunting clubs. Both foxes and jackals were sampled in roughly the same time period, the majority of animals captured from autumn to early spring (October to March). Serbian law lists the red fox and the jackal as protected species. However, their status and protection regime are regulated by hunting legislation, and hunting season lasts throughout the year for both species. The animals were sexed, and their body mass (kg) and total body length with tail (cm) were measured. Tracheas and lungs of all red foxes and jackals were carefully cut open with scissors and examined visually for the presence of respiratory parasites.

Samples of the trachea and lungs were collected from each animal, in order to test the bronchial and bronchiolar contents for the presence of *E. aerophilus* adults and eggs and *C. vulpis* adults and larvae. Tracheal samples were taken from the larynx to the tracheal bifurcation, together with the lungs. The tracheas were cut open along the frontal side with scissors, and then examined under a stereomicroscope together with the bronchi and bronchioles of the lungs. Recovered *E. aerophilus* adults and bronchial contents were immediately prepared in 50 % glycerol. Slides of the capillarid nematodes were then examined under an optic microscope, magnified 40 to 100 times. Adult stages of nematodes recovered from trachea and bronchi of the foxes were identified at the species level using morphological keys.

Samples of the nasal mucosa obtained by scarification of the nasal cavity from wild animals were tested for the presence of *E. boehmi*. After extraction, the tissue was placed in 0.9 % saline and immediately examined under stereomicroscope. *Eucoleus boehmi* adults were prepared in 50 % glycerol and examined under an optic microscope in the same way as for the previous species.

Data analysis

Examinations for the presence of *E. aerophilus* in the respiratory tract were carried out starting from 2009 and 2014 for foxes and jackals respectively. Infection data for this nematode were obtained from 351 foxes and 49 jackals, and these were the host individuals that formed the sample for quantitative analyses of infection. Examination for the remaining two species, *E. boehmi* and *C. vulpis* was conducted starting from 2013 for foxes, and 2014 for jackals. Data on quantitative parameters of infection for *E. boehmi* are based on 184 foxes and 30 jackals. The sample for *C. vulpis* consisted of 205 foxes and 49 jackals. *Angiostrongylus vasorum* was found sparingly; since nematode specimens were not precisely quantified and the heart was not examined, no further quantitative analysis was performed for this parasite species, nor was it considered in co-infection analysis.

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Quantitative parameters of infection are stated according to Bush *et al.* (1997). Data on prevalence, mean and median infection intensity, mean abundance and dispersion index are given for *E. aerophilus* and *E. boehmi*. The dispersion index was used as a measure of parasite aggregation within hosts (Shaw & Dobson, 1995). For *C. vulpis*, large numbers of larvae made precise counts of individuals difficult to obtain, and thus only prevalence is given for this nematode species. Prevalence values of *E. aerophilus*, *E. boehmi* and *C. vulpis* were compared between foxes and jackals via the exact unconditional test (Reiczigel *et al.*, 2008). Differences in mean intensities and mean abundances of *E. aerophilus* and *E. boehmi* between the two host species were tested with a bootstrap test with 20000 replications. All quantitative analyses and statistical tests were performed in Quantitative Parasitology 3.0 software (Rósza *et al.*, 2000). To calculate the percentage of animals infected with one, two or three respiratory parasite species, a subsample consisting of hosts with data for all three recurring nematodes (*E. aerophilus*, *E. boehmi* and *C. vulpis*) was created. This subsample consisted of 179 foxes and 30 jackals. Based on this data, percentages of foxes and jackals carrying specific single or combined infections were determined.

The fox sample size and high prevalence values for the two *Eucoleus* species enabled an analysis of factors influencing parasite abundance. The selected factors were year of sampling, site of sampling (the township from which the host individual originated), total length (body and tail), body mass and sex. The sample was formed exclusively from animals for which all afore mentioned data were available. This resulted in 210 foxes for *E. aerophilus*, and 150 for *E. boehmi*.

In order to determine which of the listed factors exerted influence on nematode abundance, the abundance data was fitted to a Generalized Linear Model (GLM). The initial model contained all of the factors and their two-way interactions as terms predicting the numerical response. The factors and interactions that failed to pass the significance threshold were eliminated stepwise until the most satisfactory minimal model was obtained. GLM analysis was performed in R statistical software (R core team, 2013), utilizing its standard packages.

Ethical Approval and/or Informed Consent

The animals used in this study were captured and obtained as part of routine rabies diagnosis procedure, according to a program set up by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (Official Gazette of the Republic of Serbia Nos. 43/17 and 11/18). The research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

The prevalence of *E. aerophilus* was 72.6 % in foxes, nearly double the value it had in the jackal sample. In addition, infected foxes typically carried more nematode individuals than jackals, and mean abundance of the parasite was also higher in foxes. Both values of the dispersion index d were greater than 1, signifying an aggregated distribution: the majority of the nematodes were found in a small number of hosts. The differences in prevalence, mean intensity and mean abundance of *E. aerophilus* between foxes and jackals were all statistically significant at $p < 0.0001$ (Table 1).

Prevalence of *E. boehmi* was, as in the previous species, higher in foxes than in jackals, but this difference was far more subtle than for *E. aerophilus*. Infected jackals carried more parasites on average than infected foxes, but mean abundance was higher in the fox sample. Dispersion index values point to parasite aggregation as in *E. aerophilus*, particularly evident in the jackal sample: all 6 nematodes were found in 3 host individuals, with the remainder of the jackals uninfected. The mean intensity of *E. boehmi* infection in jackals was significantly higher than in foxes ($p = 0.022$). The differences in prevalence ($p = 0.357$) and mean abundance ($p = 0.595$) of the parasite between the two host species were not significant (Table 2).

Just over 6 percent (6.1 %) of the 49 examined jackals carried *C. vulpis*. While no adult worms were found, large numbers of larvae served as evidence of presence. A higher prevalence value of 16.6 % was found in foxes, but this difference was not statistically significant ($p = 0.088$). Since precise counts of parasite individuals are unavailable, other quantitative measures were not calculated. However, while no dispersion index is given, it should be noted that all 44 adult worms were found in 14 of the 205 analyzed foxes. Both foxes and jackals displayed the same pattern of parasite community richness. Infections with a single nematode species were the most common in both hosts, with *E. aerophilus* infecting the largest number of individuals, followed by *E. boehmi* and *C. vulpis* in that order. Infections with two nematode species were

Table 1. Quantitative parameters of infection with the nematode *E. aerophilus* in foxes and jackals in Vojvodina, Serbia.

Host	Number of individuals	P%	MI	MedI	MA	d
V. vulpes	351	72.6 (67.7 – 77.1)*	8.7 (7.4 – 10.2)*	4	6.3 (5.3 – 7.4)*	17.3
C. aureus	49	32.7 (20.6 – 46.9)	1.8 (1.3 – 2.5)	1	0.6 (0.3 – 0.9)	2

P% – prevalence; MI – mean intensity; MedI – median intensity; MA – mean abundance; d – dispersion index; 95 % confidence intervals given in parentheses where applicable. Asterisks denote statistically significant differences.

Table 2. Quantitative parameters of infection with the nematode *E. boehmi* in foxes and jackals in Vojvodina, Serbia.

Host	Number of individuals	P%	MI	MedI	MA	d
V. vulpes	184	17.9 (13 – 24.1)	1.48 (1.2 – 1.9)	1	0.3 (0.1 – 0.4)	1.8
C. aureus	30	10 (2.8 – 26.3)	2*	2	0.2 (0 – 0.4)	1.9

P% – prevalence; MI – mean intensity; MedI – median intensity; MA – mean abundance; d – dispersion index; 95% confidence intervals given in parentheses where applicable. Asterisks denote statistically significant differences.

less frequent, and neither foxes nor jackals were infected with the combination *E. boehmi-C. vulpis*. Infections with all three species were rare in foxes, and altogether absent in jackals (Table 3).

The only factor that significantly influenced the variation in abundance of *E. aerophilus* in red foxes was host sex (p=0.0005). Mean abundance of the parasite was greater in males (6.22) than in females (2.47) of this host species. Sampling site (p<0.0001) and year (p=0.0002) influenced abundance of *E. boehmi* in the same host, as well as the interactions year:site (p=0.0454), year:body mass (p=0.0012) and site:total length (p=0.0033). Body mass (p=0.4301) and total body length (p=0.9716) did not influence the abundance of *C. boehmi* in foxes as independent factors. Furthermore, due to the disproportional representation of different sample categories, the fact that sampling site and year play a part in determining the abundance variation in *E. boehmi* carries little informative value. The years with a significantly higher abundance of the parasite (2013 and 2014) are also the years with the smallest number of host animals analyzed, increasing the values of the parameter. The township of Zrenjanin, which had a significantly higher *E. boehmi* abundance compared to others, contributed only 3 foxes to the total sample, skewing the result.

Aside from the three respiratory nematodes listed above, the cardiopulmonary parasite *Angiostrongylus vasorum* was also found in foxes only. Three host individuals were infected with three worms in total, resulting in a prevalence value of 1.8 %.

Discussion

Eucoleus aerophilus prevalence in Vojvodina was 72.6 % and 32.7 % in foxes and jackals respectively. It should be noted that previous studies report disparities in prevalence values obtained via necropsy and those estimated by coprological examination. The former method is often described as the golden standard for confirming respiratory nematode infection, with a much higher sensitivity than analyses of fecal matter (Magi *et al.*, 2009, 2015). The prevalence of *E. aerophilus* reported herein is relatively high when compared with infection percentages from other European countries. For example, Magi *et al.* (2015), in addition to their own results, summarize data from 12 other studies from different parts of Europe, only 4 of which report prevalence in foxes higher than 72.6 %. Lalošević *et al.* (2012), in an earlier paper concerned with the Vojvodina province of Serbia, state a prevalence of 84 %, a value far closer to the one found in the present study. On the other hand, Ilić *et al.* (2016) find a prevalence of 23.7 % in foxes, and an absence of infection in jackals. Their sample, however, consisted of animals originating south of the Sava and Danube rivers, outside of the Vojvodina region which, as data suggests, provides an ideal environment for the transmission and sustained presence of this nematode species. According to Lalošević *et al.* (2012), a higher rate of parasite transmission in Vojvodina is caused by the humid conditions of the southern part of the Pannonian basin, a consequence of an abundance of canals and tributaries between the major rivers. Tolnai *et al.* (2015) reach a similar conclusion in their analysis of climatic factors shaping the transmission of respiratory and cardiopulmonary nematodes in Hungary, which is also part of the Pannonian basin. They find that increasing humidity and decreasing average annual temperature create conditions suitable for the spreading of *E. aerophilus*, primarily due to the important role of water in the survival of the eggs which are susceptible to desiccation. The same authors report that the spatial distribution of this nematode was less localized than that of the other two species in the study (*C. vulpis* and *A. vasorum*), indicating that the proposed direct life cycle of *E. aerophilus* makes it less sensitive to the effects of climatic factors.

The sex of host foxes influenced the abundance of *E. aerophilus*, with males being more infected. Morgan *et al.* (2008) also note a larger presence of this nematode in males, but other studies did not find a connection between host sex and quantitative infection

Table 3. Percentage of host individuals infected with one (*E. aerophilus*, *E. boehmi* or *C. vulpis*), two (one of three specific combinations) or three respiratory nematode species.

Host species	Single species infections			Two species infections			Three species infections
	Ea	*Eb*	*Cv*	*Ea+Eb*	*Ea+Cv*	*Eb+Cv*	*Ea+Eb+Cv*
V. vulpes	58.2 %	3.7 %	1.5 %	14.9 %	17.9 %	0 %	3.7 %
C. aureus	66.7 %	16.7 %	0 %	8.3 %	8.3 %	0 %	0 %

Ea – Eucoleus aerophilus; *Eb – Eucoleus boehmi*; *Cv – Crenosoma vulpis*

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parameters (Saeed *et al*., 2006; Magi *et al*., 2015). The effect of host sex on parasitic burden remains a highly complex aspect of parasitological studies, with various authors reaching divergent conclusions. Nevertheless, in cases where males have more pronounced infections, an explanation based on the differential effect of natural selection on the sexes is most commonly proposed: the immunosuppressive properties of male sexual hormones and certain types of sex-specific behavior make males more likely to be infected (Zuk & McKean, 1996). Age, season and population density of the host (Saeed *et al*., 2006; Lalošević *et al*., 2012) are other factors reported as having significant roles in determining infection characteristics of *E. aerophilus*, but they were not taken into account in the present study. Additionally, results may vary depending on the author: Morgan *et al*. (2008), for example, did not find a seasonal variation in infection probability. Teasing apart the factors that determine the distribution and infective capacity of this respiratory nematode is an important task, as it allows us to make educated guesses on the patterns of its spreading and enables preventive measures. The parasite causes visible pathological changes in its hosts (Nevárez *et al*., 2005). Considered together with its complicated diagnosis, zoonotic potential and expansion into non-endemic areas, this makes it a sizeable threat to pets – domestic dogs and cats (Traversa *et al*., 2010). In Denmark, Saeed *et al*. (2006) report significantly higher prevalence of capillarid nematodes in urban foxes, compared to rural specimens. It is these urban foxes that act as a source of disease for domestic animals and people (Magi *et al*., 2009). The twelve cases of human infection found in literature, including one from Serbia (Lalošević *et al*., 2012), further emphasize the importance of raising awareness of the parasite's presence and developing precise diagnostic techniques that help differentiate between clinical signs that can easily be attributed to other respiratory conditions.

Single infections with *E. boehmi* and *C. vulpis* were far less frequent than those with *E. aerophilus*. In addition, whenever two species were found in fox and jackal hosts, the combination included *E. aerophilus*: the remaining two species were never found together, unless *E. aerophilus* was also present. This may point to a synergistic interaction, where *E. aerophilus*, by weakening or modulating the hosts' immune response, facilitates the establishment and survival of other respiratory parasites. Evidence for such synergistic interactions exists in other host-parasite systems, for example for the intestinal nematode *Heligmosomoides polygyrus* in rodents (Behnke *et al*., 2001, 2009; Maizels *et al*., 2004). Great caution needs to be taken when making assumptions such as these. Interactions and associations between parasite species remain poorly understood: experimental studies in strictly controlled conditions or analyses based on predetermined null models are necessary to determine whether any changes in abundance occur when two species coexist within the same host (Poulin, 2001), and these have yet to be carried out for respiratory nematodes. The present study focused on respiratory parasites only. It's certainly possible that the examined hosts carried other parasites (intestinal

for example), and that they are in fact the ones driving the interactions. Parasites may even behave as independent units within their hosts, their distribution determined by natural selection narrowing them down to specific predilection spots that carry the best conditions for survival. If this is true, each species would occupy its own isolated niche and interspecific interactions would be nonexistent (Poulin, 2001). In this light, the findings of the present co-infection analysis could be interpreted quite differently. For example, it could simply be that *E. aerophilus* has greater longevity than the other two species, outliving them in the host and thus more often appearing in single and multiple infections.

Eucoleus boehmi was found in the nasal mucosa of 17.9 % of examined foxes and 10 % of examined jackals, with an aggregated distribution within host individuals. Studies of extraintestinal nematode parasites of foxes in Hungary (Sretér *et al*., 2003) and Norway (Davidson *et al*., 2006) also report this species, with prevalence values of 8 % and 51 % respectively. Recently, *E. boehmi* has been receiving more attention and research has intensified. Prevalence in foxes ranges from 30.7 % in Italy (Veronesi *et al*., 2014) to 71 % in Denmark (Al-Sabi *et al*., 2013), and even 83 % in Austria (Hodžić *et al*., 2016b). On the other hand, data on jackals are far more lacking. According to a thorough review of jackal parasites by Gherman & Mihalca (2017), *E. boehmi* has, to this date, only been found in jackals in Russia, with a prevalence of 30 %. Population expansion of foxes and jackals, together with relatively high prevalence of the parasite established by contemporary research, position these canids as natural reservoirs and disseminators of the nematode. This, consequently, mandates detailed studies of nasal eucoliosis in wild animals and better diagnostic measures for domestic animals.

Foxes in Vojvodina had a relatively low prevalence of *C. vulpis* (16.6 %). In jackals, based on larvae only, the percentage of infected hosts was 6.1 %. The life cycle of this parasite is indirect, with snails as intermediate hosts. Parasite prevalence varies in different European countries: 24 % in Hungary (Sréter *et al*., 2003), 24.9 % in Austria (Lassnig *et al*., 1998), 28.2 % in the United Kingdom (Willingham *et al*., 1996), 13-18 % in Spain (Miquel *et al*., 1994), 13 % in Vojvodina province of Serbia (Simin *et al*., 2012) and 4.5 % in the Netherlands (Borgsteede, 1984). Recent data show that this nematode is the leading cause of respiratory disease in domestic dogs in Spain, Portugal, Switzerland and Germany (Unterer *et al*., 2002; Madeira de Carvalho *et al*., 2009; Barutzki & Schaper, 2009). Such conclusions highlight the importance of monitoring the occurrence of *C. vulpis* in wild animals. Presence of the parasite in a specific area should be taken into account when dogs with symptoms of inflammatory diseases are encountered.

In the current study, the cardiopulmonary nematode *A. vasorum* was not found in jackals, in accordance to Takács *et al*. (2014). However, Gavrilović *et al*. (2017) and Simin *et al*. (2014) report on pneumonia caused by this parasite in jackals and foxes. We only found the nematode in three individual foxes (1.8 %), each one carrying a single worm. Such low prevalence values are report-

ed from neighbouring countries including Croatia, Hungary and Romania. These areas appear to be the easternmost limits of its distribution, with hotspots located in Western and Central Europe (Deak *et al.*, 2017). A rise in infestation with this nematode species was noted in dogs in Italy (Traversa *et al.*, 2008), Germany and Denmark (Taubert *et al.*, 2009). Eleni *et al.* (2014) report the first occurrence of the so-called French heartworm in wolves in Italy, and Barutzki and Schaper (2009) document its rising infection trend in Germany and report on its distribution in the country. The spread of angiostrongyloidosis may occur via the transport of dogs from one country to another; indeed, it appears that the nematode was brought into Denmark via dogs originating in France. Since the disease is often fatal to domestic dogs, and its distribution is still under-documented, all researchers agree that *A. vasorum* requires greater attention as a threat to domestic and wild animal health. This is further supported by the results put forward by Gillis-Germitsch *et al.* (2017), stating that dogs seem to be developing tolerance to the parasite. This would enable prolonged survival for the heartworm and facilitate its spread, allowing it to reach new areas before being detected in its carriers. This could possibly explain new hotspots and epizootics in certain parts of Europe.

In conclusion, the present study found four species of respiratory and cardiopulmonary nematodes in foxes and jackals, two wild canids currently expanding their ranges in Europe due to their adaptability and resilience, coupled with the benefits of oral rabies vaccination. Domestic animals such as dogs and cats, as well as humans under certain circumstances, may become hosts of these parasites, the clinical consequences of these infections being far from negligible. An increase in international transport of animals and goods, with insufficient monitoring of higher levels of trade, is expected to result in a further expansion of *E. aerophilus* (Traversa *et al.*, 2010), and with it other respiratory and cardiopulmonary nematodes, rendering studies such as this one indispensable in the near future. All of the above explicitly points to the significance of such research and the necessity for its intensification.

Conflict of Interest

Authors state no conflict of interest.

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