



Angiostrongylus vasorum in golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) from Northern Bosnia and Herzegovina

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Abstract

In this study, we conducted post-mortem examinations of golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) from the northern region of the Republic of Srpska (Bosnia and Herzegovina) to detect the presence of *Angiostrongylus vasorum*. An epidemiological survey was conducted in the densest golden jackal population in the country. Over two time intervals, we examined a total of 30 jackals and 16 foxes. The presence of *A. vasorum* was confirmed in two jackals (6.6%) and one fox (6.25%). These findings strongly suggest that the spread of *A. vasorum* can be facilitated by the active expansion and migration of golden jackals in Southeastern Europe. The morphological and molecular detection of *A. vasorum* in jackals and foxes confirmed its presence and active circulation in wildlife, while the phylogenetic analysis of the ITS-2 gene indicated a low sequence distance from European isolates.

Keywords Angiostrongylosis · Epidemiological survey · Morphological examination · PCR

Introduction

Angiostrongylus vasorum (Baillet, 1866), or “French heart-worm,” is a metastrongyloid parasite of the right cardiac side and pulmonary arteries. It has a complex (indirect) life cycle and wide range of animal hosts including both domestic and wild carnivores (Morgan et al. 2021). Epidemiological studies have indicated that this species is widespread in Europe, as well as in certain geographical areas of North America, South America, and Africa (Ferdushy and Hasan 2010). The use of new molecular and serological diagnostic methods

has significantly enhanced the detection of *A. vasorum* infections, contributing to the understanding that this species is widespread across most of the European continent. In areas of high prevalence, typical endemic foci or “hotspot” areas with high number of clinical cases in dogs have been reported, for example in Italy (Traversa et al. 2013).

In general, prevalence of canine angiostrongylosis, caused by *A. vasorum*, in the dog population ranges between 0.5 and 3% in Europe (Morgan et al. 2021). However, a remarkable increase in prevalence towards 81.8% has been noted in the fox population in Switzerland (Gillis-Germitsch et al. 2020). For this reason, it is believed that foxes are the main natural reservoir of this parasite, significantly contributing to the complex and rather dynamic epidemiological status of canine angiostrongylosis in most European countries. In contrast, golden jackals are a highly adaptive species with an increasing population in Southeastern Europe (Trbojević et al. 2018), but their role as hosts for *A. vasorum* was confirmed only in Hungary, Serbia, and Italy (Takács et al. 2014; Gavrilović et al. 2019; Orioles et al. 2024). Data on *A. vasorum* presence in Bosnia and Herzegovina are scarce. A clinical case of autochthonous canine angiostrongylosis was reported by Stevanović et al. (2020a). Studies by Hodžić et al. (2016) and Stevanović et al. (2020b) failed to record *A. vasorum* in foxes and wolves, respectively.

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The aim of this study is to analyze the epidemiological status of *A. vasorum* in red foxes and golden jackals in northern Republic of Srpska (Bosnia and Herzegovina). Since it has been proven that this region is densely populated with golden jackals (density of 0.33 groups/10 km²) (Trbojević et al. 2018), we hypothesized that there is a high possibility of detecting the presence of *A. vasorum* in this area.

Material and methods

The epidemiological survey covered the northern geographical regions of the Republic of Srpska (Bosnia and Herzegovina), predominantly lowlands along the Sava River valley and the border region with Croatia. Data on jackal population density and the number of jackal groups were obtained from Trbojević et al. (2018). Sampling of legally hunted animals was performed in two intervals.

The first group of hunted jackals ($n = 16$) was examined in the period from 2018 to 2020 in the area of the cities/municipalities of Banja Luka ($n = 4$; coordinates: 44.772182, 17.191000), Kozarska Dubica ($n = 1$; coordinates 45.17667, 16.80944), Derventa ($n = 4$; coordinates 44.97750, 17.90750), Gradiška ($n = 4$; coordinates 45.14583, 17.25453), and Prnjavor ($n = 2$; coordinates: 44.87021, 44.87021). The *post-mortem* examination of jackal carcasses and sampling from the first group were conducted under field conditions. The second group, consisting of hunted jackals ($n = 14$) and foxes ($n = 16$), was sampled in 2024 in Derventa (coordinates 44.97750, 17.90750) and Brod (coordinates 45.13747, 17.98722). A total of 10 jackals and 10 foxes were sampled in Derventa, while carcasses of 4 jackals and 6 foxes were sampled in Brod. All examined jackals and foxes from the second group were delivered to the Public Veterinary Institute of the Republic of Srpska “Dr. Vaso Butozan,” Banja Luka, Bosnia and Herzegovina. Hearts and pulmonary arteries were dissected, rinsed with water in glass cups. The supernatant was discarded, and tap water was added which visualizes better the sediment. The sediment was visually examined for adults of *A. vasorum*. Adult worms were counted, with only males being cleared with Amman’s lactophenol. Two female specimens ($n = 1$ from a jackal; $n = 1$ from a fox) were stored in 70% ethanol for further molecular study. Morphological identification of male worms at the species level was performed using available keys and descriptions by Guilhon and Cens (1973) and Costa et al. (2003). In our PCR study, we first extracted genomic DNA from adult worms using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). Next, we amplified the Internal Transcribed Spacer 2 (ITS-2) region using NC1/NC2 primers, as reported by Gasser et al. (1993). The resulting amplicons of approximately 500 bp were purified and subjected to Sanger sequencing

in both directions by a commercial company (Macrogen, Amsterdam, Netherlands).

To analyze the generated sequences, we employed MEGA7 software and the BLAST tool available at NCBI (<http://blast.ncbi.nlm.nih.gov>) (Kumar et al. 2016). For comparative purposes, 17 partial ITS-2 sequences of *A. vasorum* from GenBank was included. Additionally, we obtained a partial ITS-2 sequence from an *A. vasorum* isolate detected in a fox from Romania, generously provided by Georgiana Deak. Analogous sequences of *Angiostrongylus cantonensis* and *Angiostrongylus chabaudi* were used as outgroups. To construct the phylogenetic tree, the neighbor-joining method in MEGA7, applying the Tamura-Nei model and performing bootstrap analysis with 1000 reiterations, was used. The pairwise distances were calculated using MEGA7 software. The obtained sequence was deposited in GenBank under the accession number PQ014732.

Results and discussion

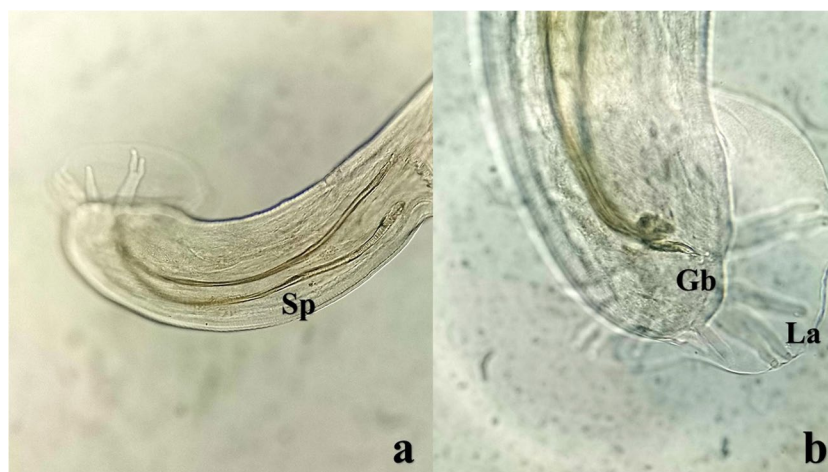
A. vasorum was not recorded in the hearts and pulmonary arteries in the first examined group of 16 golden jackals during 2018–2020. In the second group, consisting of 14 jackals from Derventa and Brod, the presence of adult forms of *A. vasorum* was detected in two adult male jackals (14.3%). Out of a total of 16 examined foxes from Derventa and Brod, adult forms of *A. vasorum* were found in one adult female fox (6.25%). A mixed infection of *A. vasorum* and *Dirofilaria immitis* was found in one jackal from Derventa.

Regarding the intensity of the infection, two male and three female *A. vasorum* were found in the first jackal. In the second positive jackal from Derventa, one male and one female worm were identified. In the female red fox from Brod, one male and two female worms were found. Macroscopically, no pathological changes in the pulmonary arteries, hearts and lungs of positive animals were observed.

Morphological examination of adult forms isolated from golden jackals and foxes identified *A. vasorum* according to the descriptions and specific keys by Guilhon and Cens (1973) and Costa et al. (2003). In males, the most typical morphological features are a well-developed copulatory bursa with whip-like, long, and transversely striated spicules. The bursal rays were short, and the lateral rays had a common trunk. A triangular and well-developed gubernaculum was evident (Fig. 1). From a lateral projection, the gubernaculum was clearly separated from the distal endings of the spicules. Females exhibited the typical “barber’s pole” appearance and were significantly larger than males.

PCR amplification of ITS-2 gene was only successful in one case of female worm in red fox from Brod. The obtained ITS-2 sequence of (469 bp) from Bosnia and Herzegovina was aligned with analogous sequences from various locations worldwide, revealing mean distances ranging

Fig. 1 **a** Copulatory bursa with spicules (Sp) in male *Angiostrongylus vasorum*, $\times 200$. **b** Gubernaculum (Gb) and lateral rays (La) in male *A. vasorum* (La), $\times 400$



from 0.7% compared with most European sequences to 1.2% and 1.6% in comparison to sequences from the USA (OQ702321.1 and OQ702322.1) and UK (GU045374.1), respectively. The phylogenetic tree based on the compared ITS-2 sequences reflects the grouping of *A. vasorum* isolates from Europe and Canada separately from isolates from the USA. Notably, overall genetic diversity among the analyzed sequences was relatively low (Fig. 2).

The morphological and molecular detection of *A. vasorum* in jackals and foxes from northern Bosnia and Herzegovina confirms the presence and active circulation of this cardiorespiratory nematode in wildlife. This supports earlier findings that jackals and foxes can serve as natural reservoirs for transmission to other hosts (Gavrilović et al. 2019; Gills-Germitsch et al. 2020). From an epidemiological standpoint, it is highly suggestive that the spread of *A. vasorum* to Bosnia and Herzegovina follows the immigration of the golden jackal population from Serbia and Croatia along an east–west continuity. This immigration, expansion to new areas, and the rapid population increase of golden jackals in northern Bosnia and Herzegovina have been documented by Trbojević et al. (2018). This hypothesis is further supported by the increased prevalence of *A. vasorum* in golden jackals in Serbia (Gavrilović et al. 2019), negative findings of *A. vasorum* in foxes and wolves from earlier surveys (Hodžić et al. 2016; Stevanović et al. 2020b), and the absence of *A. vasorum* in the first group of jackals examined between 2018 and 2020. A limitation of the study is that cut and opening of hearts and pulmonary arteries applied as sampling methodology has lower sensitivity in detection of *A. vasorum* than recommended methods of washing and squeezing and artificial digestion of lung parenchyma (Arcenillas-Hernández et al. 2023). Furthermore, *A. vasorum* was found in a geographical area where the largest jackal groups in Bosnia and Herzegovina were recorded (Trbojević et al. 2018). The obtained results do not follow the claims of other authors who stated that the distribution pattern of *A. vasorum* is from the west to

the southeast (Simin et al. 2014). The first regional study on the spread of angiostrongylosis in foxes in neighboring Croatia indicates that the prevalence was 1% (Rajković—Janje, 2002), while later studies in Serbia and Romania found that the prevalence increases over time. In Serbia, South Banat, the prevalence of angiostrongylosis was proven to be 54.17% in foxes (Gavrilović et al. 2018) and 24% in jackals (Gavrilović et al. 2019). In Romania, the prevalence of *A. vasorum* infection in foxes was found to be 4.2% (Deak et al. 2017).

We speculate that endemic hotspot areas can form under conditions of dense populations of definitive hosts, the presence of gastropods, and optimal environmental factors. The further expansion of the golden jackal population in Bosnia and Herzegovina, alongside a stable population of foxes, would change the dynamics of *A. vasorum* spread to new areas, indicating the need for greater veterinary awareness regarding French heartworm in dogs and more precise diagnostic methods in daily practice. Only one case of canine angiostrongylosis was recorded in Banja Luka, Bosnia and Herzegovina (Stevanović et al. 2020a). This finding is hard to link with negative jackal cases from this region obtained in our study. It should be unequivocally concluded that *A. vasorum* was present in the investigated area before this study, but the extent of the infection was epidemiologically negligible or angiostrongylosis was underdiagnosed. There is a high risk that, in the near future, the epidemiological status of *A. vasorum* infection in Bosnia and Herzegovina will become more complicated.

In recent years, the genus *Angiostrongylus* has been the subject of systematic research in Bosnia and Herzegovina. *A. vasorum* has been found in jackals, foxes (this study), and dogs (Stevanović et al. 2020a). Additionally, *Angiostrongylus chabaudi* has been identified in wild cats (Stevanović et al. 2019a), and *Angiostrongylus daskalovi* in badgers (Stevanović et al. 2019b). These findings suggest that more congeneric species may be present in Bosnia and Herzegovina, contributing valuable epidemiological

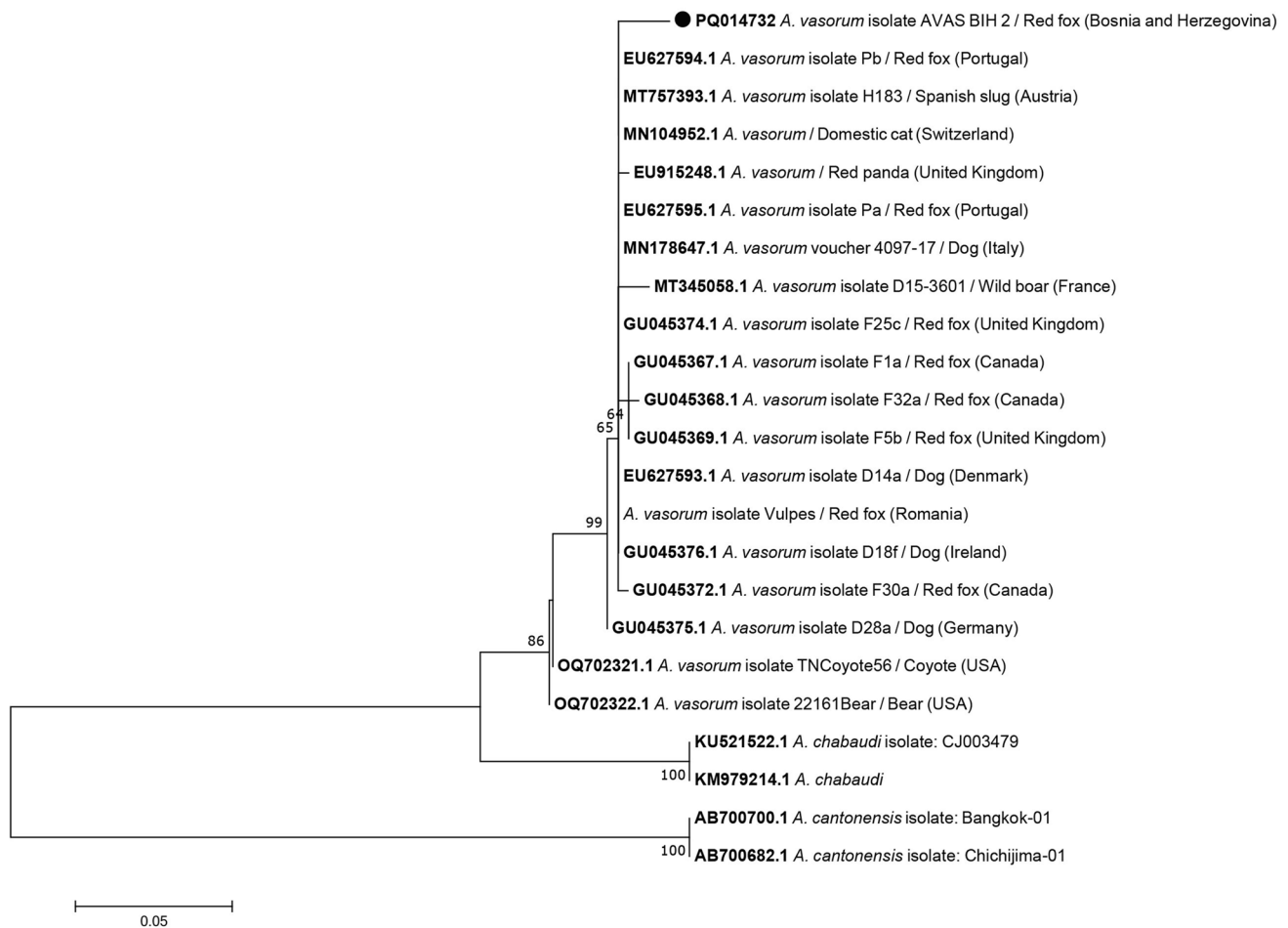


Fig. 2 The phylogenetic tree is based on the nucleotide sequences of the partial ITS-2 sequence of *Angiostrongylus vasorum*. *Angiostrongylus cantonensis* and *Angiostrongylus chabaudi* were included as outgroups. The numbers associated with the nodes represent the per-

centage of 1000 bootstrap iterations supporting the nodes (only percentages greater than 50% are shown). Nucleotide sequence obtained in this study is marked with a black circle. GenBank accession numbers, isolate names, hosts, and the country of origin are indicated

data on cardiorespiratory nematodes in Europe. Diversity within the genus *Angiostrongylus* is notable. Studies on the molecular typing of ITS-2 and COI genes indicate intraspecific genetic variations in *A. vasorum* (Jefferies et al. 2010). Isolates of *A. vasorum* from South America and Europe show higher pairwise distance differences in partial ITS-2 sequences, suggesting the potential presence of separate, possibly cryptic, species (Jefferies et al. 2009).

In addition to the genetic diversity of *A. vasorum*, some studied specimens exhibit variable morphology of the lateral rays in the copulatory bursa (Costa et al. 2003). These variations highlight the importance of combining diagnostic methods (both morphological and molecular), especially when describing a new species in a new geographical area and host.

In conclusion, we confirm the presence of the metastrongyloid species *A. vasorum* in golden jackals and red foxes from northern Bosnia and Herzegovina. For the first time,

we have confirmed this species using molecular methods, with phylogenetic analysis of the ITS-2 gene indicating that the *A. vasorum* isolate from Bosnia and Herzegovina has a low ITS-2 sequence distance from many European isolates. Finally, our work undoubtedly indicates that *A. vasorum* is spreading through the active expansion and immigration of the golden jackal in Southeastern Europe.

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Author contribution O.S performed the sampling, parasitological analysis, designed the study and wrote the manuscript. D.D performed the sampling, parasitological analysis and edited the manuscript. T.I and N. J edited and revised the manuscript. I.T edited and revised the manuscript. A.R performed phylogenetical study and edited the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Authors disclose any financial or non-financial interests that are directly or indirectly related to the manuscript submitted for publication in Parasitology Research.

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Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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