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Red fox (*Vulpes vulpes*) as a potential reservoir host of cardiorespiratory parasites in Bosnia and Herzegovina

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ABSTRACT

Red fox (*Vulpes vulpes*) is considered as reservoir of different cardiorespiratory parasites of veterinary and medical importance. Since data on cardiorespiratory parasites in foxes in Bosnia and Herzegovina are still lacking, the aims of the present study were to (*i*) investigate the prevalence and geographical distribution of these parasites, (*ii*) determine genetic diversity of detected parasite species, and (*iii*) to estimate the role of foxes in the transmission cycle to companion animals and humans. Four species, morphologically and molecularly identified as *Eucoleus boehmi* (64.6%; 51/79), *Eucoleus aerophilus* (69.7%; 154/221), *Crenosoma vulpis* (45.7%; 101/221) and *Linguatula serrata* (1.3%; 1/79) were retrieved from nasal cavity and lungs in 184 (83.3%) animals. The occurrence of heartworms, *Angiostrongylus vasorum* and *Dirofilaria immitis* was not detected by necropsy or PCR. Furthermore, three distinct haplotypes of *E. aerophilus* (I, III, XV) and two of *C. vulpis* (I, II) previously reported in pet animals and wild carnivores were confirmed in this study. A new haplotype of *C. vulpis* (designated as haplotype V) was also identified based on 12S rRNA gene for the first time. The present study indicates a high prevalence and wide distribution of nasal and lung nematodes in fox population in Bosnia and Herzegovina, and supports the existence of transmission patterns between wildlife and pet animals.

1. Introduction

Red fox (*Vulpes vulpes*) is the most widely distributed wild canid species in the world (Duscher et al., 2014) and is considered as major reservoir host and spreader of nasal and cardiorespiratory nematodes, such as *Eucoleus boehmi* (syn. *Capillaria boehmi*) Supperer, 1953, *Eucoleus aerophilus* (syn. *Capillaria aerophila*) Creplin, 1839, *Crenosoma vulpis* Dujardin, 1845, *Angiostrongylus vasorum* Baillet, 1866 and *Dirofilaria immitis* Leidy, 1856 (Traversa et al., 2010; Otranto et al., 2015; Latrofa et al., 2015). Recently, these parasites have also been recognized as an emerging disease threat to dogs and/or cats in several European countries (Traversa et al., 2010). The rising populations of red fox, along with climate changes, pet movements, urbanization, destruction and reduction of wildlife habitats, all these increase the risk of infections in animals and humans (Deplazes et al., 2004; Veronesi et al., 2014; Plumer et al., 2014). These also, may be the main driving factors responsible for the emergence and spreading of cardiorespiratory parasites in non-endemic areas (Traversa et al., 2010; Veronesi et al., 2014; Otranto et al., 2015).

In Europe, *E. boehmi*, *E. aerophilus* (Enoplida, Capillaridae) and *C. vulpis* (Strongylida, Crenosomatidae) are the most important nematode parasites infecting the respiratory system of wild and domestic carnivores (Magi et al., 2015; Tolnai et al., 2015). Adult stages of *E. boehmi* infect the upper respiratory tract (i.e. nasal cavity, sinuses), whilst *E. aerophilus* and *C. vulpis* inhabit the trachea and bronchi; they can cause respiratory distress in dogs (*E. boehmi, E. aerophilus* and *C. vulpis*) and cats (*E. aerophilus*) with varying degrees of severity and clinical signs, e.g. cough, sneezing, reverse sneezing, epistaxis, wheezing, nasal discharge and impaired sense of smell (Traversa et al., 2010; Veronesi et al., 2013, 2014; Di Cesare et al., 2015; Morganti et al., 2015; Alho et al., 2016). Nonetheless, *E. boehmi* has recently been documented as a potential cause of chronic meningoencephalitis in dogs, due to aberrant intracranial migration of the parasite (Clark et al., 2013). Moreover, foxes may also serve as definitive hosts for a worm-like parasite *Linguatula serrata* (Pentastomida, Linguatulidae), that lives in the upper respiratory tracts of various vertebrates (Yılmaz et al., 2011).

Heartworms *A. vasorum* (Strongylida, Angiostrongylidae) and *D. immitis* (Spirurida, Onchocercidae) are highly pathogenic nema-

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todes affecting the cardiopulmonary system of domestic and wild canids (*A. vasorum*, *D. immitis*) and felids (*D. immitis*). Adult worms occur in the right chamber of the heart and pulmonary arteries causing respiratory and circulatory distress, coagulopathy, neurological clinical signs and even sudden death (Morgan et al., 2009; Traversa et al., 2010; Tolnai et al., 2014). Foxes represent the main wildlife reservoir hosts for *A. vasorum* in many European countries (Otranto et al., 2015), whereas their role in the natural transmission of *D. immitis* remains speculative (Marconcini et al., 1996; Tolnai et al., 2014).

Almost all of the abovementioned parasites have an indirect life cycle, with gastropods (*C. vulpis*, *A. vasorum*), mosquitoes (*D. immitis*) and various vertebrates (*L. serrata*) serving as intermediate hosts and vectors (Traversa et al., 2010; Otranto et al., 2015). Therefore, their occurrence and distribution mainly depends on the availability of competent intermediate hosts and culicid vectors (Traversa et al., 2010; Traversa and Di Cesare, 2014). Conversely, the life cycle and route of transmission of *E. boehmi* and *E. aerophilus* are still poorly known, and both species may either develop directly in the environment, or in earthworms acting as facultative paratenic hosts (Di Cesare et al., 2012a, 2014; Veronesi et al., 2013).

Due to their pathogenic potential and diagnostic and control challenges, the importance of infections caused by cardiorespiratory parasites is even higher in pet animals (Traversa et al., 2010; Traversa and Di Cesare, 2014). Additionally, human cases of *E. aerophilus*, *D. immitis* and *L. serrata* infections have been described worldwide (Lalosević et al., 2008; Koehsler et al., 2011; Yılmaz et al., 2011; Traversa et al., 2010; reviewed in Otranto et al., 2015).

Red fox population in Bosnia and Herzegovina is presumably increasing, mostly as a consequence of an efficient anti-rabies vaccination program, which was started in October 2011 (Hodžić et al., 2014a). Since then, foxes have been intensively investigated for the occurrence of different human and pet relevant pathogens (Hodžić et al., 2014a, 2014b, 2015; Alić et al., 2015). However, data on cardiorespiratory parasites in foxes are still lacking, the aims of the present study were to (*i*) investigate their prevalence and geographical distribution, (*ii*) determine genetic diversity of retrieved parasite species, and (*iii*) to estimate the role of foxes in the transmission cycle to companion animals and humans.

2. Materials and methods

2.1. Sample collection and parasitological examination

During the hunting season from January to April 2015, carcasses of 221 red fox (98 females and 123 males), legally shot in six different regions in Bosnia and Herzegovina (Fig. 1), were collected by hunters and delivered to the Department of Pathology at the

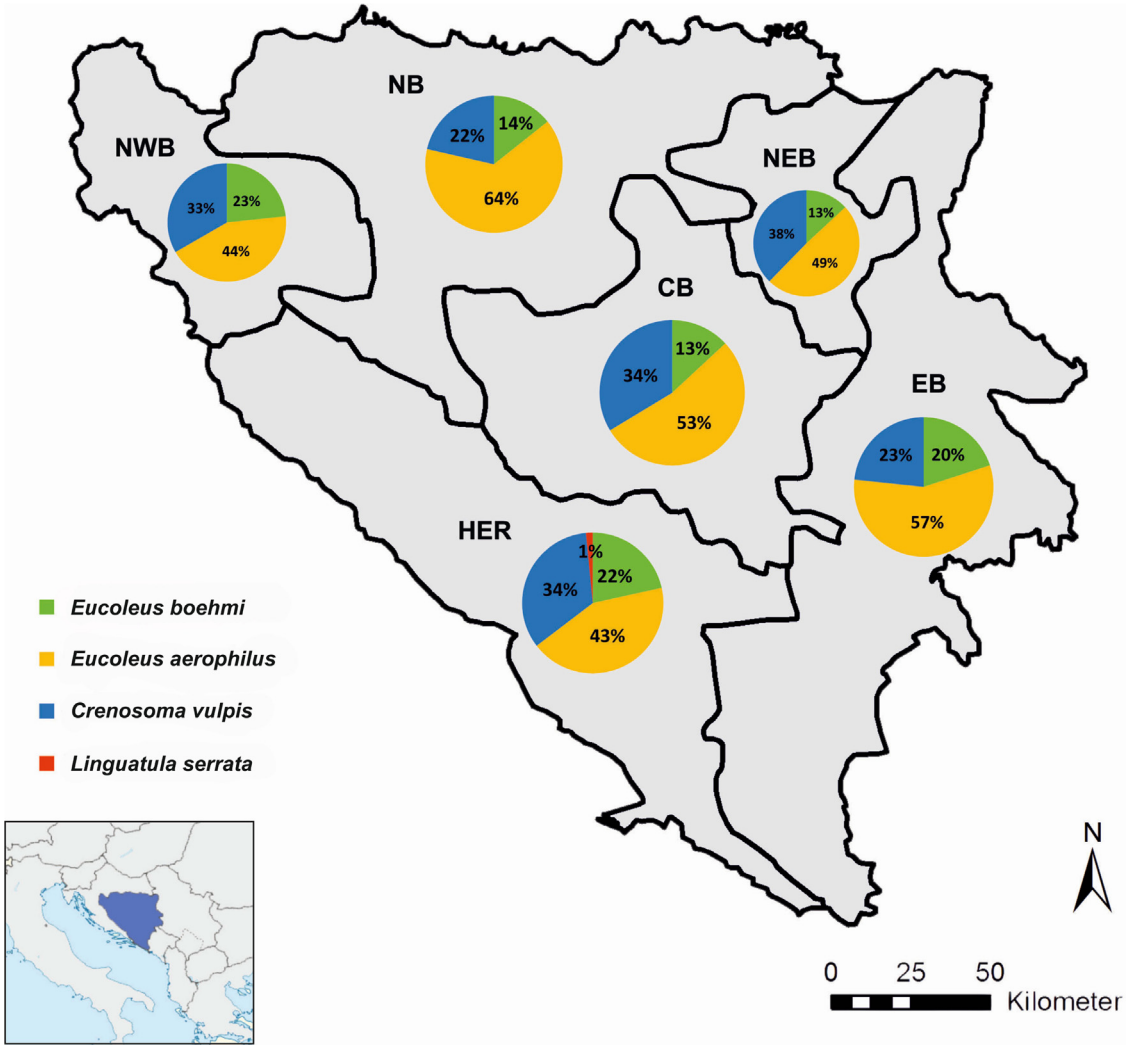

Fig 1. Map of Bosnia and Herzegovina showing the regional distribution and prevalence rate of infection with *Eucoleus boehmi*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Linguatula serrata* in red foxes. Legend: CB = Central Bosnia; NB = North Bosnia; NEB = North East Bosnia; EB = East Bosnia; NWB = North West Bosnia; HER = Herzegovina.