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***Crenosoma vulpis* in red foxes (*Vulpes vulpes*) in Northern Italy**

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ABSTRACT: *Crenosoma vulpis*, the fox lungworm, is a nematode parasite of wild and domestic canids belonging to the super-family *Metastrongyloidea*. A survey of infection was carried out examining 88 red foxes (*Vulpes vulpes*) obtained during the regular hunting season (2014-2015) from the EmiliaRomagna region of Italy. Carcasses were stored frozen (-21°C) prior to necropsy. Lungs were examined for the presence of adult worms by dissection of the trachea, bronchi and bronchioles and then the lung tissue was examined for first-stage larvae (L1) by the Baermann method. No adult stages were detected, but L1, identified based on morphology as *Crenosoma vulpis* were recovered from 28.4% (25/88) of the fox lungs. No significant differences in infection were found based on sex or geographical distribution. A brief review on *C. vulpis* report in red foxes in Italy and other European countries was also carried out.

KEY WORDS: Red fox, *Vulpes vulpes*, *Crenosoma vulpis*, Emilia-Romagna, Italy.

Introduction

Red foxes (*Vulpes vulpes* L. 1758) are wild canids worldwide in distribution. They are opportunistic predators, whose varied diet includes: lagomorphs, small and medium-sized rodents, domestic and wild birds, reptiles, invertebrates, but also fruit and vegetables especially in summer and autumn and, if available, also garbage containing food scraps, carrion of domestic and wild ungulates, and aquatic animals such as fish and amphibians (Kidawa and Kowalczyk 2011). Prey availability and geographical setting could affect the parasitic fauna of these animals, including the helminths of the respiratory tract; such as *Crenosoma vulpis* (Dujardin, 1845) (Nematoda, Metastrongyloidea) and others (Segovia et al. 2004). According to the faunistic and zoogeographical maps (Toso et al. 1999), the red fox is widely distributed in Emilia-Romagna region (Italy), with peaks of 1.38 pairs/km² in some areas of the Forlì-Cesena province, but literature about the presence of fox lungworms in this territory, is still lacking. The objective of this study was to provide data about lungworm infection in red foxes in this part of Italy.

The survey area included the provinces of Forlì-Cesena (2,378.4 km² 44°13'N; 12°02'E), Rimini (864.88 km² 44°03'N; 12°34'E) and Ravenna (1,859.44 km² 44°25'N; 12°11'E) for a total area of 5,102.72 km² which represents about a quarter of the Emilia-Romagna region (22,452.78 km², see Fig.1). The northern side of these provinces, which is the more populated and industrialized, is characterized by cultivated and flat lands (Pianura Padana) whereas the southern part is rich in hills and mountains, the top altitude is 1,654 m above sea level (a.s.l) reached by Falterona mount, belonging to the Forlì-Cesena province. These territories have a subcontinental temperate climate with warm to hot summers and cold winters. In this region the swine industry has great economic importance and must demonstrate the total absence of *Trichinella* spp. even in wildlife, in order to avoid exportation restrictions, so red foxes, either shot during the regular hunting season or found dead, were delivered to “Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna” (IZSLER) for trichinosis surveillance. From September 2014 to January 2015, 88 carcasses of red foxes, were delivered to the Forlì IZSLER section and stored at -21°C before necropsy. The respective lungs (with

or without trachea) were re-stored at -21°C and handed to our laboratory. Each respiratory tract was labeled with information regarding the geographic origin and gender when available. Fifty-one red foxes came from Forlì-Cesena province, thirty-five from Rimini province and just two from Ravenna province. Before analysis, the samples were thawed at room temperature and the trachea, when present, was inspected for macroscopic lesions, then bronchi and bronchioles were carefully dissected, washed and observed for the presence of adult lungworms. Lungs were then chopped into small pieces (5mm) and examined for the presence of lungworms first-stage larvae (L1) using the Baermann method according to Lacorcia et al. (2009). Larvae recovered by Baermann examination were identified based on size and morphology. The L1 were measured and the tails were examined under an oil immersion lens and photographed. All microscopic images and measurements were taken using a digital software image processing system NIS-Elements D 4.10.01 64-bit. Each element was identified according to the morphological keys as suggested by McGarry and Morgan (2009) and Conboy (2009).

Prevalence with a 95% Confidence Interval (CI) was estimated. Range, mean length, standard deviation and median of the detected larvae were also computed. *Chi-square* test was used in order to assess any statistical difference between gender, provinces and geographical features based on the frequency of parasites, using the software EpiInfo 3.5.1, accepting a statistical significance level for p-value less than 0.05.

Gender was known for 63 of the 88 foxes collected, with 32 males and 31 females. No adult parasites were found but in 25 foxes, first-stage metastrongyloid L1 were recovered, giving a prevalence of 28.4% (95% CI: 19-37.8). A majority of the larvae appeared to be dead but many were recovered alive showing vigorous motility. The range of larvae, detected from each positive lung, run from 1 to >1000. Overall, 271 larvae were collected and measured, covering a mean of 10.8 larvae per fox. The mean length of the detected larvae (including the nonviable L1) was 274.48 µm (SD 44.29 µm), with a range from 116.8 to 333.8 µm. There was a cephalic button at the anterior-end, a sub-dorsal mouth opening, the esophagus was about a third to almost half the length of the larvae and the tail ended in a simple point but had a slight deflection. The larvae were all identified as *C. vulpis*. Larvae of other lungworms which could be found by Baermann examination, such as *Oslerus osleri*, *Filaroides hirthi*, *F. milksi* and *Angiostrongylus*

vasorum can be discounted based on morphology. *Capillaria aerophila*, shedding eggs, is not detectable by Baermann technique.

With respect to gender, L1 were detected in 9/32 males (28%) and 7/31 females (22.6%), with no significant differences based on *chi*-square test ($\chi^2= 0.26$, $p=0.613$). No statistical difference ($\chi^2= 0.41$, $p=0.523$) was found comparing the prevalence based on the provinces. For this purpose we took into account just two provinces, Forli-Cesena and Rimini, because only two foxes were from Ravenna province. In addition, we looked for any difference with respect to geographical features, comparing animals from either flatland or hill/mountain regions. Once again no statistical differences were detected $\chi^2= 1.85$, $p=0.174$ (Tab.1).

C. vulpis infects wild -mainly foxes- domestic canids and several other carnivores, dwelling in the bronchioles, bronchi and trachea of the definitive hosts, that acquire the infections by ingesting infective third-stage larvae (L3) present in the tissues of the infected gastropod intermediate hosts (Anderson, 2000). In our work no adult helminths were macroscopically recovered in the trachea, bronchi and lower airways. The frequent lack of the trachea may have contributed to determine an underestimation of the adult presence, even if it has been already reported in dogs by Bihl and Conboy (1999). Moreover, no macroscopic lesions were observed in the trachea, when present, and in the lung parenchyma: this could suggest that the red fox and *C. vulpis* are well adapted to each other. Infections were detected by the recovery and identification of L1 based on morphology. The presence of a slight deflection to the tail but lacking a kink or dorsal spine and the presence of a subdorsally opening mouth are definitive for *C. vulpis*. Although the measurements of the L1 differ from those observed by McGarry and Morgan (2009) the morphology was consistent with that of *C. vulpis*. The small size of the larvae recovered in this study is likely due to shrinkage occurring during the post-mortem damaging. Despite the freezing at -21°C for more than two weeks, some L1 were found still alive. This could explain the survival of this parasite in colder climates, such as Prince Edward Island (Conboy et al. 2017) and Newfoundland (Jeffery et al. 2004).

Overall, high prevalence values for *C. vulpis* in foxes have been detected in several European countries, which range from 4.5% in the Netherlands to 58% in Norway (Tab. 2). In Italy, previous surveys

performed along the years in other regions using different methods, show prevalence of *C. vulpis* in red foxes ranging from 3% in Tuscany region to 17,3% in Trentino Alto Adige (Tab. 2). In the present study, the highest prevalence (28,4%) was reported, despite a modest number (88) of examined lungs. It is important to consider the possible role played by foxes as reservoir hosts for domestic animals, as suspected by Tolnai et al. (2015) and Hodžić et al. (2016). Bihr and Conboy (1999) suggested a significant difference in the infection proportions in dogs residing in rural and urban areas, assuming an overlapping in the wild/domestic interface. The spread of foxes into urban areas of European and North American countries and the high prevalence of *C. vulpis* infections may increase the exposure risk of this nematode in companion animals (Traversa et al. 2010); indeed, though neglected in the past, *C. vulpis* infections in dogs have been more often detected and/or recognised as a cause of chronic respiratory disease in various countries in the last fifteen years (Barutzki and Schaper 2011; Barutzki 2013; Conboy et al. 2013; Maksimov et al. 2017). In two recent Italian surveys, one dog out of onethousand was positive (Morelli et al. 2018) as well as five dogs out of 1748 (0.3%) found positive by Brianti et al. (2018). Recently, the spontaneous shedding of L3 in the feces of infected slugs and their survival for up to 120 days free in the environment was reported (Conboy et al, 2017). This suggests the possibility that exposure could also occur through ingestion of L3 present in the environment by the consumption or licking of grass and other plant material, increasing the possibility of transmission. Studies on fox lungworms have relevance in order to deeply understand the role of these wild carnivores in the epidemiology of these parasites related to companion animals. In the near future, veterinary clinicians should consider the possibility of lungworm infections not only in those dogs sharing a high degree of environmental overlap with foxes (such as hunting or truffle dogs) but also in dogs living in urban and suburban areas.

Conflict of interest

Benedetto Morandi, Sofia Bertaso, Gary Conboy, Andrea Gustinelli, Roberta Galuppi, Giovanni Tosi and Giovanni Poglayen, all together, declare that they have no conflict of interest .

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Tables

	strata	n° for strata	n° of positive	frequency		
gender	male	32	9	0.28	$\chi^2= 0.26$	p=0.613
	female	31	7	0.23		
province	Forlì-Cesena	51	11	0.22	$\chi^2= 0.41$	p=0.523
	Rimini	35	13	0.37		
geographical features	flatland	37	12	0.32	$\chi^2= 1.85$	p=0.174
	hill/mountain	51	13	0.25		

Table 1. Foxes found positive for *C. vulpis* larvae, stratified on the basis of gender, province and geographical features. χ^2 test does not show any statistical difference.

%	European country	<i>C. vulpis</i>		Sample size
		95% (CI)		
	Netherlands (Borgsteede, 1984; Z Parasitenkd 70: 281-285)	4.5	-	139
	Portugal (Carvalho-Varela and Marcos, 1993; Acta Parasitol	1.3	-	306
	Croatia (Rajković-Janje et al., 2002; Z Jagdwiss 48: 151-	>20	-	85
	Hungary (Sréter et al., 2003; Vet Parasitol 115: 329-334)	24	-	100
	Denmark (Saeed et al., 2006; Vet Parasitol 139: 168-179)	17.	-	748
	Norway (Davidson et al., 2006; Vet Parasitol 136: 307-316)	58	-	181
	Lithuania (Bružinskaitė-Schmidhalter et al., 2012; Parasitol	53.	43.8-63.7	310
	Hungary (Tolnai et al., 2015; Vet Parasitol 207: 355-8)	24.	23.2-26	937
	Bosnia and Herzegovina (Hodžić et al., 2016; Vet Parasitol	45.	39.3-52.3	221
	Italy			
	Piomonte (Rossi et al., 1983; Parassitologia 25: 340-343)	9.1	-	33
	Tuscany (Poli et al., 1985; Erkrankungen der Zootiere, 507–	3	-	355 42
	Trentino Alto Adige (Manfredi et al., 2003; J Mt Ecol 7:	17.3	-	129
	512) Tuscany (Magi et al., 2009; J Wildl Dis 45: 881261-	14.7	-	
	263) -885)			
	Liguria and Piemonte (Magi et al., 2014; J Helminthol 89:	15.8 8.7	10.2-21.3	165
	Southern Italy (Latrofa et al., 2015; Parasitol Res 114:	28.4	-	138
	3611506-511) Present		19-37.8	88
	study3617)			

Table 2. Prevalence of *C. vulpis* in different European countries and in different Italian regions.

Figure captions:

Figure 1. Study area: Forli-Cesena, Rimini and Ravenna provinces, located on the eastern side of the Emilia-Romagna region. The yellow drawing pins indicate negative foxes; red drawing pins refer to positive foxes.