

RESEARCH NOTE

# ***Angiostrongylus vasorum* infection in red foxes (*Vulpes vulpes*) in southern Italy**

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## **Abstract**

*Angiostrongylus vasorum* (Nematoda: Angiostrongylidae) infection was detected at post-mortem examination in the pulmonary arteries and hearts of 34/102 (33,3%) of red foxes (*Vulpes vulpes*) from the Campania Region in southern Italy. Pathological changes consisted of granulomatous interstitial pneumonia caused by larvae and intravascular pulmonary adult nematodes. These changes confirm that angiostrongylosis infection in red foxes has a mainly chronic course, in which the infected host may disperse parasite larvae in the environment over its lifetime. Results suggest that the life cycle of *A. vasorum* is well established in the red fox in the Campania Region representing a potential infection risk for dogs.

## **Keywords**

*Angiostrongylus vasorum*, red fox, *Vulpes vulpes*, cardiopulmonary infection

Angiostrongylosis is a snail/slug-borne disease of dogs and wild carnivores caused by the metastrongyloid nematode *Angiostrongylus vasorum*. The adult nematodes are found in the pulmonary arteries and right side of the heart of the definitive host where they cause verminous pneumonia and coagulopathy. *A. vasorum* occurs in tropical, sub-tropical and temperate regions of Europe, Africa, North and South America (Bolt *et al.* 1994; Koch and Willesen 2009; Barbosa *et al.* 2005; Demiaszkiewicz *et al.* 2014). In Europe, the red fox (*Vulpes vulpes*) is considered its natural reservoir being able to support the infection generally without serious morbidity or mortality (Poli *et al.* 1984, 1991; Koch and Willesen 2009). Its distribution has been characterized by isolated endemic foci with only sporadic occurrence outside a few circumscribed known areas. However, in the last two decades the spread of *A. vasorum* to new geographical areas has been documented (Bolt *et al.* 1994; Koch and Willesen 2009; Demiaszkiewicz *et al.* 2014). In Italy, since the first documented cases in red fox from the Tuscany Region (Poli *et al.* 1984), more recent

studies reported the occurrence of the parasite in other areas of central and northern Italy (Magi *et al.* 2009, 2014; Eleni *et al.* 2014). Studies on *A. vasorum* infection in dogs also suggest that the disease is spreading in Italy (Traversa *et al.* 2010; Di Cesare *et al.* 2011). Monitoring distribution and defining new endemic foci of infection have been considered a priority to improve our understanding of the epidemiology of angiostrongylosis (Bolt *et al.* 1994; Koch and Willesen 2009). Here we report the first record of *A. vasorum* infection in red foxes from southern Italy. Its pulmonary pathological changes are also described.

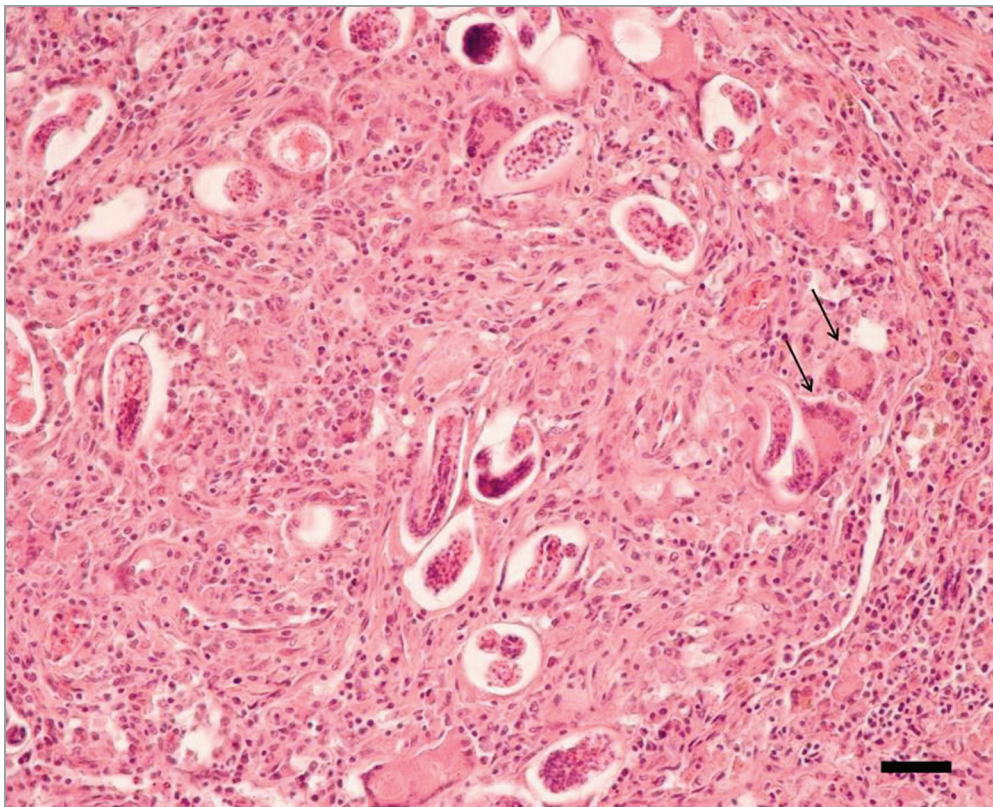
A total of 102 red foxes from the Campania Region of southern Italy, obtained between October 2012 and February 2014 were examined for *A. vasorum* infection. Most of the red foxes were collected by local hunters (n = 75), and a few (n = 27) were road-killed specimens. Hosts were sexed and on the basis of tooth examination, they were classified as juveniles (<12 months old) or adults (>12 months old). During necropsy examination, the heart and pulmonary arteries were

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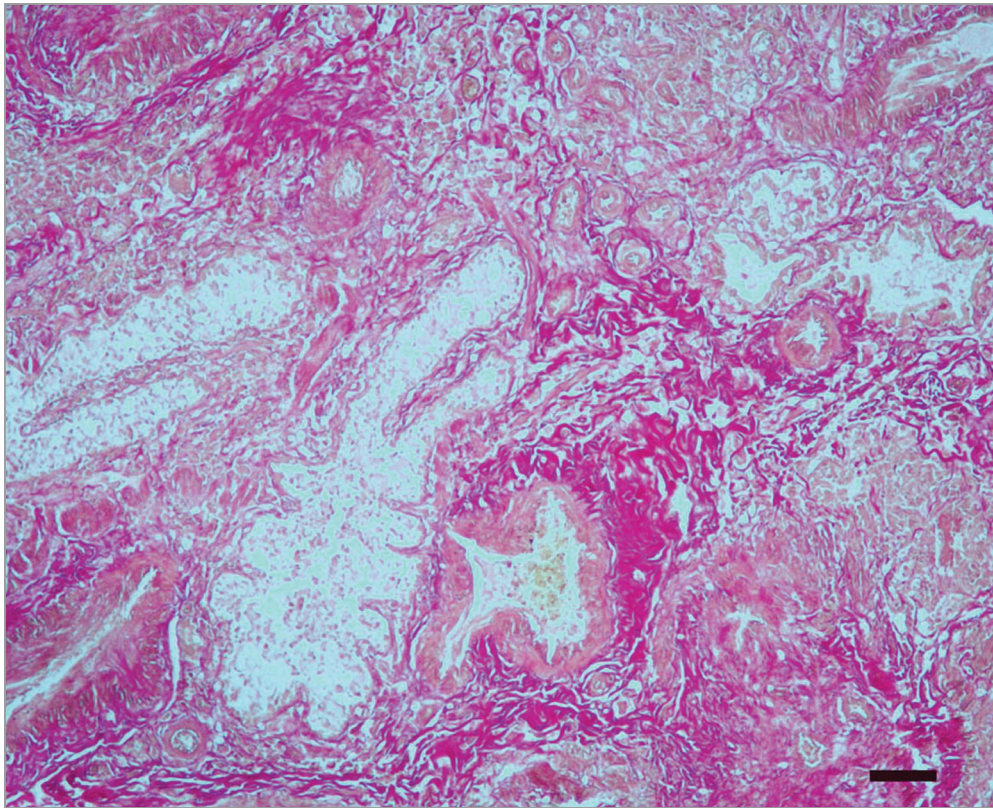
opened and examined for helminths, and worms were collected and identified. Differences in prevalence between age classes and gender were assessed by the two-sided Chi-square test. A  $P$  value  $<0.05$  was regarded as statistically significant. Tissue samples from lungs of red foxes positive for *A. vasorum* were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin and Van Gieson.

Of the 102 red foxes studied, 43 were females (including 19 juveniles and 24 adults) and 59 males (including 14 juveniles and 45 adults). A total of 34 (or 33,3%) individuals were positive for *A. vasorum* including 19 males (15 adults and 4 juveniles) and 15 females (6 adults and 9 juveniles). No significant differences were found between age classes and/or gender groups. Most parasites were found in the lungs and in two cases in the right heart. Lungs of positive individuals showed varying severity of inflammatory responses consisting of reddish-brown or yellow-brown foci with increased resistance to slicing and scattered areas of emphysema. Microscopically, pulmonary tissues showed pathological changes ranging from mild to severe. Lesions consisted of granulomatous interstitial pneumonia with a number of eggs, larvae, and intravascular pulmonary adult nematodes. Granulomas associated with *A. vasorum* and its eggs were characterized by aggregates of epithelioid macrophages, lymphocytes, plasma cells, fibroblasts and eosinophil granu-

locytes, often surrounding small deposits of amorphous necrotic debris (Fig. 1). In some cases multinucleated giant cells were also evident. Inflammatory cells were organized to form nodular lesions but in more severe cases large areas of interstitial fibrosis (Fig. 2) contained mild to severe infiltrates of lymphocytes, plasma cells, macrophages with hemosiderin, eosinophilic granulocytes and multinucleated giant cells. According to Koch and Willeßen (2009) the prevalence of infection in the European red fox ranges from 5 to 49% with a peak of 92.9% in Denmark (Tønsberg *et al.* 2004). Prevalence of infection found here suggests that the life cycle of *A. vasorum* is well established in red foxes in the Campania Region. The generalist diet of red fox varies greatly among different geographical areas, ranging from fruits to carrion, including a wide variety of invertebrates and some medium or small-sized mammals (Serafini and Lovari 1993). Unfortunately, no data exist on red fox diet in southern Italy, however the *A. vasorum* prevalence of infection here observed suggests that snails and slugs (acting as intermediate hosts of *A. vasorum*) constitute an important constituent in its diet, representing a potential reservoir for infection in dogs. On the other hand, the lack of differences between age classes and gender groups suggests that juveniles and adults and females and males of red fox in Campania Region feed in the same way on the invertebrate intermediate hosts of *A. vasorum*.



**Fig. 1.** Interstitial pneumonia with a number of eggs and larvae surrounded by epithelioid cells, macrophages, lymphocytes, plasma cells, fibroblasts and multinucleated giant cells (arrows) are showed. Haematoxylin and eosin. Bar 25  $\mu$ m



**Fig. 2.** Severe interstitial fibrosis surrounding vessels and bronchial structures. Van Gieson stain. Bar 40  $\mu$ m

Most of the foxes here examined were from rural areas of Salerno Province. This geographical area is characterized by mean month temperatures ranging from 4° to 29°C, and high annual humidity rates with a mean annual rainfall more than 1100 mm (data from meteorologic station of Salerno Province). Environmental factors are important in determining occurrence and prevalence of infection of helminths in the red fox (Gortázar *et al.* 1998; Segovia *et al.* 2004; Barbosa *et al.* 2005) being able to influence for example the viability of parasite eggs or larval longevity of helminths which may be susceptible to temperature or humidity conditions (Stromberg 1997). In addition, the availability and behaviour of both intermediate and definitive hosts can also be affected by environmental factors and therefore influence the occurrence and persistence of parasites (Stromberg 1997; Gortazar *et al.* 1998; Barbosa *et al.* 2005). For instance, high humidity levels positively influence the survival of parasite larvae in infected faeces and factors such as the mobility of slugs and snails may, in turn, increase the availability of these intermediate hosts for red foxes. Actually more than 25 species of terrestrial and aquatic snails and slugs have been demonstrated to be intermediate hosts for *A. vasorum* (see Koch and Willeßen 2009). It is of particular concern in areas with dense populations of both red foxes and dogs (including mainly feral, vagrant and hunting dogs) which live in close proximity (Stromberg 1997; Koch and Willeßen 2009) in the area studied. According to

data from the Italian Health Ministry, the Campania Region holds the record in Italy for the number of feral dogs with about 66,500 individuals (see <http://burc.regione.campania.it>). Studies on helminths of feral dogs in Campania Region are lacking, but it is plausible to hypothesize that because of their great density in this area, feral dogs may play an important role in environmental dispersion of *A. vasorum* within the wild and from wild to urban suburbs (Stromberg 1997).

In dogs *A. vasorum* infection may show a wide spectrum of clinical manifestations, ranging from no or minor clinical signs to life-threatening disease (Koch and Willeßen 2009). In red foxes, generally clinical signs are usually milder, and only in presence of concurrent diseases, high worm burdens can provoke significant lesions in lung parenchyma (Poli *et al.* 1991; Morgan *et al.* 2008), however fatal infections in red foxes have been recently described (Eleni *et al.* 2014). Pathological changes here observed confirm that infection in the red fox has a mainly chronic course in which the infected host may disperse parasite larvae in the environment for its lifetime.

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