First report of Angiostrongylus vasorum *in a wild red fox (*Vulpes vulpes*) from Apulia (Italy)*

Giuseppe Passantino[1], Fabio Marino[2], Gabriella Gaglio[2], Rosa Patruno[3], Giovanni Lanteri[2*] and Nicola Zizzo[1]

[1] *University of Bari, Department of Veterinary Medicine, 70010 Valenzano (BA), Italy.*
[2] *University of Messina, Department of Veterinary Sciences, Viale Annunziata, 98168 Messina, Italy.*
[3] *Department of Animal Health, ASL BAT, Via Paradiso 33/H, 70026 Modugno (BA), Italy.*

[*] *Corresponding author at: University of Messina, Department of Veterinary Sciences, Viale Annunziata, 98168 Messina, Italy. Tel.: +39 090 3503707, e-mail: lanterig@yahoo.it.*

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Angiostrongylus vasorum,
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Summary
Severe lung strongylosis was detected in a wild red fox (*Vulpes vulpes*) (1/12) from Apulia (Italy). We performed routine diagnostics on 12 foxes found dead in Apulia. Eleven of them showed lesions consistent with a vehicle collision. However, the remaining fox appeared to have died from other causes. At necropsy we observed, catarrhal enteritis, fatty liver, lung congestion with some areas firm in consistence and brain haemorrhages and malacia. Histopathology revealed lung fibrosis with mononucleate cells infiltration, thrombosis a several larval nematodes spread in the parenchyma, interstitial nephritis, interstitial myocarditis, encephalitis, encephalomalacia, and a brain granuloma. The larvae recovered from the lung parenchyma were identified as the first stage larvae of *Angiostrongylus vasorum*. This is the first documented report of angiostrongylosis in a fox in Southern Italy.

Angiostrongylus vasorum *in una volpe (*Vulpes vulpes*) in Puglia (Italia)*

Parole chiave
Angiostrongylus vasorum,
Polmone,
Puglia,
Volpe.

Riassunto
In una volpe selvatica, su un totale di 12 volpi trovate morte in Puglia, sono state eseguite indagini diagnostiche di routine. Tali indagini hanno evidenziato enterite catarrale, steatosi epatica, congestione polmonare con aree di aumentata consistenza, emorragie cerebrali e malacia. L'esame istopatologico rilevava fibrosi polmonare con infiltrati mononucleati, trombosi e forme larvali di nematodi diffusi nel parenchima, nefrite interstiziale, miocardite interstiziale, encefalite, encefalomalacia e un granuloma cerebrale. Le larve recuperate dal parenchima polmonare sono state identificate come il primo stadio larvale di *Angiostrongylus vasorum*.

Lung parasitic diseases generally are not so common in canids, although several cases have recently been reported in Italy as well as in the other European countries, reaching percentages > 40%. Among these diseases, angiostrongylosis is due to a metastrongyloid nematode *Angiostrongylus vasorum* (French heartworm), affecting domestic and wild carnivores (Morgan *et al.* 2008, Eleni *et al.* 2013). The basic life cycle of *A. vasorum* is well described (Bolt *et al.* 1994), albeit doubts persist over some epidemiological details (Morgan *et al.* 2008). The life cycle is indirect, with dogs becoming infected by eating snails, and sometimes also frogs. Adult worms live in the pulmonary arteries and in the right ventricle of the heart. Eggs released by female worms are transported to the pulmonary capillaries, where they hatch into the first stage larvae (L1). Larvae (L1) penetrate the capillary and alveolar walls to the airways, reach the pharynx and then are excreted through the faeces. Canids acquire infestation by the ingestion of the third stage larvae (L3) contained in intermediate hosts (mainly gastropods). After ingestion, the infective stage travel to the lymph nodes adjacent to the alimentary tract, where both parasitic moults (fourth and fifth stage larvae) take place, and then to the vascular predilection site. Larvae (L5) have also been found in the liver of canids. The prepatent period is 28 to 108 days. Wild canids, especially foxes, are likely to have a role in the epidemiology of canine infestation, and the parasite could also affect fox health and population dynamics (Morgan

et al. 2008). In Italy, the first report of such parasitic disease was described in Tuscany in 2002 (Della Santa *et al.* 2002), followed by sporadic reports in Lazio, Abruzzo, Umbria, and Apulia. In recent years, the parasite has been increasingly reported in dogs and foxes, in association with geographical range expansion and possibly increasing transmission in endemic areas.

The infestation can be asymptomatic. However, persistent cough, respiratory rattles, apathy, lethargy, scarce blood coagulation, spontaneous haemorrhages, bleeding from nose and mouth, mucosal anemia, anomalous behavior, back pain, convulsions, anorexia, weight loss, nausea, vomit, and diarrhoea can be registered at clinical evaluation.

The aim of this short communication is to report the presence of *A. vasorum* in foxes from Southern Italy, to describe histopathological features, and to compare our findings to data reported in the extant literature.

From 2011-2013, 12 adult wild red foxes (*Vulpes vulpes*), which died in the territory of Apulia (Italy), were examined during routine diagnostic at the section of Comparative Pathology and Oncology of the Department of Veterinary Medicine (DMV) of the University of Bari, Italy.

After age estimation, autopsy was conducted on each fox approximately 24 hours after death. Samples of lung, heart, liver, tracheobronchial lymph node, kidney, and brain were fixed in 10% buffered formalin solution and then routinely processed for paraffin embedding. We obtained 4 µm thick transverse and longitudinal sections, which were then dewaxed, dehydrated, and stained with haematoxylin-eosin and Masson trichromic techniques.

For scanning electron microscopy (SEM), small samples of lung parenchyma of foxes were microdissected and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, for 48 hours. The samples were washed in tap water, dehydrated in a graded series of alcohol, dried by the method of the critical point and sputter coated with 20 nm gold-palladium. Samples were then examined under a Cambridge Stereoscan 240 to 20kV.

Routine virology was carried out. The fox was examined for rabies virus, canine distemper virus, and canine parvovirus.

The cause of death of 11/12 foxes was due to trauma related to collisions with vehicles. In 1 fox, which showed good general body condition, the main gross lesions were attributable to the following factors: catarrhal enteritis, fatty liver, the heart muscle, and kidney were discolored. The lung in particular appeared hardened and of a dark red colour, and, showed multiple focal consistent areas with a diameter of 5-8 mm near the edges of the lobes. The brain showed dark red foci of haemorrhage and malacia. Routine virology did not detect any pathogen.

The histopathological examination revealed a focal pulmonary fibrosis with a large or moderate infiltration of macrophages, lymphocytes, and plasma cells, occasionally eosinophiles and multinucleated giant cells. There could be haemosiderin-laden macrophages in areas with presence of numerous red blood cells. Several nematode larvae were detected in alveolar spaces causing a thickening of the perialveolar, peribronchiolar, and perivascular connective tissue (Figure 1). Larvae within some pulmonary arteries of medium caliber cause a thickening of the wall. Pulmonary artery was partially occluded by a thrombus (Figure 2). In regional lymph nodes of the lung, medullary sinuses were lying with many hemosiderin-laden macrophages.

The myocardium showed a slight interstitial

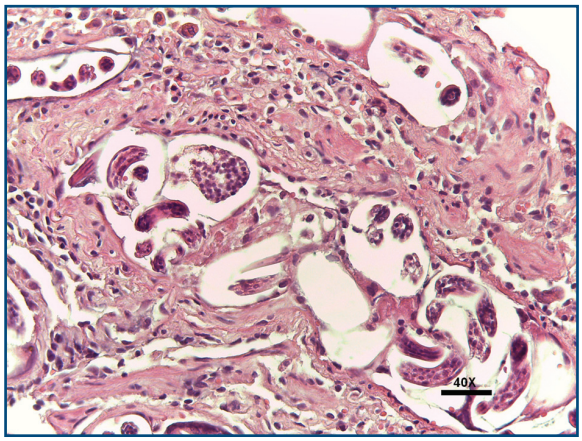

Figure 1. *Fox: lung. Nematode larvae in alveolar spaces causing a thickening of the perialveolar, peribronchiolar, and perivascular connective tissue (haematoxylin-eosin 40X).*

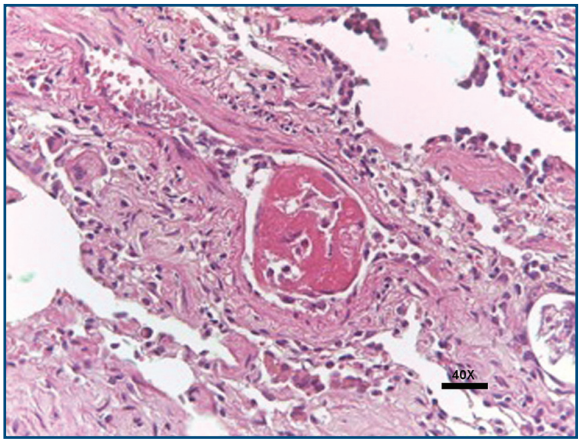

Figure 2. *Fox: lung. Pulmonary artery partially occluded by a thrombus. (haematoxylin-eosin 40X).*

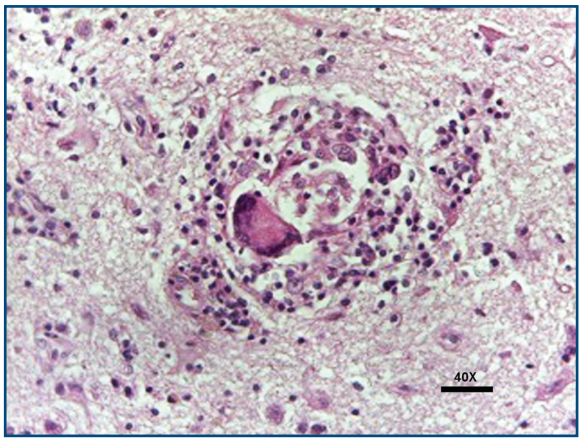

Figure 3. *Fox: brain: granulomatous reaction with giant cells and signs of malacia of the white substance (haematoxylin-eosin 40X).*

reactivity. In the kidney, multifocal interstitial infiltrates of macrophages, lymphocytes, and plasma cells were scattered within the renal cortices. The brain showed encephalitis with areas of lymphoplasmacytic infiltration in the perivascular tissue. A granulomatous reaction with giant cells and signs of malacia of the white matter were also detected (Figure 3).

The larvae recovered from the lung parenchyma, of the size 330-380 µm in length and 14.2-14.8 µm in width, were identified using the morphometrical keys available in literature (McGarry and Morgan 2009) as the first stage larvae of *A. vasorum* (Figure 4).

By SEM, the lungs showed particular features that cannot be demonstrated by light microscopy. In particular, SEM analysis let us better understand the 3-dimensional distribution of larvae surrounded by the severe proliferation of interstitium, which deeply modified the lung architecture (Figure 5).

This short communication reports for the first time the presence of *A. vasorum* in fox living in Apulia region (Southern Italy).

Figure 4. *First stage larvae of* Angiostrongylus vasorum.

In Northen and Central Italy, *A. vasorum* presence was investigated in foxes from Liguria, Piedmont, Tuscany, with values of prevalence of 80%, 70%, 7%, respectively. In Southern Italy, the nematode was found only in dogs from Apulia region, and no data are available on its presence in foxes (Sasanelli *et al.* 2008) from Abruzzo, Basilicata and Umbria (Lepri *et al.* 2011, Tieri *et al.* 2011, Traversa *et al.* 2008, Traversa *et al.* 2013).

In the reported case, no adult parasites were found either in pulmonary arteries or in the heart, although the detection of a thrombus as well as the damage of the endothelium and activation of the intrinsic and extrinsic coagulation may imply their presence. According to Morgan and colleagues (Morgan *et al.* 2008), the failure in finding adults could be due to a different localization of the parasites, probably detectable with other specific techniques, such as flushing. In contrast to the lesions found in dogs by Koch and Willesen (Koch and Willesen 2009) and Tieri (Tieri 2011), no granulomatous formations were observed in the kidney, but only multifocal interstitial infiltrates of macrophages, lymphocytes, and plasma cells.

The weight of the heart in relation to the body weight was 0.89%, within the normal range for red foxes (Cavallini *et al.* 1997). Regarding tissue changes detected in the nervous system, such as encephalitis, malacia, and granuloma, they could

Figure 5. *Fox: lung. 3-dimensional distribution of larvae of* Angiostrongylus vasorum *(Scanning Electron Microscopy).*

be, in our opinion, the result of aberrant migration of larvae (Bourque *et al.*,2008).

The positivity of a single fox over a total of 12 animals may indicate a low circulation of the nematode in fox population of the Apulia region, although, it has already been found in dogs from the same region (Sasanelli *et al.* 2008). Moreover, Jefferies and colleagues (Jefferies *et al.* 2010) showed that there was no evidence of genetic segregation of *A. vasorum* isolated from dogs, foxes, and coyotes, supporting the hypothesis that transmission occurs between wild and domestic canids.

Some authors attribute the increase of the *A. vasorum* infestation in dogs and foxes to predisposing factors such as mild temperature and moist air, proliferation of gastropods (Magi *et al.* 2009, Morgan *et al.* 2008). Further research is needed to estimate the importance of infestation due to *Angiostrongylus* in Apulia and to provide important information on its genetic lineage following the spread of this parasite through Europe, South America, Canada, and the United States (Jefferies *et al.* 2009). Bolt and colleagues (Bolt *et al.* 1992, Bolt *et al.* 1994) assert that the direct transmission between foxes and dogs is possible even if it is not fully demonstrated whether foxes act effectively as reservoirs of infestation for dogs.

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