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First report of *Spirocerca lupi* infection in red fox *Vulpes vulpes* in Greece

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During the necropsy of a red fox *Vulpes vulpes* that had died from poisoning, we found a nodule of 2×3 cm in size in the gastric wall, which was caused by the nematode *Spirocerca lupi*. The histological features of the parasite include a smooth cuticle, large chords of the hypodermis, muscle cells of the polymyarian/coelomyarian type and large intestine cells with prominent microvillus border. The nodule was infiltrating different layers of the gastric wall and consisted of granulation tissue, with various inflammatory cells and active fibroblasts. This is the first report of *S. lupi* infection in the red fox in Greece.

Key words: gastric nodule, Greece, histology, red fox, *Spirocerca lupi*, *Vulpes vulpes*

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Spirocerca lupi (Spirurida, Spiroceridae) is a nematode infecting dogs *Canis lupus familiaris* and wild carnivores in tropical and subtropical areas (Bailey 1972, Fox et al. 1988). The adult parasite is usually found in large nodules in the wall of the oesophagus. It is less commonly found at other sites of the animal, among which are the stomach, intestine and trachea (reviewed by van der Merwe et al. 2007). In this article, we describe the presence of a nodular lesion on the stomach wall of a red fox *Vulpes vulpes*, which was caused by *S. lupi*.

An adult red fox was found dead and was brought to the Necropsy Service, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, for autopsy. At necropsy, typical lesions of anticoagulant rodenticide poisoning were found, which made it possible for us to determine the cause of death. These lesions were characterised by the

presence of unclotted blood in the abdominal and the thoracic cavity, multifocal hemorrhages in the subcutaneous tissue and the gastrointestinal tract, diffuse pulmonary hemorrhages, subepicardial and subendocardial petechial hemorrhages and ecchymoses. Furthermore, we saw a nodule of 2×3 cm in size in the gastric wall (*fundus*) without any ulceration or penetration of the gastric mucosa or serosa.

On the cut section of the gastric nodule, we observed large sized coiled red nematodes embedded in a greyish-white granulomatous tissue. We collected and submitted parasites to the Laboratory of Parasitology and Parasitic Diseases for identification. Also, we fixed tissue of the nodule in a 10% neutral-buffered formalin, processed routinely, embedded in paraffin wax, sectioned at 4-6 mm and stained with haematoxylin and eosin (H&E) and Masson's trichrome methods.

Figure 1. Morphologic features in sections of *Spirocerca lupi* include large lateral chords extending into body cavity (arrows), prominent gut (arrowheads) and uterine tubes filled with developing eggs (asterisks). Granulomatous tissue (open arrows) developed in the gastric wall is surrounding the coiled nematode.



Histological examination indicated that a thick-walled nodule, which consisted of parasitic granulomas and granulation tissue, was located in the outermost connective tissue (*adventitia*) of the stomach wall and focally infiltrating into the layers of smooth muscles (*muscularis externa*), resulting in extension of the granulomatous tissue to the submucosa. Granulomas consisted of centrally located worms (Fig.1), surrounded by degenerated and viable eosinophils and neutrophils and necrotic cellular debris admixed with bacilli, rimmed by a well vascularised, collagenous rich connective tissue infiltrated predominantly by mononuclear cells, specifically histiocytes, plasma cells and lymphocytes. We also observed isolated or foci of parasite eggs which were surrounded by degenerated and viable eosinophils, neutrophils and necrotic cellular debris. Into the collagenous connective tissue, presumably adjacent to the worm tract, diffuse infiltrates or foci of the above mentioned inflammatory cells were present. We also saw particularly active fibroblasts without marked cellular atypia.

The worms in the granulomas exhibited characteristic features of spirurid nematodes. Multiple sections of a coiled, large, gravid female worm were present. The cuticle was smooth without any special ornamentation and the hypodermis was thin and inconspicuous except at the areas of the lateral chords, which were very large and projected far into the body cavity (see Fig. 1). The muscle cells were prominent, numerous (polymyarian type) and extended into the body cavity (coelomyarian type). The intestine was large and distinctive; the cells were

columnar and slender, the nuclei all tended to lie at the same level in the cells, and a very prominent microvillus or brush border was present. The paired uterine tubes were large and filled with characteristic small, long and slender spirurid eggs with a thick shell that contained a developing larva (Fig. 2).

First, we studied the morphological features of the parasites isolated from the nodule macroscopically, and then microscopically using a stereoscope and a microscope. The worms were bright red nematodes, 1 mm in maximum diameter and 4 cm (males) to 7.5 cm (females) long. Their mouth was surrounded by six lobes. The hind end of the male was spirally coiled, had lateral alae and papillae and unequal spicules. In the female, the vulva opened close to the hind end of the oesophagus and the uterus contained characteristic thick and smooth shelled eggs, $30-37 \times 11-15 \mu\text{m}$ in size. We did not perform a post-mortem faecal examination for detection of parasitic ova. According to their morphology, the parasites were identified as *S. lupi* (Soulsby 1968, Yamaguti 1961).

S. lupi predominantly occurs in species belonging to Canidae and has a worldwide distribution, but it is more prevalent in warm climates (i.e. tropical and subtropical regions). In Greece, *S. lupi* has been reported in 0.4-24.2% of the dogs (Himonas 1968, Haralabidis et al. 1988, Mylonakis et al. 2001) but not in wild carnivores.

The adult worms are found coiled within granulomatous nodules formed mainly in the oesophagus, where they deposit embryonated (L1) eggs, which pass into the gastrointestinal lumen through small fistulous tracts which the parasite opens through the

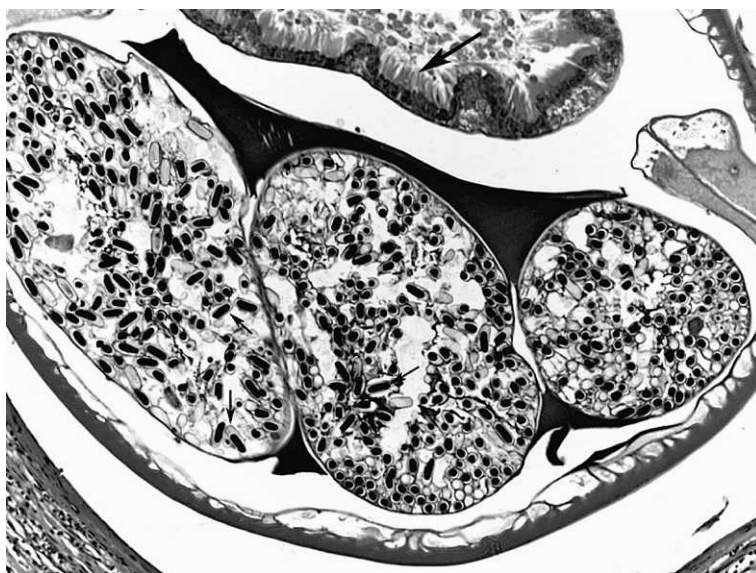


Figure 2. High-power magnification of *Spirocerca lupi* section, illustrating the nature of the gut, including the prominent brush border (large arrow), and the uteri filled with typical, thick-shelled spirurid eggs (small arrows) containing a developing larva. When viewed in cross section, the eggs appear round.

oesophageal wall. The eggs are passed in the faeces or may be shed in vomitus. Eggs are ingested by the intermediate host (a variety of species of arthropods, mainly coprophagous beetles) and the larvae encyst within the tissues and develop to infectivity (L3) within two months. The beetle gets ingested by the final host (a carnivore) or a paratenic host (e.g. reptiles, birds and small mammals; Bailey 1972, Soulsby 1968, Fox et al. 1988, Urquhart et al. 1996).

Spirocercosis is usually subclinical, but it may also be the cause of oesophageal/stomach granulomas and sarcomas, aortic scarring and aneurysms, thoracic discospondylitis/spondylosis, hypertrophic osteopathy and salivary gland necrosis (Mylonakis et al. 2008). In the case we describe here, we observed no other lesions relevant to the parasite's presence, apart from the gastric nodule. Diagnosis of spirocercosis can be made by demonstrating the characteristic eggs of the parasite in faeces applying an appropriate method, by endoscopy or by radiography (Mylonakis et al. 2008). In the case of foxes and other wild animals, diagnosis is usually made *post mortem*, i.e. by necropsy.

Gastric lesions in foxes due to *S. lupi* have been reported in Sicily (Italy), where 9.16% of the animals harboured these nematodes exclusively in gastric nodules (Ferrantelli et al. 2010). There is also a report of gastric nodule caused by *S. lupi* in a domestic cat in Florida, USA (Mense et al. 1992). Another parasite of the same family (Spirocercidae), *Cylicospirura* spp. was reported to cause similar lesions in wild Felidae (Ferguson et al. 2011). Pence & Stone (1978), on the other hand, found that aortic involvement

(scarring and aneurysms) are more frequent in wild carnivores than esophageal nodules.

In a total of 32 helminth species found in red fox in southern Belarus, *S. lupi* was present in 2.1% of the faecal samples (Shimalov & Shimalov 2003). This parasite was also found in red fox in Belgrade (Pavlović et al. 1997) and the Iberian Peninsula (Segovia et al. 2004). In all the above-mentioned cases, the animals examined were dead, so possible clinical signs could not be observed.

Despite the great variety of helminth species found in previous studies of red fox in Greece (Diakou 1996, Papadopoulos et al. 1997), our observation is the first report of *S. lupi* in this animal species.

Infection by *S. lupi* can in some cases be fatal. This is an important fact to be considered in countries where wild carnivores (e.g. the grey wolf *Canis lupus* and the golden jackal *C. aureus* in Greece) are vulnerable or threatened by extinction. Moreover, parasites such as *S. lupi* which can be found both in domesticated and wild animals are of particular epidemiological significance as cross infection between the two animal groups is possible.

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