Spirocercosis and its complications in stray dogs in Shiraz, southern Iran

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ABSTRACT: *Spirocerca lupi* is accepted in Veterinary Medicine as the cause of true malignancies in dogs. Aortic lesions and esophageal nodular granulomas are pathological lesions of spirocercosis that are considered to be pathognomic for this disease. *Post mortem* and histopathological studies of 105 stray dogs both male and female of different age groups from various residential areas were performed and their esophagus were examined for any lesions and helminthic parasites. Twenty dogs (19.04%) were infected with *S. lupi*. The infection rate was significantly higher in dogs greater than one year old (23.5%) and in western areas (36.4%) with similar prevalence rate in males and females. At histopathologic level, two isolate but closely related fibrosarcoma masses were situated at the distal portion of the thoracic esophagus of one dog and in the site of junction of esophagus and stomach of another one. Metastasis were observed in the lung and mediastinal lymph nodes of one of the dogs. Despite presence of large population of stray dogs little information is available about *S. lupi* in this country, so this study was undertaken to obtain an overall picture of this infection in stray dogs of Shiraz area, southern Iran.

Keywords: spirocercosis; dog; esophagus; fibrosarcoma; Iran

Spirocercosis due to *Spirocerca lupi* (a parasitic nematode) is worldwide and is endemic in some warm climates. Despite the importance of *S. lupi* in causing dysphagia, vomiting, esophageal neoplasia, aortic aneurysm or rupture, and secondary pulmonary osteoarthropathy very few studies are conducted on the epidemiology and pathologic lesions of this infection. The important factors affecting prevalence of this disease are proximity to intermediate and paratenic hosts and the population density of intermediate and infected adult hosts (Brodley et al., 1977; Johnson, 1992; Kagira and Kanyari, 2001). This nematode is infective for many species including human beings, but affects mostly carnivores (Bailey, 1972; Lobetti, 2000; Dvir et al., 2001; Mazaki-Tovi et al., 2002). The clinical manifestations due to spirocercosis vary greatly, depending on the stage of the disease, aberrant migrations and possible complications. Clinical signs, in endemic areas include vomiting or regurgitation, dysphagia, dyspnoea, pyrexia and anorexia (Bailey, 1972; Jubb et al., 1993; Lobetti, 2000). Definitive diagnosis is made by detection of typical embryonated eggs in fecal smears (Markovics and Medinski, 1996), and/or finding the parasite in the granulomatous lesions (Fox et al., 1988).

In their life-cycle, the female lays embryonated eggs that are transferred through a tract in the nodule and are passed out in the feces or vomitus of the host and hatch only after they are ingested by a coprophagus beetle. The larvae develop to the infective stage in the beetle within two months and become encysted there. The beetle in turn is swallowed by another intermediate and/or paratenic

host or by the final host especially from the canidae family (Ramachandran et al., 1984; Jubb et al., 1993; Dvir et al., 2001; Donald et al., 2007). Carnivores are infected by ingestion of beetles or a variety of paratenic hosts including numerous amphibians, reptiles, lizards, domestic and wild birds and small mammals such as hedgehogs, mice and rabbits (Soulsby, 1986; Anderson, 2000). In the definitive host, the larvae usually follow a specific migratory route, reaches to the stomach of the dog and then penetrate the gastric mucosa of the host and begin a predictable migratory route. They migrate along arteries, follow their life cycle in the thoracic aorta and about three months post-infection, the larvae leave the aorta and migrate to the caudal esophagus where they provoke the development of granulomas as they mature to adults over the next three months (Soulsby, 1986; Urquhart et al., 1996). Here they form granulomatous tumor-like growth in which they reach maturity and reproduce eggs. The eggs are passed via a small opening (or openings) into the esophagus and their cycle repeats itself (Evans, 1983; Lobetti, 2000).

The most reported pathological lesions of spirocercosis include aortic scars and aneurysms, and esophageal nodular granulomas (Fox et al., 1988; Dvir et al., 2001; Moulton, 2002). Pathologic lesions may result from normal or aberrant nematode migration. In the normal migration route along the gastro-aortic arterial system, the lesions include rupture of the aorta resulting haemothorax and sudden death, or rupture of other major blood vessels, that cause haematomas (Hamir, 1984; Riberio et al., 1994; Dvir et al., 2001; Kagira and Kanyari, 2001). Migration through the aortic wall to the oesophagus may result in mediastinitis, pneumomediastinum, pleuritis or pyothorax with clinical sign of dysponea (Stephens et al., 1983; Hamir, 1986; Dvir et al., 2001; Klainbart et al., 2007). Necrosis, haemorrhages and exudation followed by fibrosis, scar formation and sometimes mineralisation and aneurysm formation in the aortic and other blood vessels are other usual histopathologic findings (Johnson, 1992; Jubb et al., 1993). Aberrant migration of larvae result from nodules containing worms in many sites, including the trachea and lungs (Stephens et al., 1983), diaphragm (Harrus et al., 1996), heart (Garg et al., 1989), stomach, intestine and mediastinum (Dvir et al., 2001), rectum (Georgi et al., 1980), kidneys, subcutaneous tissues (Singh et al., 1999) and urinary bladder (Thanikachalam et al., 1984). Additional reported complications of spi-

rocercosis include aortic thromboembolism (Gal et al., 2005), aberrant migration to the vertebral canal (Smith and Knottenbelt, 1989; Tudury et al., 1995), spondylitis (Ramachandran et al.,1984), discospondylitis (Fox et al., 1988), myelomalacia and paraplegia (Tudury et al., 1995), sialoadenosis and salivary gland necrosis (Schroeder and Berry, 1998), secondary megaoesophagus (Matros et al., 1994; Dvir et al., 2001; Londono et al., 2003), haemopericardium (Pereira et al.,1995), oesophageal obstruction or perforation (Hamir,1986; Klainbart et al., 2007), gastro-oesophageal intussusceptions (Rallis et al., 1995), septic polyarthritis (Dvir et al., 2001), endocarditis and interstitial nephritis (Harrus et al., 1996).

Spirocercosis has generated considerable interest among pathologists and oncologists because of the hyperplastic and neoplastic changes that arise secondarily as a result of infection with this parasite (Jubb et al., 1993; Campbell, 1997). The development of esophageal osteosarcomas and fibrosarcomas has been noted in dogs infected with *Spirocerca lupi*, specifically in areas endemic to this nematode (Bailey, 1972; Moulton, 2002). Neoplasms of the canine esophagus are exceedingly rare, but canine esophageal tumors associated with *S. lupi* have been reported in the United State (Bailey, 1972), India (Parthasarathy and Ghandrasekharan, 1966; Kumar et al., 1981; Ramachandran et al., 1984), Brazil (Levine, 1980), Kenya (Wandera, 1976; Brodley et al., 1977) and Iran and Iraq (Ivoghli, 1977). In addition to dogs chronic helminthiasis is recognized as a significant factor in cancer development in human beings too (Herrera and Ostrosky-Wegman, 2001).

Esophageal lesions are the most common findings in dogs exhibiting clinical signs and the search for *S. lupi* eggs is not rewarding (Evans, 1983; Fox et al., 1988; Dvir et al., 2001). There are many stray dogs, and concomitant environmental problems in this area. Little is known about the prevalence of *S. lupi* and its pathologic changes in southern provinces of Iran. This study was undertaken, in order to find the prevalence and pathologic changes due to this nematode which has veterinary and public health importance in this area.

MATERIAL AND METHOD

A total of 105 stray dogs of both sexes (58 males and 47 females) and of different age groups (24 less

than one and 81 more than one year old) from various residential areas of Shiraz in Fars Province, southern Iran were randomly captured, sacrificed and *post mortem* examination was then carried out. The capture was conducted under permission of Fars Environment Protection Organization and the euthanasia was performed under Ethics Code of Iranian Veterinary Organization. After ligation the junction of the stomach with duodenum the esophagus and stomach were separated from other gastro-intestinal tract organs and were removed and examined for any macroscopic lesion. Tissue sections of 1 cm^3 in thickness from the lesions were fixed in 10% neutral buffered formalin. The tissues were washed and dehydrated in graded ethanol and embedded in paraffin wax and 5 μm in thickness sections were stained with hematoxylin and eosin for light microscopic examination.

RESULTS

Twenty of 105 captured stray dogs (19.04%) were infected with *S. lupi*. The prevalence of this infection was variable at different geographical localities and ranged from 36.4% in western and 28.6% in northern areas to 13.9% and 12.5% in eastern and southern areas, respectively. Nine of females (19.1%) and 11 of male dogs (19%) were infected with this spirurid. The prevalence rate was greater in dogs older than one year old (23.5%) compared to those of less than one year old dogs (4.2%).

One to six granulomas were usually found in the oesophagus and mostly the mucous membrane over the lesions was ulcerated and covered with a necrotic and fibrino-purullent membrane and fibrino-

necrotic cellular debris were present on the serosal surface of the injured esophagus. The granulomatous lesions varied in size from less than 1 cm to more than 5 cm in diameter and usually were situated at the distal portion of the thoracic esophagus and in two cases in the cardia of stomach. The esophageal nodular mass usually protruded into the lumen of the esophagus and partially obstructed their lumen. One or more irregular tracts opened the lumen of the granulomatous lesion into the esophageal lumen. The nodules not only bulged into the lumen of the esophagus but also distorted the esophageal wall and extended into the surrounding mediastinal tissues. Two to fifteen nematodes were recovered from the pocket, sinus tracts under and around of each ulcerated nodule. The males were up to 51 mm and the females were up to 75 mm long.

Histopathological examination of formaline-fixed tissues revealed extensive diffuse fibrous tissue proliferation surrounding the nematodes and their jelly impregnated eggs. The granulomas showed different pictures according to their constituents and possibly the stage of development. The newly developed granulomas showed neovascularization, hyperemia, haemorrhages, tissue necrosis and infiltration of neutrophils (Figure 1), macrophages, lymphocytes, plasma cells and eosinophils between myofibroblasts and unorganized fibrous connective tissue (Figure 2). The fibroblasts present in these early granulomatous lesions were plump and embryonal-like with many mitotic figures. The mediastinal surface was covered in patchy areas by fibrinonecrotic cellular debris, large numbers of neutrophils and few lymphocytes, plasma cells and macrophages. However, in the chronic lesions, no neutrophils were observed and very few mononu-

Figure 1. *Spirocerca lupi* in the wall of esophagus in an acute lesion. Polymorphonuclear cells with cellular debris that formed pus are seen in the right side. See the typical eggs in the left (H and E, 180×)
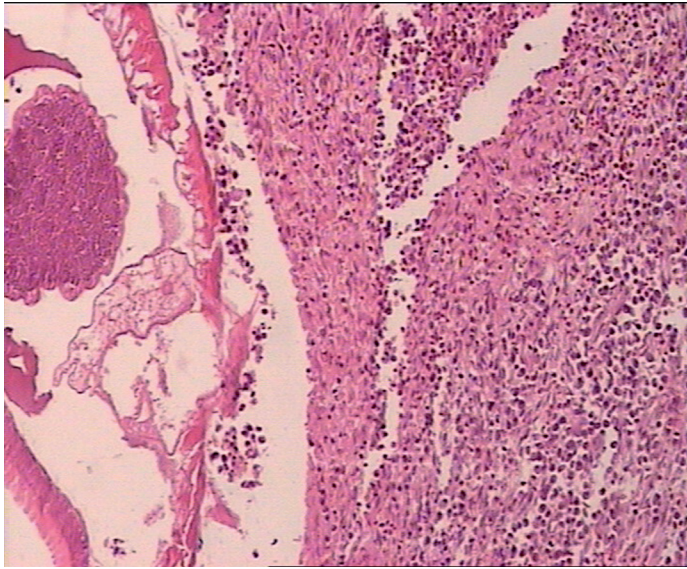
Figure 2. Many mononuclear cells are infiltrated between muscle fibers and fibrous connective tissue surrounding the nematode (H and E, 180×)
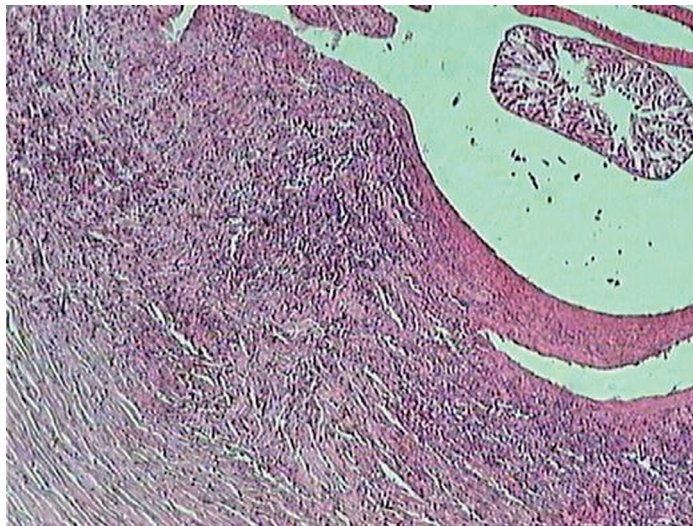

Figure 3. A thick fibrous connective tissue wall with minimal inflammatory cell infiltration between the collagen fibers in a chronic lesion (H and E, 180×)
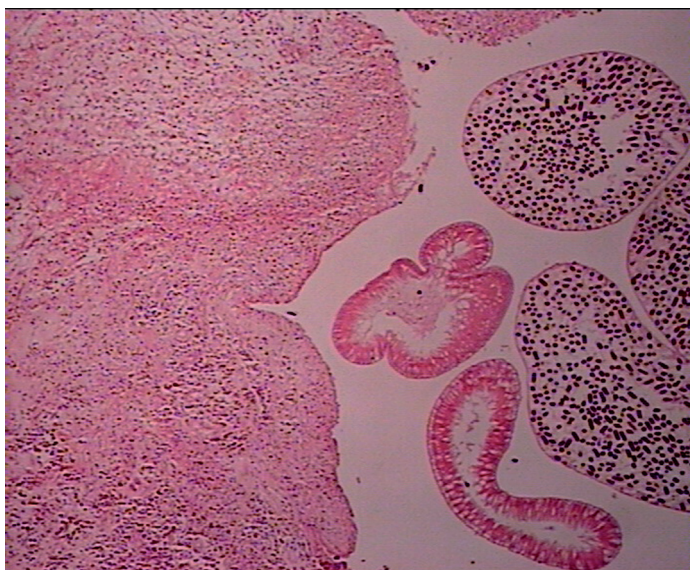

Figure 4. The eggs of the *Spirocerca lupi* are present in mucoid jelly in the diverticulum's of the granuloma in the right side. Mild infiltration of mononuclear cells is seen in the newly formed fibrous connective tissue wall. Two cross-sections of the nematode are also seen (H and E, 180×)
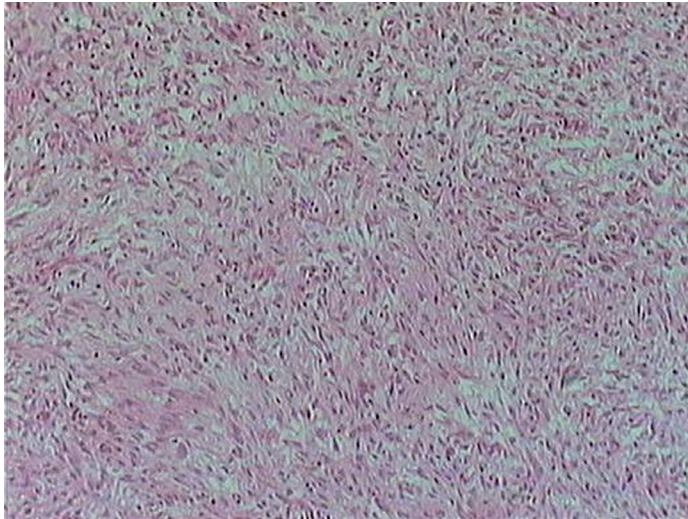
Figure 5. Section from a fibrosarcoma mass in the wall of the esophagus of a dog infected with *Spirocerca lupi* (H and E, 180×)

clear cells were seen in the lesions, and the fibrocytes and the mature connective tissue (Figure 3) that occasionally showed mineralization foci were the main constituent of these granulomas.

Mostly many eggs were present in a mucoid jelly in the diverticulum or sinus tracts (Figure 4). Two dogs developed fibrosarcoma (Figure 5), one of them with three masses of this tumor in esophagus and the metastasis were present in the ventral portion of the left lung and caudal mediastinal lymph nodes. The tumors in the esophagus were not located in direct association with the granulome but rather they were situated in their vicinity.

DISCUSSION

S. lupi is a nematode with a worldwide distribution in regions with a warm climate. The majority of reports are from Greece (Mylonakis et al., 2001), Turkey, India (Kumar, 1981; Ramachandran et al., 1984), Pakistan (Anataraman and Krishna, 1966), Israel (Mazaki-Tovi et al., 2002), the southern United States (Dixon and McCue, 1967), Brazil (Oliviera-Sequeira et al., 2002), Kenya (Brodley et al., 1977) and South Africa (Lobetti, 2000). The prevalence of Spirocercosis of dogs in these endemic regions varies from 10% to 85%. Based on necropsy results of stray dogs prevalence rates of 23.5% in India (Ramachandran et al., 1984), 3–78% in Kenya, 13% in South Africa (Minnaar et al., 2002) and 85% in Kenya (Brodley et al., 1977) is previously reported. In a survey on the incidence of intestinal helminths and protozoa in house-dogs in Teheran, Iran, *Toxocara canis, Taenia multiceps* and *Spirocerca lupi* were the most common helminths in dogs (Mirzayans et al.1972). In another study in North of Iran, a total of 65.2% of the dogs were infected with *S. lupi* (Sadighian, 1969). In the present study, high prevalence of this disease in Shiraz area, southern Iran during the course of this study suggests that an outbreak of this disease has occurred in this area. The prevalence ranged from 36.4% in western and 28.6% in northern areas to 13.9% and 12.5% in eastern and southern areas respectively. The variability in prevalence is attributed to degree of rural development, utilization of pesticides, proximity to intermediate and/or paratenic hosts, efforts at disease control and nutritional habits. High prevalence of spirocercosis in this area could also be attributed to the large number of stray dogs in this area, easy contact with Assyrian's sheep dogs and wild life carnivores and beetles. As wild carnivores population might serve as important reservoirs of the disease. Research in to this aspect greatly enhances the understanding of the complex relationship between host and parasite. Such knowledge would support useful preventive measures.

In this study, the prevalence rate was greater in dogs older than one year old (23.5%) than dogs less than a year (4.2%). After infection it takes a long time the nematode to burden the esophageal wall and initiate a granulomatous reaction and it is reported that the infected dogs under six months of age, have not developed esophageal lesions and the clinical signs (Fox et al., 1988).

There was no significant difference between males and females. This finding is comparable with other studies that showed no sex predilection for infection (Wandera, 1976; Fox et al., 1988). All the dogs' studied in this investigation were stray dogs

of local breeds and it was practically impossible to study the prevalence of disease in different breeds. However, a breed predilection is reported and it is showed that the larger breeds such as German shepherd dogs were more infected than smaller breeds (Ramachandran et al., 1984 and Dvir et al., 2001).

In this study, the nodules vary from 0.5–4.5 cm in diameter and not only bulged into the lumen of the esophagus but also distort the esophageal wall and extended into the surrounding mediastinal tissues. The lesions were of granulomatous nature and usually were situated at the distal portion of the thoracic esophagus and in the cardia of stomach. One to six granulomas each containing two to fifteen worms was located in each nodule. The adult worms found coiled within nodules in the esophageal wall. The lesion normally develops from migration of the infective larvae, continued presence of larvae and adult worms in tissue and secondary bacterial infection. Scarring and granulomatous formation with one or more sinus tracts in the thoracic esophagus and the cardia of stomach of dogs are pathognomic lesions of spirocerca infection (Jubb et al., 1993 and Donald et al., 2007). In some of the granulomatous lesions initially the worms were surrounded by highly vascularised loose connective tissue, which contained fibrin-rich fluid, neutrophils, eosinophils and foci of necrosis, hyperemia, hemorrhages, and infiltration of mononuclear cells including macrophages, lymphocytes and plasma cells between myofibroblasts and unorganized connective tissue. Some other lesions were composed mostly of actively dividing fibroblasts with an embryonal appearance, sometimes resembling granulation tissue and sarcoma.

Two dogs developed fibrosarcoma, one of them with three masses of this tumor in esophagus and the metastasis in the ventral portion of the left lung and caudal mediastinal lymph node. Esophageal sarcomas in dogs are, almost exclusively, found in areas endemic to *S. lupi* infection. The association between spirocercosis and neoplasia was first reported by Seibold et al. (1955) and then by other investigators including Bailey (1972), Johnson (1992), Moulton (2002) and Donald et al. (2007). In a report from Kenya, from the 206 dogs infected with spirocercosis 42 cases had sarcomas, that 17 of these sarcoma were fibrosarcomas and 25 of them osteosarcomas (Wandera, 1976) and from 15 cases of a retrospective study in the Hebrew University of Jerusalem nine sarcomas were diagnosed as os-

teosarcoma, five as fibrosarcoma and one as an undifferentiated sarcoma (Ranen et al., 2004). The pathogenesis of *S. lupi*-induced tumors has not been elucidated (Johnson, 1992). It is possible that the uncontrolled proliferation of the fibroblasts undergo malignant transformation. These neoplasms are true malignancies since metastasis, particularly to the lungs, is frequently noted (Bailey, 1972).

This disease has been shown to be fatal; however, proper surgical treatment could prolong the animal's lifespan for months and improve its quality of life. The typical pathological findings indicate that the dogs in this study were heavily infected with *S. lupi*, and that the development of esophageal sarcomas in these dogs was induced by this worm. This study shows the high risk of *Spirocerca lupi* infection in the stray dog population in Shiraz area. Though spirocercosis is of difficult diagnosis in stray dogs and wild canidae shouldn't be ignored and a restrict control program should be conducted by the city authorities. Education of the population on the epidemiology of the parasite should reduce the infection risk and any measures taken in this direction may also help to diminish other parasitic infections. Removing dogs' feces from the public places, control of the beetle's population and preventing dogs from ingesting beetles and paratenic hosts, periodic fecal examination and antihelminthic preventive treatment are required measures in reducing the incidence of canine spirocercosis in endemic regions.

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