

# Unraveling the enigma of *Spirocerca lupi* infection in dogs: A hidden menace

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## ABSTRACT

*Spirocerca lupi* is a nematode responsible for causing true malignancies in dogs. Esophageal nodular granulomas are pathological lesions of Spirocercosis. Spirocercosis is a disease occurring predominantly in Canidae, caused by the nematode *Spirocerca lupi*. The present case was noted in 3 years old German shepherd dog with esophageal nodular growth having neoplastic transformation following *S. lupi* infection was histologically elaborated with possible pathologic mechanism. Typical clinical signs are regurgitation, vomiting and dyspnoea. Grossly, there was presence of round nodular masses on wall of esophagus occupied almost obliterating the whole lumen of the esophagus. On section, red-colored worms were noticed in the mass and were identified as *Spirocerca lupi* by parasitological examination. The nodular masses were processed routinely and stained by Hematoxylin and Eosin (H & E) and microscopically, esophageal mass revealed numerous parasitic eggs (*Spirocerca lupi*) encircled by proliferating fibrous connective tissue with infiltration of polymorphonuclear cells, predominantly eosinophils and histopathologic lesions of other organs were recorded. Based on parasitic gross, microscopic and immunohistochemical evaluation, the case was diagnosed as Spirocercosis in the dog with acute renal failure.

**Key words:** Dog, *Spirocerca lupi*, glomerulonephritis, nodule

## INTRODUCTION

Spirocercosis is a helminthic disease caused by the nematode *Spirocerca lupi*. The infection is typically characterized by persistent nodular lesions in the oesophagus and aneurysmal changes in the aorta. These lesions may subsequently undergo neoplastic transformation, leading to sarcoma development and can also cause complications such as hemothorax, predominantly reported in dogs and various wild carnivore species<sup>1</sup>.

Aside from canids, *S. lupi* infects wild felids, goat, donkey and man by ingesting either the intermediate host (coprophagous beetle) or the paratenic hosts (i.e. reptiles, amphibians, birds and small mammals)<sup>2,3</sup>. After ingestion of infective larvae, it migrates from the stomach *via* the gastric arteries, to the aorta from where they migrate to the caudal thoracic aorta, then, adjacent oesophagus (parasite ectopic migration). In esophagus they mature to adult form and form an inflammatory fibroblastic nodule which may progress to an esophageal sarcoma<sup>4</sup>. The typical clinical signs are related to the presence of esophageal nodules which include regurgitation, vomiting, dysphagia and weight loss, together with non-specific signs like pyrexia<sup>5,6</sup>.

This study documented histopathology of esophageal tumour induced by *Spirocerca lupi* parasite along with kidney damage (glomerulonephritis).

## Clinical history

Three year old, male, German shepherd dog was presented for postmortem to the necropsy hall, Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar for postmortem with history of weakness, anorexia in the past 7 days, vomiting and sudden onset of hind leg paralysis in the past 10 days.

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## MATERIALS AND METHOD

At necropsy, the animal appeared weak, emaciated, pale mucous membrane, dehydration with rough hair coated skin. On flaying the skin, the overall appearance of visceral organs appeared markedly congested. The esophagus showed single large oval shaped firm nodule with mixed fibrous tissue texture, grown outwardly. Esophageal granuloma was located at the distal part of the esophagus about 3 cm from stomach inlet. This lesion was protruded into the lumen causing stenosis of esophagus almost obliterating the lumen. On cut section, single,

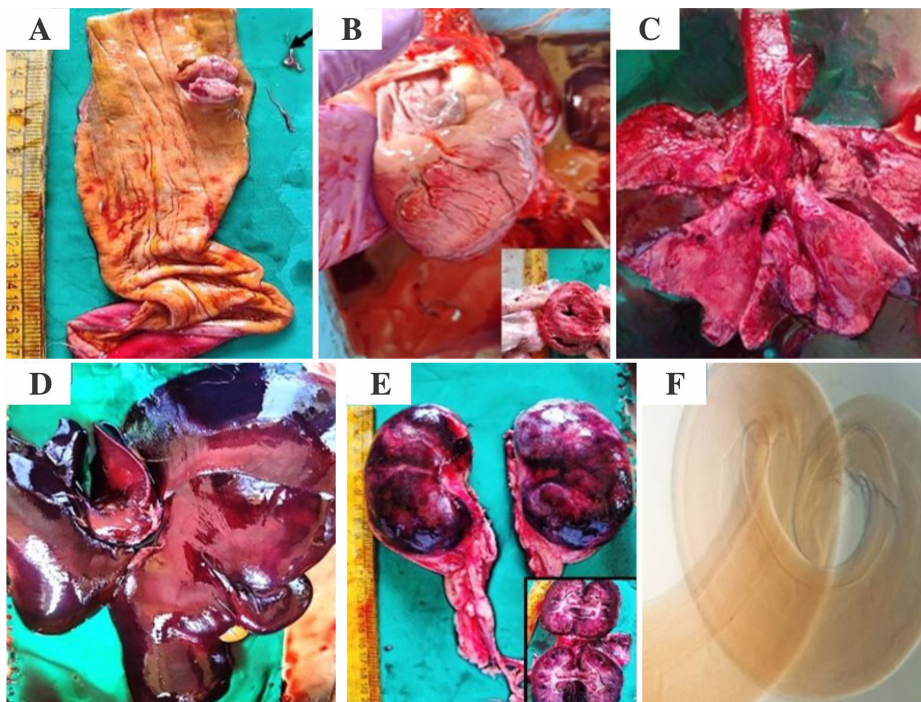
well-circumscribed, raised round to oval nodular mass projecting into the lumen and measures approximately 3-5 cm in diameter. It is pink to reddish in colour and mucosal surface over the nodule is ulcerated with firm texture and appears fibrous in consistency. A worm-like structure is visible adjacent to the lesion, suggestive of *Spirocerca lupi* infection. The worms were pulled out from the central cavity and were collected in normal saline. For morphological identification, the individual parasites were transferred to hot 70% alcohol and then to glycerol alcohol for clearing. Esophagus, lungs, heart, liver and kidneys were collected in 10% neutral buffered formalin for fixation, subsequently processed, embedded in paraffin wax and sectioned at 4-5  $\mu$  thickness. Sectioned tissues were stained with Hematoxylin and Eosin (H&E)<sup>7</sup> to demonstrate eggs in impression smear, immunohistochemical staining (IHC) for demonstrating proliferating nuclear antigen (PCNA) and Heller's method for proteinuria<sup>8</sup>.

## RESULTS

Grossly, esophages has nodular lesion occupying lumen, severe congestion of lungs, rounding of heart, severe congestion of liver, lungs and kidneys (Fig.1). On morphology, posterior end of *Spirocerca lupi* showing

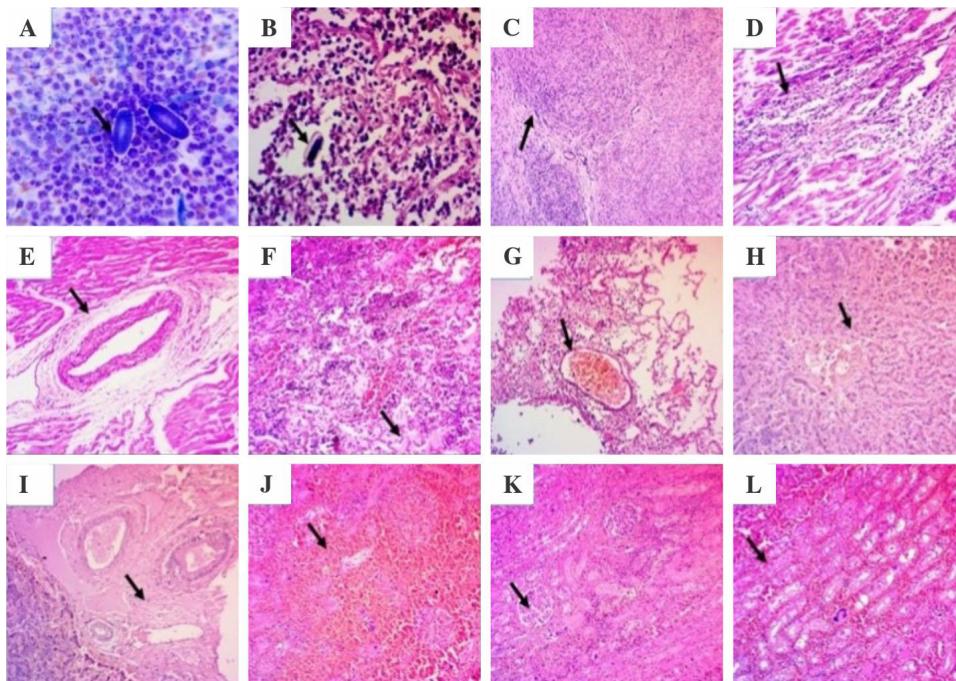
blunt and rounded, tapering slightly towards the tip and vulval slit was located near the posterior end of the body. Female parasite communicates into esophageal lumen through nodule opening (Fig.1F).

On impression smear of the esophageal nodule, the cytology shows numerous inflammatory cells, predominantly composed of neutrophils, lymphocytes and eosinophils suggestive of chronic active inflammation with elongated, oval shaped *Spirocerca* eggs (Fig.2A). Histopathological evaluation of the esophageal tissue revealed a well-developed granulomatous inflammatory response. The granulomas were characterized by concentric layers of proliferating fibrous connective tissue encapsulating parasitic ova, which were surrounded by a dense infiltrate of inflammatory cells. The inflammatory population consisted predominantly of polymorphonuclear leukocytes, chiefly eosinophils, along with lymphocytes and plasma cells, consistent with a chronic active eosinophilic granulomatous esophagitis (Fig. 2B). Within the granulomatous lesions, there was marked fibroblastic proliferation exhibiting cellular atypia. The neoplastic cells were spindle-shaped and arranged in interlacing bundles and whorled patterns. These cells demonstrated anisocytosis, anisokaryosis, hyperchromatic nuclei and frequent mitotic figures, including atypical

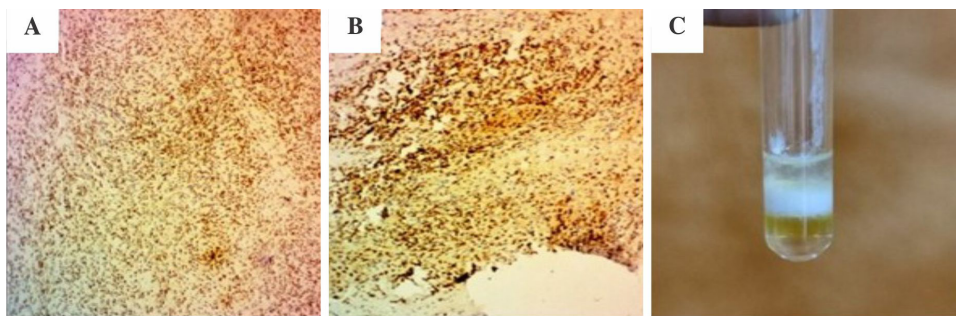


**Fig.1A.** Gross image of oesophagus showing focal nodular mass occupying oesophageal lumen in a dog. *Spirocerca lupi* worms in an opened nodule. **Arrow**-An individual Worm; **B.** Heart showing rounding due to hypertrophy of left ventricle; **C.** Lung showing edema with severe congestion; **D.** Liver showing severe congestion and rounding of borders; **E.** Kidneys showing haemorrhages and patchy areas of necrosis; **F.** Posterior end of *Spirocerca lupi* showing vulval slit. Female parasite communicates into esophageal lumen through nodule's opening.

forms, indicative of anaplasia. Such histomorphological features are consistent with fibrosarcoma (Fig. 2C). Additionally, multifocal nodular areas displayed densely cellular zones composed of closely packed spindle cells arranged in streaming fascicles, with occasional mitotic figures. The overall histomorphological architecture and cytological characteristics support a diagnosis of esophageal fibrosarcoma arising in association with chronic granulomatous inflammation. These pathomorphological changes are coincides with strong immunopositivity of PCNA marker which indicate proliferation of fibroblastic cells (Fig.3 A, B). The granulomas showed different the stage of development with newly developed granulomas showed neovascularization, hyperemia, hemorrhages, tissue necrosis and infiltration of eosinophils and



**Fig.2. (arrow)** A. Impression smear of oesophageal nodule showing *Spirocercus lupi* eggs; B. Esophageal nodule showing parasitic eggs surrounded by profused infiltration of eosinophils, MNCs and PMNs (H&E x400); C. Esophagus wall showing diffuse infiltration of fibroblast in "whorl" pattern (H&E x100); D. Heart showing degeneration of cardiac myocytes with infiltration of PMNs (H&E x100); E. Heart section showing perivascular and intermuscular fibrosis with necrosis and rupture of cardiac myocytes (H&E x100); F. Lung section showing haemorrhages, oedema with infiltration of PMNs especially eosinophils (H&E x100); G. Lung showing emphysema and distortion of alveoli, congestion of pulmonary blood vessels (H&E x100); H. Liver showing severe congestion and haemorrhages, degenerative and atrophic hepatocytes, infiltration of inflammatory cells, distorted hepatic cords with increasing in sinusoidal space (H&E x100); I. Liver showing ectasia of portal vein with congestion, oedema and perivascular fibrosis of portal traid (H&E x100); J&K. Section of kidneys showing severe necrosis, haemorrhages and presence of hylane cast in degenerating tubules (H&E x100); L. Section of kidney showing necrosis of tubular epithelium with degenerated tubules (H&E x100).



**Fig. 3. A-C.** Severe nuclear immunopositive reaction with PCNA marker (IHC x100); C. Urine sample positive for protein in Heller's method as white ring at the junction.

neutrophils present around the eggs. Further, lymphocytes along myofibroblasts are proliferation occurs along with unorganized fibrous connective tissue.

Heart showed severe infiltration of inflammatory cells with degeneration and necrosis of cardiac myocytes along with perivascular and intermuscular fibrosis (Fig.2E), necrosis and rupture of cardiac myocytes (Fig.2D,E). Where as microscopic picture of lung showing haemorrhages, congestion of pulmonary blood vessels,

oedema with infiltration of PMC's especially eosinophils with emphysema and distortion of alveoli (Fig.2F,G). Liver showing extensive congestion and haemorrhages, degenerative and atrophic hepatocytes, mild to moderate infiltration of inflammatory cells, distorted hepatic cords with increasing in sinusoidal space with ectasia of portal vein with congestion, oedema and mild fibrosis of portal traid (Fig.2H,I).

Microscopic picture of kidney showed severe coagulative necrosis of tubular epithelium along with vacular degeneration with infiltration of inflammatory cells, hylane cast in tubules (Fig.2J-L).

To assess kidney function, we went for Heller's method and observed formation of white ring at the junction indicated presence of protein (protenuria) (Fig.3C).

## DISCUSSION

We report here a case of esophageal tumor due to Spirocercosis in dog with acute renal failure. The occurrence of acute renal failure (acute kidney injury, AKI) is most likely a secondary complication rather than a direct effect of the parasite. Chronic esophageal tumouration can result in dehydration and hypovolemia due to dysphagia, vomiting, regurgitation, anorexia and reduced water intake, ultimately leading to decreased renal perfusion and ischemic acute tubular necrosis. Spirocercosis in dogs were reportedly found to be associated with pulmonary lesions due to perforation of parasite laden esophageal

growth/ tumor along with acute renal failure due to parasite visceral larval migration. The tumor developed in esophagus showed a tendency to undergo metaplastic changes towards osteoma and myxomatous growth<sup>9</sup>.

The molecular pathogenesis of neoplasia associated with *Spirocerca lupi* infection is believed to involve chronic inflammation induced oxidative stress, persistent fibroblast stimulation, and dysregulation of tumor suppressor genes and oncogenes (including altered p53 expression), leading to genomic instability, uncontrolled cellular proliferation and malignant transformation of esophageal mesenchymal cells. The diagnosis of *S. lupi* is not straight forward compared to other nematodes. Diagnostic technique is hampered by the short and unpredictable period of oviposition, relative ineffective direct fecal preparations and routine flotation and the maturity of the parasite where female inside the nodule produced a passage in the esophageal lumen<sup>10</sup>. Therefore, effective clinical diagnosis is required to diagnosis infection. The condition occurs due to acquiring the infective stage third larvae (L3) by eating either small bird, lizards and dung beetles<sup>3</sup>. After ingestion, L3 enters the body by penetrating the gastric wall and migrates through gastric, gastroepiploic and celiac arteries reaching the caudal thoracic aorta, where they moult into L4<sup>4</sup>. This results in outpouching aneurysm of the thoracic aorta adjacent to the affected esophagus. Later on, the immature adults migrate from aorta to the caudal esophagus and form nodules at the tunica submucosa and adventitia. When the parasite matures, it creates an opening into esophageal lumen where eggs normally pass and forms nodular structure<sup>9</sup>. The present study identified significant sequelae of Spirocercosis, including fibrosarcoma and acute renal failure. Chronic and longstanding infection with *Spirocerca lupi* is well recognized to predispose affected animals to neoplastic transformation, most commonly resulting in sarcoma formation. Among these, fibrosarcoma is the predominant tumour type associated with *S. lupi* infection. Fibrosarcoma is a malignant mesenchymal neoplasm arising from fibroblasts of fibrous connective tissue and is characterized by invasive growth and variable metastatic potential<sup>4</sup>.

The probable cause of a death in the present case is as a result of acute renal failure and cardiomyopathy because of degeneration, necrosis of cardiomyocytes in addition to pneumonia leading to generalized toxemic conditions. Other associated pathological changes that contributed in the death of the animal was serious systemic circulatory disturbance which was due to esophageal tumor resulted in vascular constrictions besides acute renal failure. Despite the pathologic effect of spirocercosis, the parasite is often neglected and underestimated by many veterinary practitioners and

researchers. Nevertheless, *S. lupi* prevalence worldwide ranges from 10% to 85% where reports are common in tropics and subtropics.

## CONCLUSION

The present report should stimulate awareness to all veterinarians in the country about Spirocercosis. It can be regarded as one of the important parasitic infections in canines that usually unnoticed until death and thus act as a silent killer. Spirocercosis should be differentiated from other disease diagnosis while dealing with cases showing clinical signs of vomiting or regurgitation, dyspnoea, persistent coughing in dogs greater than 6 months old. During routine deworming, the anthelmintic effective against Spirocercosis should be used.

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