# FINE NEEDLE ASPIRATION BIOPSY FROM OESOPHAGEAL NODULES IN A DOG

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Veterinary Clinical Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>VCP-09-1372.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>What Is Your Diagnosis?</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td></td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>De Lorenzi, Davide; Veterinary Clinic San Marco Furlanello, Tommaso; Veterinary Clinic San Marco</td>
</tr>
<tr>
<td>Key Words:</td>
<td>cytology, Spirocercosis, Fine needle biopsy</td>
</tr>
</tbody>
</table>
What is your diagnosis?

FINE NEEDLE ASPIRATION BIOPSY FROM OESOPHAGEAL NODULES IN A DOG

Davide De Lorenzi¹, Tommaso Furlanello²

1) “San Marco” Private Veterinary Clinic, Padova, Italy

2) “San Marco” Private Veterinary Laboratory, Padova, Italy

Reprint requests: Davide De Lorenzi, DVM, DECVCP, “San Marco” Private Veterinary Clinic, via Sorio 114/C – 35141 Padova, Italy; e-mail: davide.delorenzi@fastwebnet.it
Case Presentation

A 15-month-old intact female mixed breed dog had been referred because of progressive cough and weakness in the last two weeks. The dog had been recently rescued in Mauritius Islands (southwest Indian Ocean). At the physical examination the dog presented dyspneic with a distended abdomen. A multidetector computer tomography (LightSpeed 16, GE Healthcare, Milwaukee, WI) showed a large (10 cm diameter) parietal oesophageal cystic mass, deep venous thrombosis and abdominal effusion.

Endoscopic findings included a locally narrowed oesophagus with two small, flat, pinkish nodules (0.5 and 1 cm) and a larger, smooth (2 cm) pedunculated nodule, all localized in the distal oesophageal tract, between heart and lower oesophageal sphincter. Several fine needles biopsies were performed from the two smaller nodules by using a Wang needle inserted in the working channel of the endoscope. Some attempts to collect tissue specimens for histopathologic evaluation only gave mucoid and bloody material that was considered unsuitable for formalin fixation process. All the samples were air-dried and stained with May-Grünwald-Giemsa (MGG) stain in automatic slide stainer (Figure 1).

Cytologic Interpretation

Six smears were examined. The sample was markedly cellular and contained mainly predominantly nondegenerate neutrophils and activated macrophages intermixed with a small amount of mucus and amorphous debris. Scattered throughout the smears were small (30 x 15 μm) oval egg-like structures with a thick capsule (Figure 1) and a smooth surface or with a surface containing a longitudinal fold on a different focus plan (Figure 2 a, b); many of these structures were partially or totally filled with dark blue or purple circumvoluted filamentous material (Figure 2 c) in contrast with others that appeared totally filled with homogeneous dark blue material (Figure 2 d). The described structures were interpreted as eggs.
On the basis of imaging, endoscopic and cytologic findings the final diagnosis was pyogranulomatous inflammation due to *Spirocerca lupi* infestation.

**Additional test results**

The esophageal cystic mass was drained by minimally invasive, thoracoscopic technique and the patient was treated with doramectin (Dectomax®, Pfizer Italia) at the dose of 500 µg/kg injected subcutaneously and repeated after 7 days.

Three weeks after the second injection thoracic radiogram showed normal pulmonary fields with no mass or other pathological findings as the owner reported a full recovery from disease. A control endoscopy was declined by the owner.

**Discussion**

Spirocercosis is a disease caused by the nematode *Spirocerca lupi* which has a variety of clinical presentations and regurgitation and vomit are common. This nematode has a worldwide distribution in regions with a warm climates and has been found in many species, affecting mostly carnivores, especially Canidae\(^1\); in the present case the parasitic infections had been likely acquired in Mauritius Islands, that is a short distance to Reunion Island, where spirocercosis is thought to be endemic.

The life-cycle involves an intermediate (coprophagous beetle) and a variety of paratenic hosts (including poultry, wild birds, lizards and rabbits). Larvae follow a specific migratory route, penetrating the gastric mucosa of the host, migrating along arteries, maturing in the thoracic aorta to L4 stage before eventually moving to caudal oesophagus where they mature to adults over the next three month. Here the worm lives in esophageal nodules and passes larvated eggs\(^2\).

Usually there are one to four worm-containing nodules in the sub-mucosa of the wall of the oesophagus, a few centimetres cranial to the diaphragm\(^3\). The nodules vary from <1 to >4 cm in
diameter and not only bulge into the lumen of the oesophagus but also distort the oesophageal wall and extend into the surrounding mediastinal tissues.

A presumptive diagnosis of spirocercosis is largely dependant upon thoracic radiography, which demonstrates the caudal oesophageal soft tissue masses, caudal thoracic vertebral spondylitis and aortic undulation due to aneurysm formation but oesophageal endoscopy has a greater diagnostic sensitivity than radiography, showing one to several nodule that may vary in shape and size, depending on the progression of the disease.

A definitive diagnosis can be made by demonstrating the characteristic small (11-15 x 30-37 \( \mu \)m), elongated eggs that contain larvae in the feces, but several factors can affect fecal egg detection. Eggs will only be present in feces when the female worm has a patent passage to the esophageal lumen and for this reason the maturation of the nodule is essential. Passage of eggs occurs for a relatively short period in the lifespan of the worm. Eggs are often difficult to detect both in direct fecal preparations and routine fecal flotation devices using sugar and salt solutions; for this reason special laboratory techniques are needed.

While improved diagnostic sensitivity has been shown using sodium nitrate solution with a specific gravity of 1.36 or supersaturated 33% zinc sulphate to concentrate the eggs, several fecal floats need may need to be done to detect the eggs.

To the authors’ knowledge this is the first description of *Spirocerca lupi* eggs in air dried smears stained with a Romanowsky stain and shows a possible alternative to definitive diagnosis of spirocercosis by identifying eggs in fine needle biopsy from oesophageal nodules when fecal flotation is not diagnostic.

Eggs in unstained fecal flotation samples have a monomorphic appearance while in MGG stained smears they show different characteristics, probably due to different maturation stages of the eggs and potentially drying and staining artefacts. Working knowledge of the variation in the morphologic features of the *Spirocerca lupi* eggs can help cytologist accurately diagnose these cases.
The oesophageal nodule may undergo malignant neoplastic transformation and the association between *Spirocerca lupi* infection with oesophageal sarcoma is well known since 1955. While evaluation for malignant transformation can be performed with endoscopic biopsy and histopathologic evaluation, the histopathologic results of endoscopic biopsies are often non-diagnostic. In early nodules, the intact stratified squamous epithelium of the oesophageal mucosa resists biopsy efforts. In the larger, more cauliflower-like lesions, where the possibility of neoplastic transformation may alter treatment strategies, the biopsy often does not include diagnostic tissue; from this point of view endoscopic guided fine needle aspiration biopsy could differentiate between malignant neoplasm-bearing cases and benign cases by collecting cells from deeper tissue, assisting the clinician in diagnosis, treatment and prognostication.

In summary, cytologic identification of *Spirocerca lupi* eggs from fine needle biopsy from oesophageal nodules could aid in more easily confirming a clinical suspect of spirocercosis.

If further studies will prove our findings, endoscopic fine-needle biopsy can be used with fecal flotation to improve diagnostic accuracy in presence of *Spirocerca lupi* infestation.
REFERENCES


Figures

Figure 1- Direct smear from fine needle aspiration of an oesophageal nodule in a 15 month old mongrel dog. May-Grüenwald-Giemsa; bar: 20 µm

Figure 2: *Spirocerca lupi* egg (a): on a different focus plan (b) is well evident a longitudinal fold on eggshell surface; (c, d) different morphologic aspects in stained samples (see text) May-Grüenwald-Giems; bar 20 µm
Figure 1- Direct smear from fine needle aspiration of an oesophageal nodule in a 15 month old mongrel dog.
May-Grüenwald-Giemsa; bar: 20 µm

174x131mm (150 x 150 DPI)
Figure 2: Spirocerca lupi egg (a): on a different focus plan (b) is well evident a longitudinal fold on eggshell surface; (c, d) different morphologic aspects in stained samples (see text) May-Grüenwald-Giemsa; bar 20 µm

138x164mm (150 x 150 DPI)