

## Gastric mass aspirate from a young cat

F. Ibba <sup>1</sup>, G. Cancedda <sup>2</sup>, E. Lepri <sup>3</sup>, S. Paltrinieri <sup>4</sup>, D. Traversa <sup>5</sup>

<sup>1</sup> DVM, Capoterra, Italy

<sup>2</sup> DVM, Carbonia, Italy

<sup>3</sup> DVM, PhD, Dipl. ECVP, Dipartimento di Scienze Biopatologiche ed Igiene delle Produzioni Animali ed Alimentari Sezione Patologia e Igiene Veterinaria, Università di Perugia, Italy

<sup>4</sup> DVM, PhD, Dipl. ECVP, Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria Sezione di Patologia Generale Veterinaria e Parassitologia, Università di Milano, Italy

<sup>5</sup> DVM, PhD, Dipl. EVPC, Dipartimento di Scienze Biomediche Comparete, Università di Teramo, Italy

**Signalment:** domestic shorthair, male, young (probably 1 or 2 years old)

**History:** Unknown history. The cat was found hit by a car. The cat was very thin, dirty and had a poor hair coat.

**Clinical findings:** The cat mainly presented neurological signs probably of traumatic origin (anisocoria, stupor, seizure) and several wounds in the facial region. There was a palpable mass in the cranial abdomen. An ultrasound examination was performed to rule out thoracic and abdominal bleeding. During ultrasound analysis the mass revealed a solid texture with no cavitation or fluid accumulation. The mass was grossly spherical and involved the gastric wall, with disruption of gastric wall layers. An ultrasound-guided fine needle biopsy of the mass was performed. The cytologic sample was stained with Diff Quick stain (Fig. 1, 2, 3, 4, 5, 6, 7).

**Cytological examination:** The sample was moderately cellular and had a finely granular necrotic background (Fig. 1). A mixed population of stromal mesenchymal cells and inflammatory cells was present (Fig. 2, 4, 5). The inflammatory population was mainly composed by eosinophils (Fig. 5) and degenerated neutrophils with occasional mast cells (Fig. 6), lymphocytes, plasma cells and macrophages. Some neutrophils contained intracytoplasmic round coccoid bacteria about 2 µm in diameter. The stromal mesenchymal cells were large (about 20-30 µm in diameter) with moderate anisocytosis and showed several features of atypia as anisokariosis, multiple irregularly shaped and sized nucleoli and vacuolized cytoplasm (Fig. 3, 4, 5, 7 and 8).

This cytological pattern was consistent with the diagnosis of eosinophilic inflammation with severe reactive fibroplasia, although a mesenchymal neoplasia could not be completely ruled out on a cytological basis alone.

An additional cytologic sampling was performed immediately after euthanasia. Only ovoid structures resembling nematode eggs were found. The eggs were ovoid with clear thick refringent capsule, and some of them contained small basophilic ovoid nuclei (embryonated eggs) (Fig. 9).

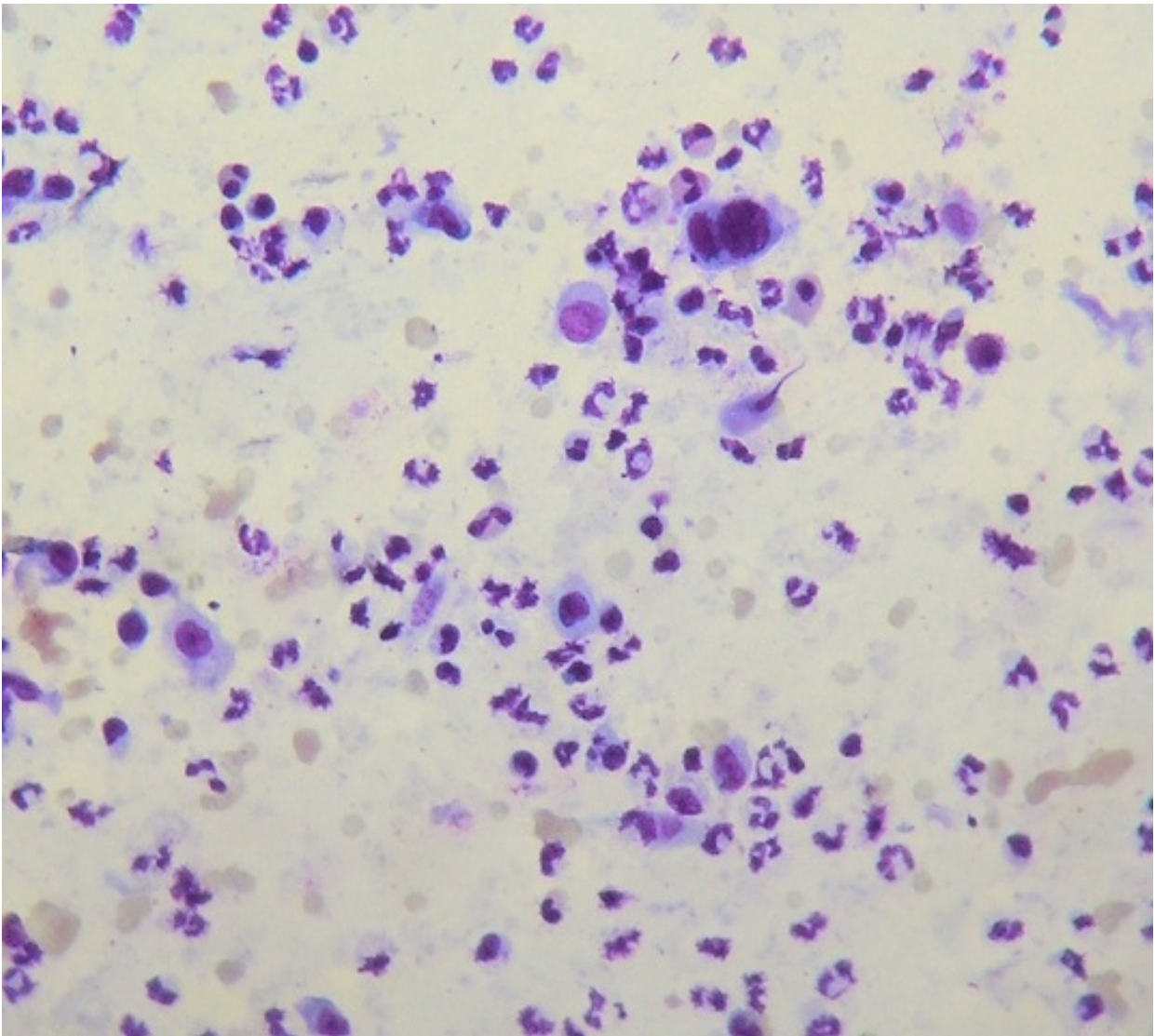


Fig. 1 Overall cytologic appearance of the gastric mass

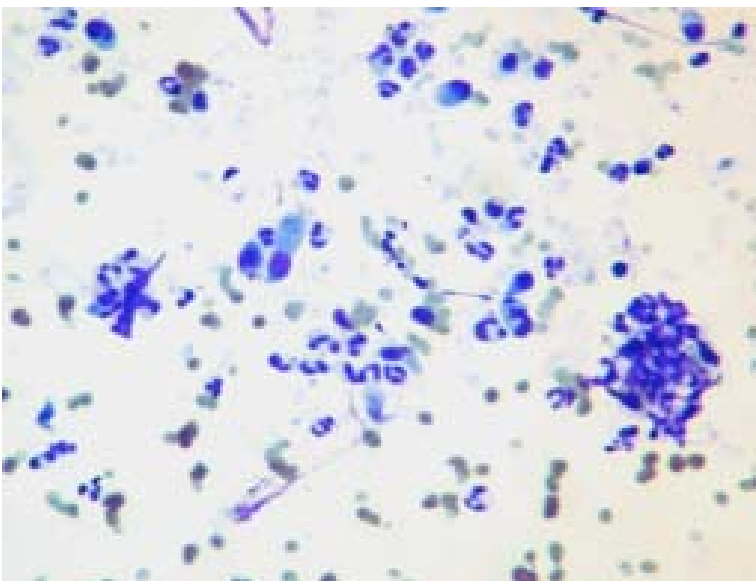


Fig. 2 Mixed population of inflammatory cells

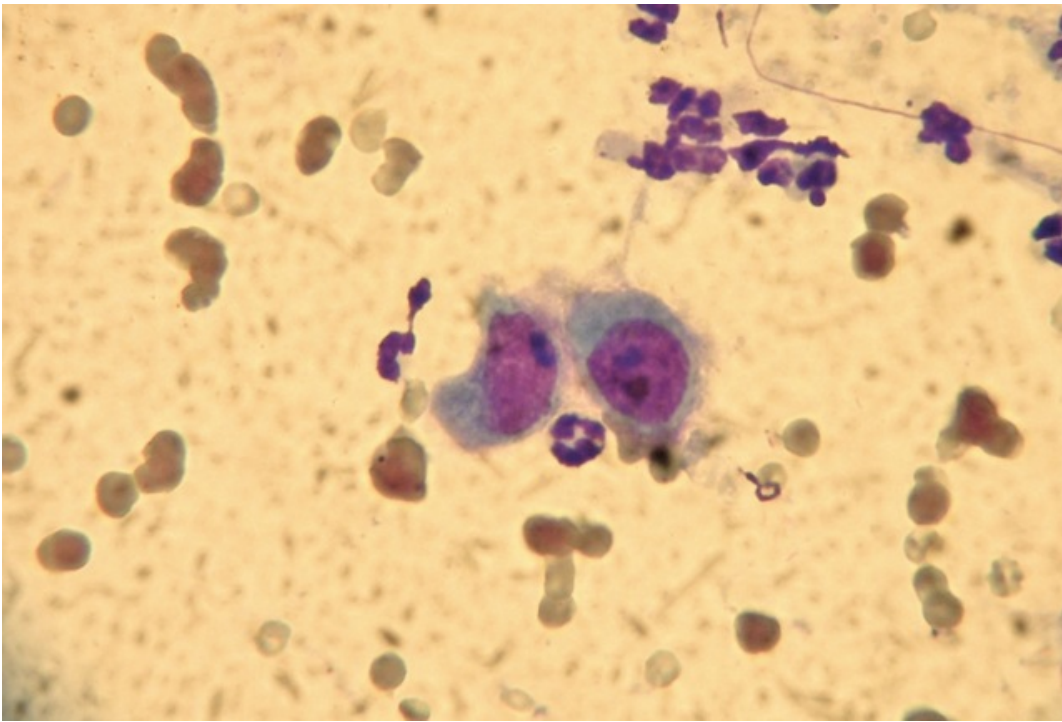


Fig. 3 Degenerated neutrophils with spindle cells

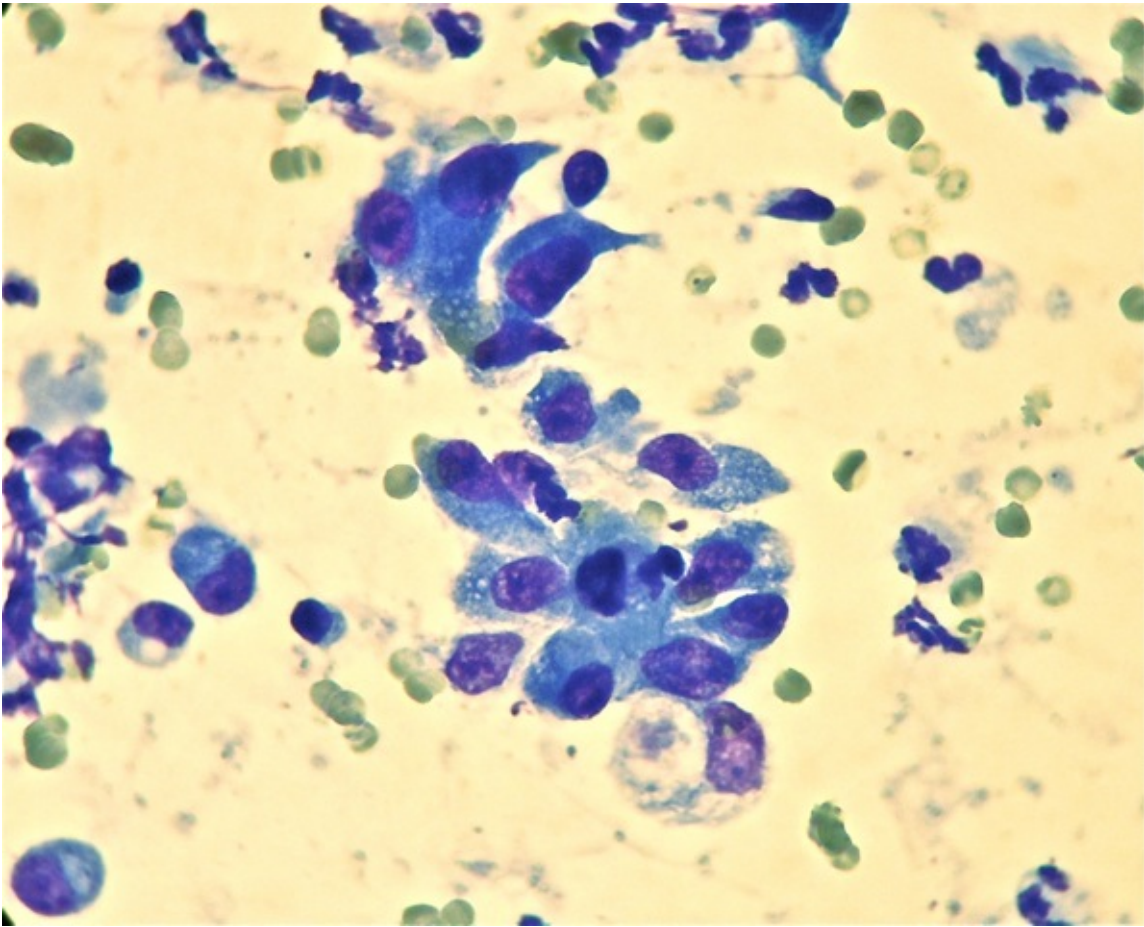


Fig. 4 Mixed population of stromal mesenchymal cells and inflammatory cells



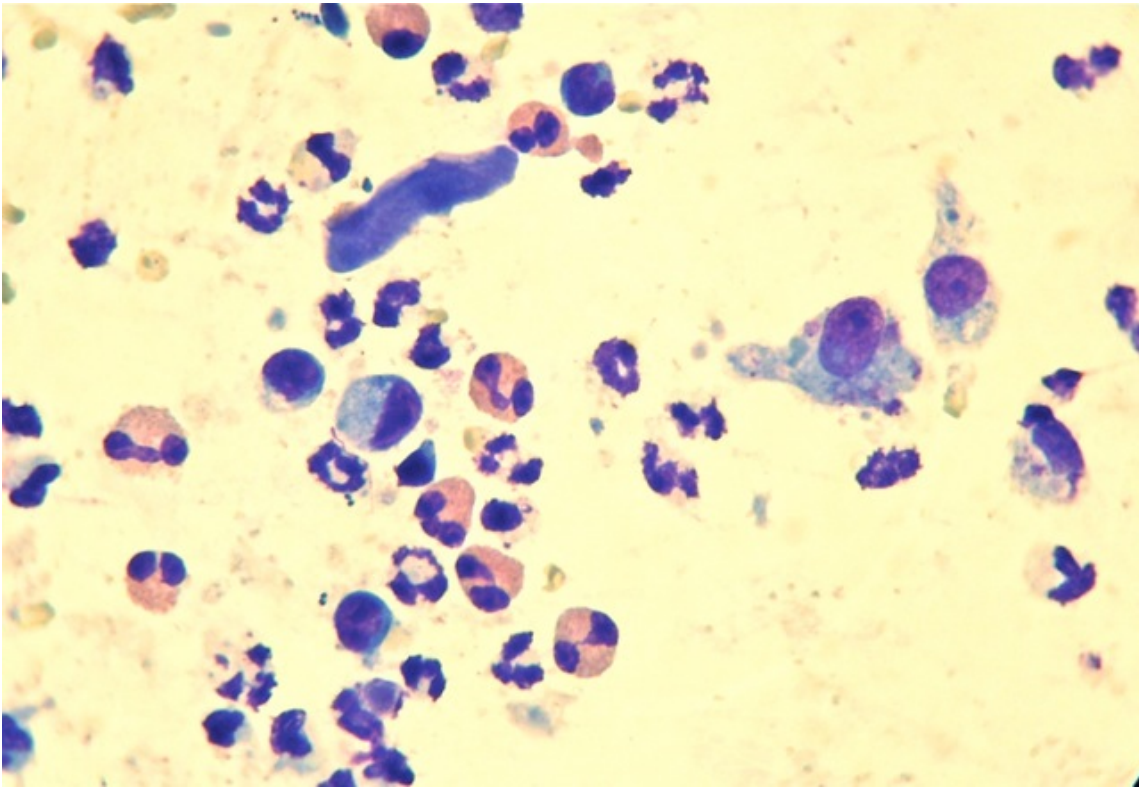


Fig. 5 The eosinophilic population was predominant.

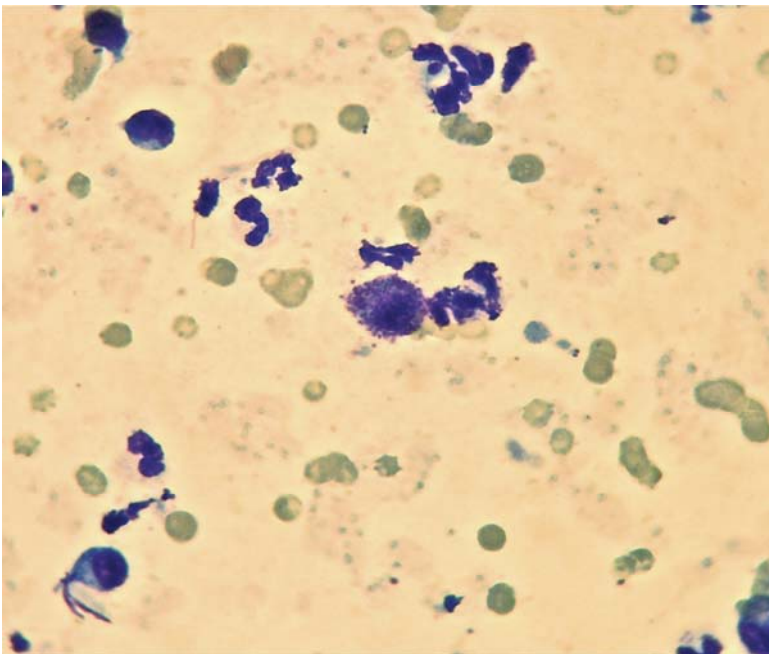


Fig. 6 Occasional mast cells were present

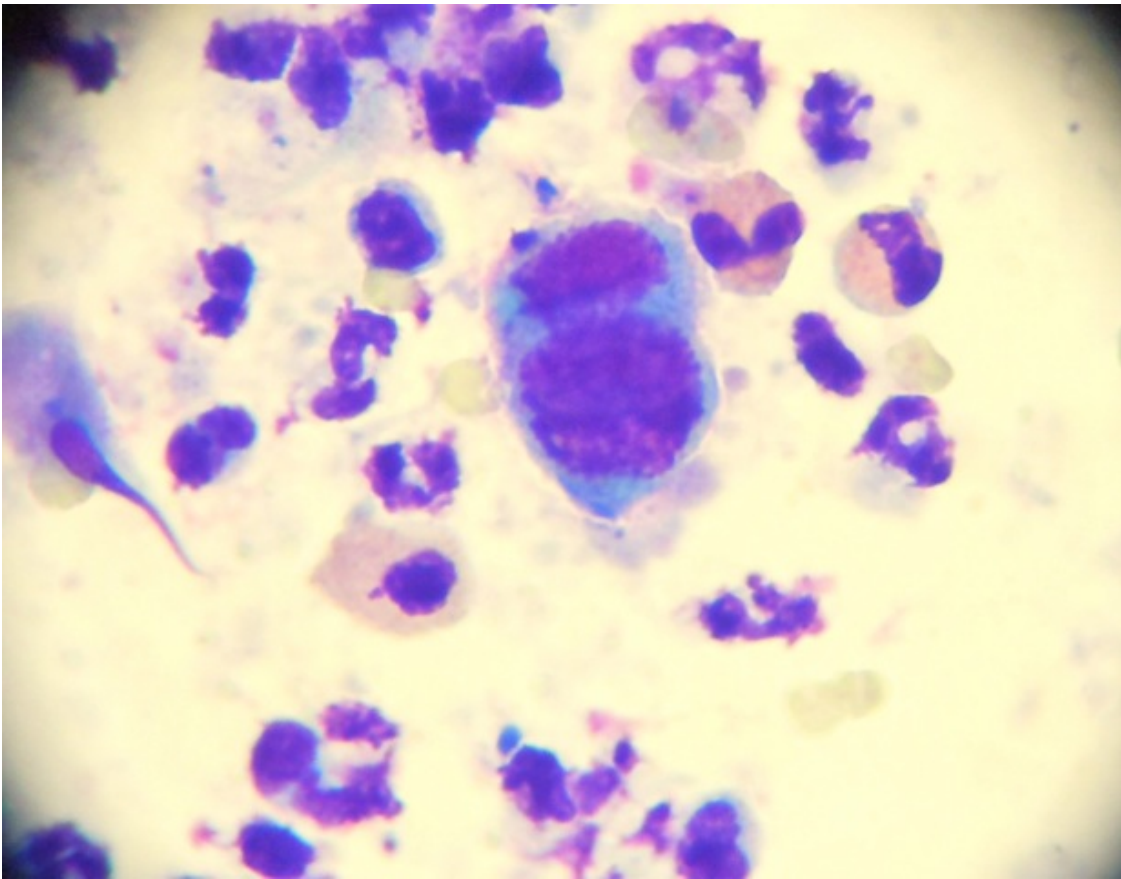


Fig. 7 Mixed inflammatory pattern with atypical spindle cells

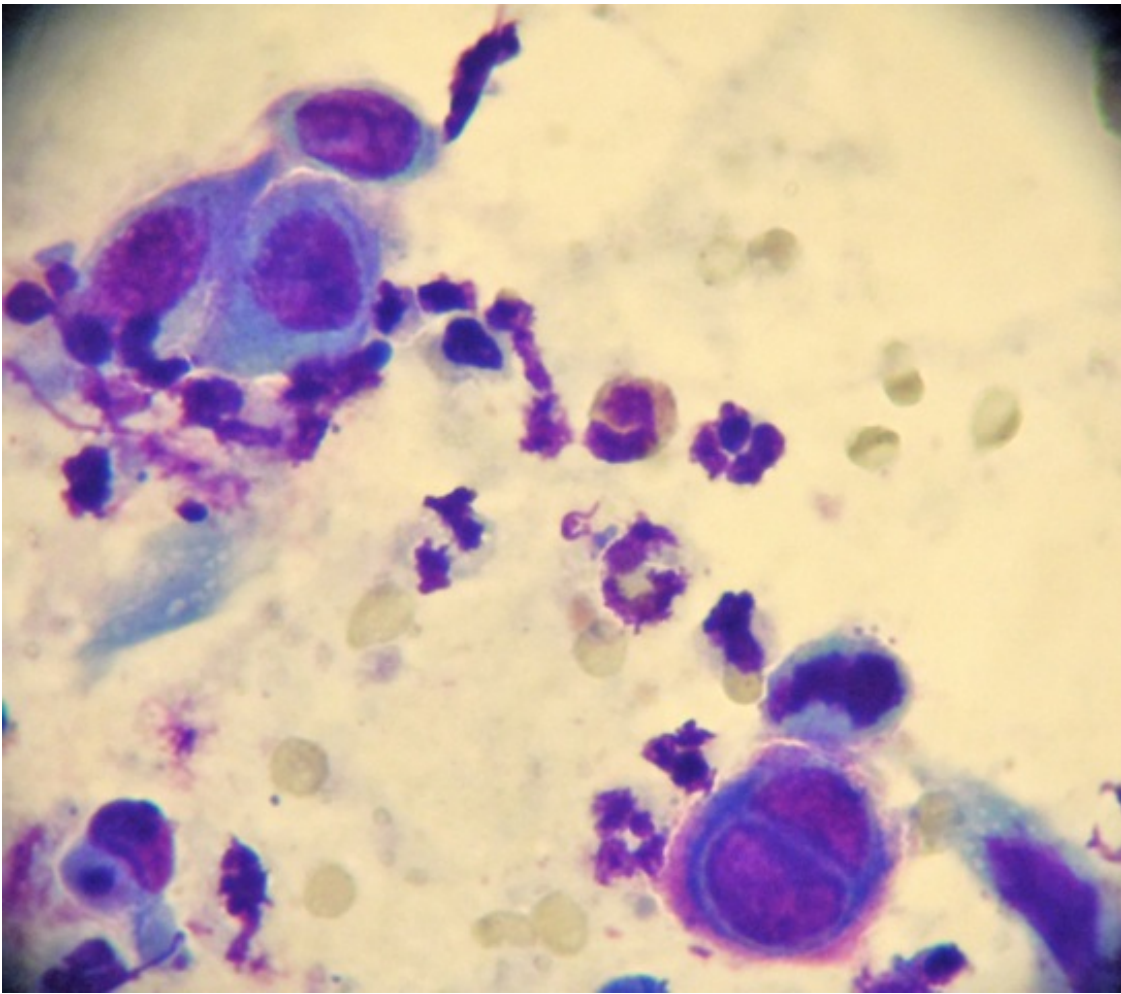


Fig. 8 Mixed inflammatory pattern with spindle cells showing moderate to severe atypia

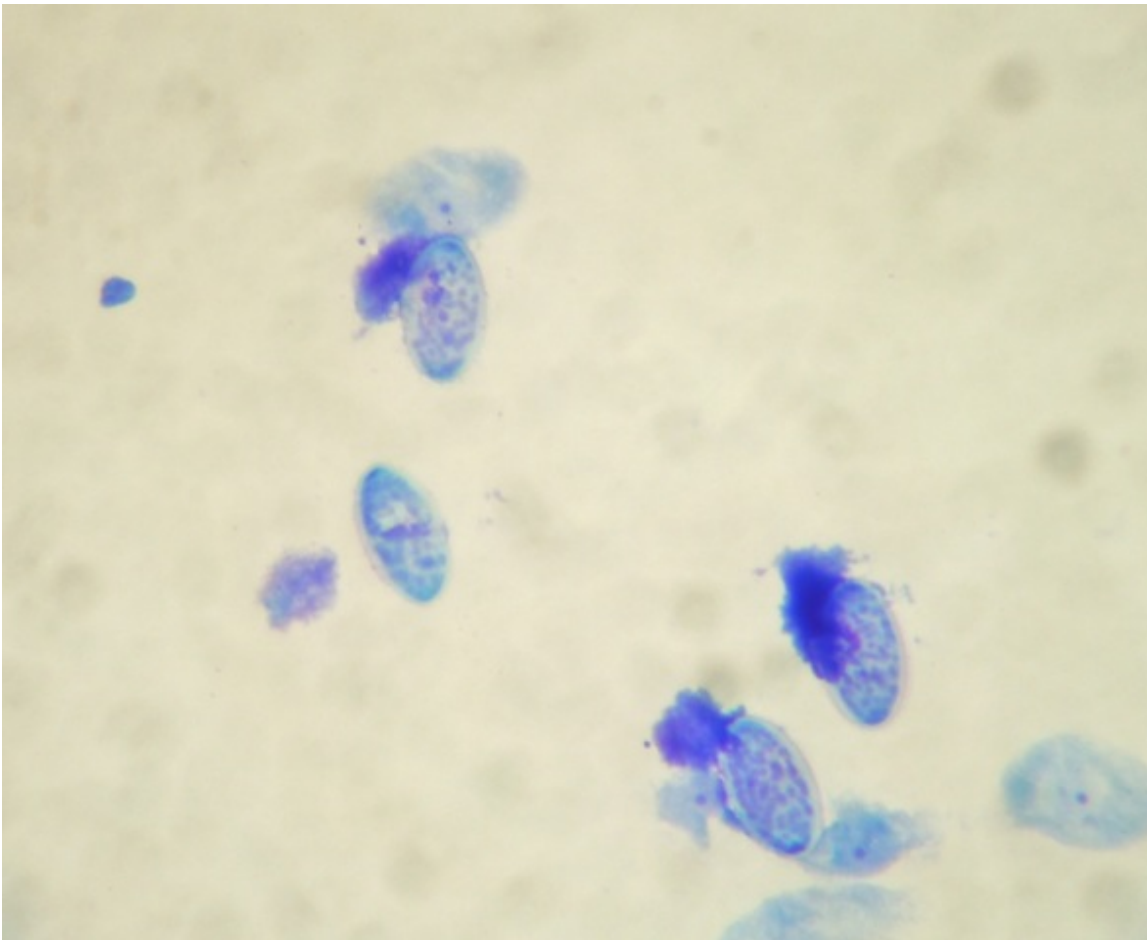


Fig. 9 Ovoid elements resembling nematode eggs

**Necropsy findings:** A spherical, well defined, nodule (3x2 cm) was excised from the gastric wall. No signs of peritoneal involvement were observed. The overlying gastric mucosa was intact, except for a small fissure 2 mm in length (Fig. 10); On the cut surface the nodule was smooth, white-yellow, and contained some reddish nematodes (Fig. 11).





Fig. 10 Mucosal surface of the mass



Fig. 11 Cut surface of the mass

Detailed pictures of a single worm extracted from the mass are showed in Fig. 12 (gross appearance of the nematode), 13 and 14 (microscopical details).



Fig. 12 Gross appearance of single nematode extracted from the mass



Fig. 13 Cephalic end of one worm extracted from the gastric mass



Fig. 14 Spiculated caudal end of the same worm

### **Histopathology:**

Histologically the nodule was composed by interlacing boundless of mature dense collagen interspersed with perivascular to coalescing accumulation of inflammatory cells in different proportions (Fig. 15, 16). Some aggregates were composed mainly by lymphoplasmacytic cells (Fig. 17), while in other there was a prevalence of eosinophils (Fig. 18) or macrophages with large clear foamy cytoplasm and oval peripheral nuclei, with scattered neutrophils (Fig. 19). The inflammatory infiltration was centered around several sections of adult nematode with thick hyaline cuticle, intestine and uterus filled with myriads of oval eggs, morphologically consistent with those observed in cytological specimens (Fig. 20). Occasionally scattered necrotic areas with eosinophilic amorphous material (eosinophilic granules) were present, probably around dead degenerated nematodes (Fig. 21). The inflammatory infiltrate was embedded in a well differentiated mature collagen stroma with cords and whorls of spindle cells showing moderate atypia, consistent with ongoing fibrotic disease (Fig 22-23). Scattered spindled mast cells were present (Fig. 24).

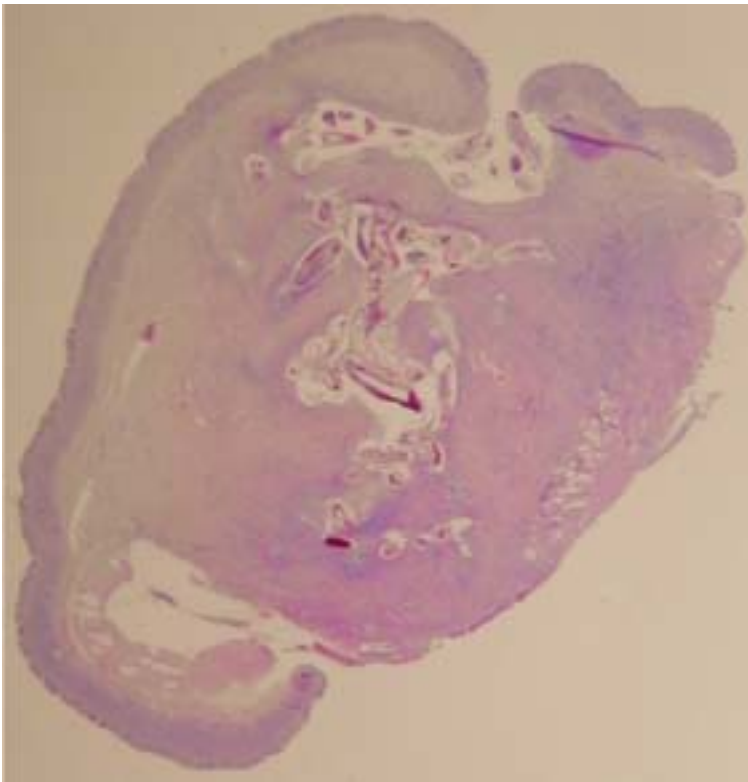


Fig.15 Distinct well defined nodule with mucosal opening and several sections of adult nematodes; Hematoxylin and eosin (HE), 1x

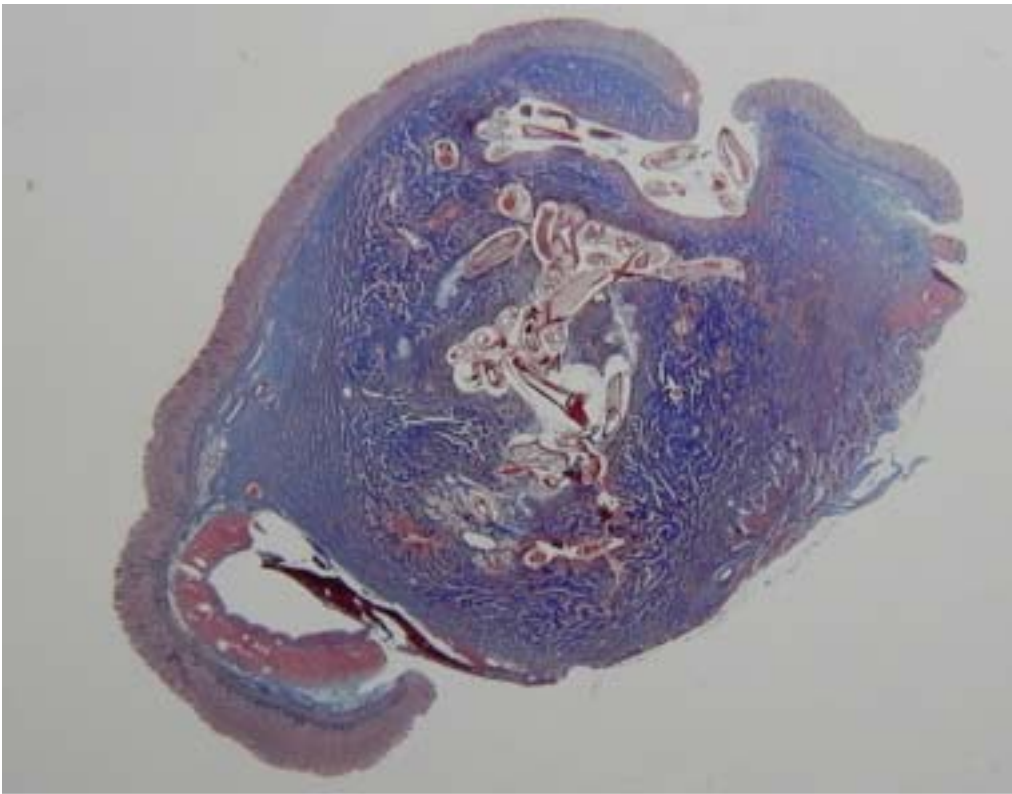


Fig. 16 The nodule is mainly composed by mature collagen tissue; Masson trichrome, 1x

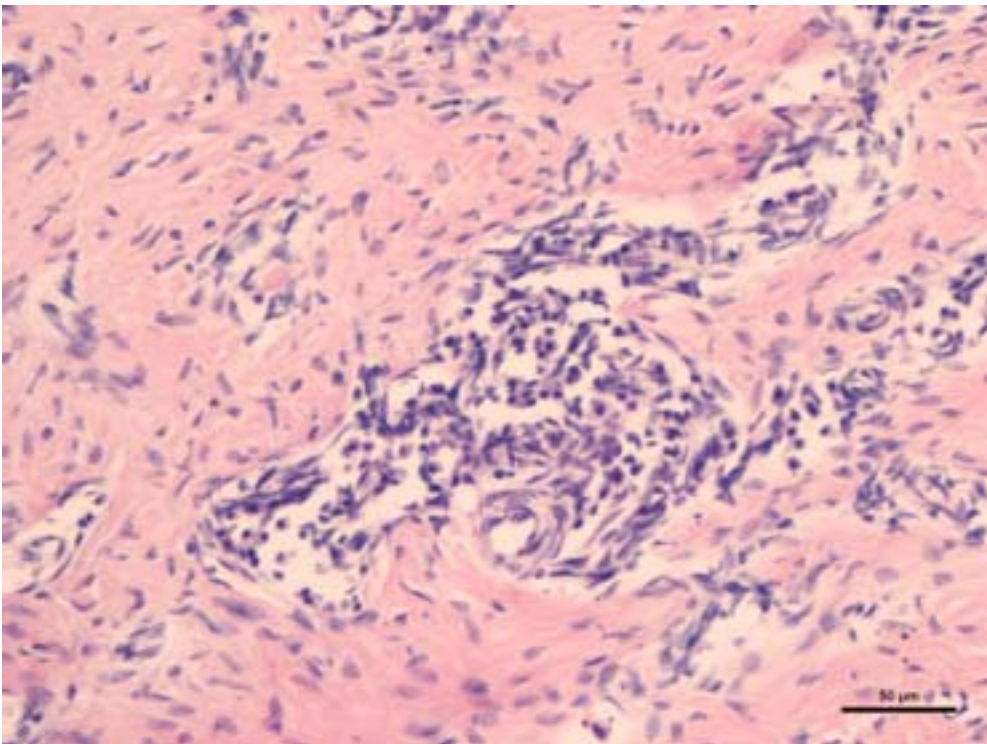


Fig. 17 Lymphoplasmacytic aggregates, HE stain



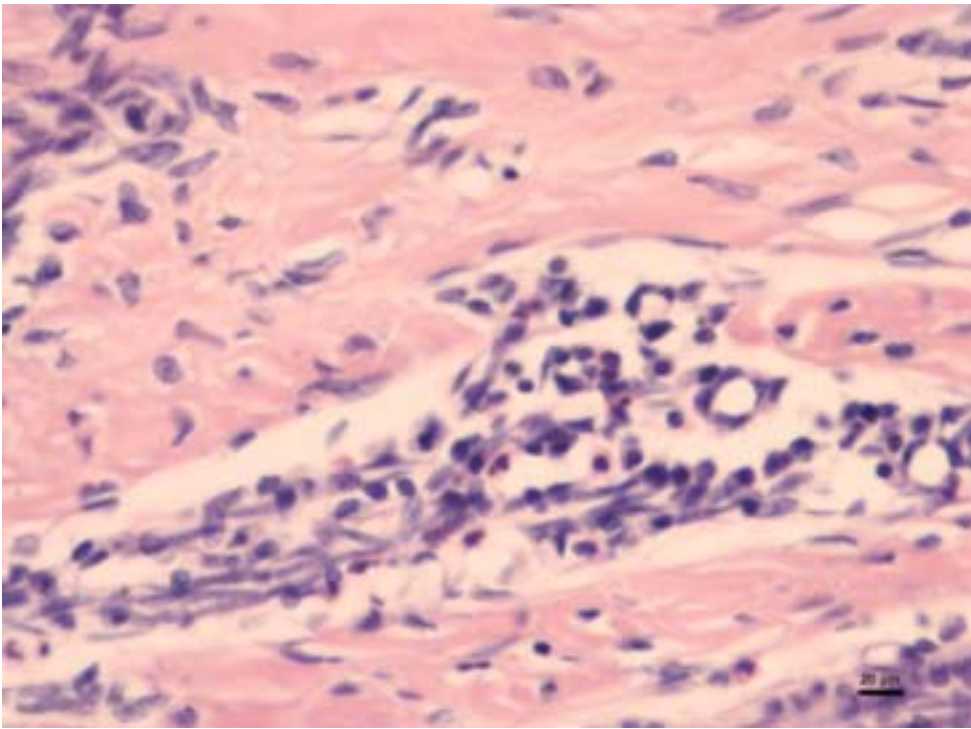


Fig. 18 Eosinophilic infiltration, HE stain

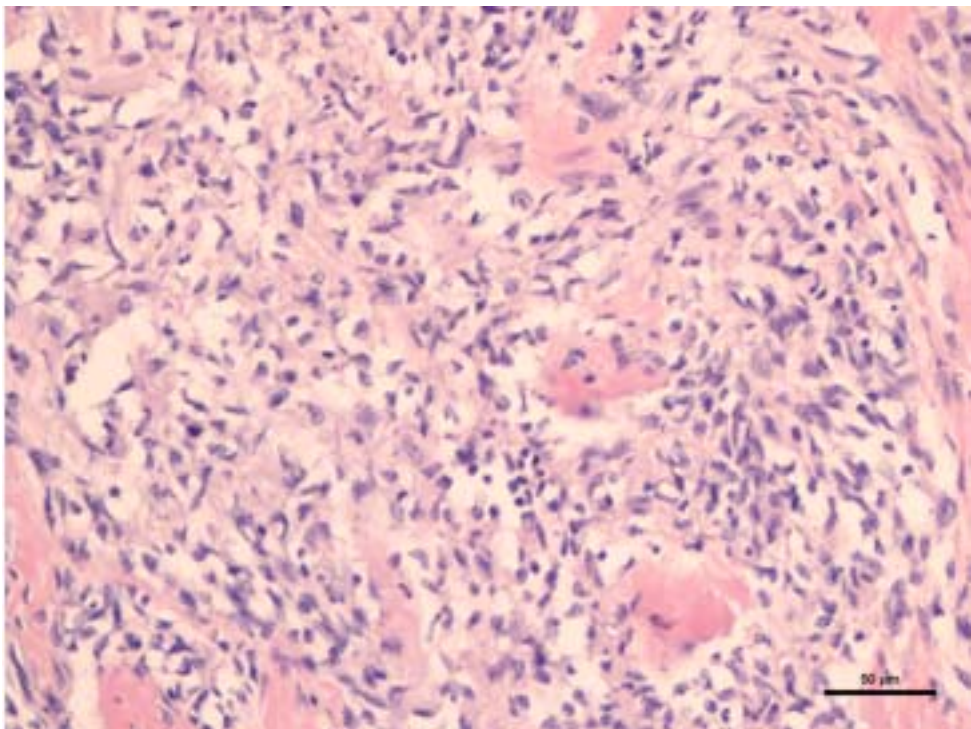


Fig. 19 Macrophages with large clear foamy cytoplasm with scattered neutrophils, HE stain

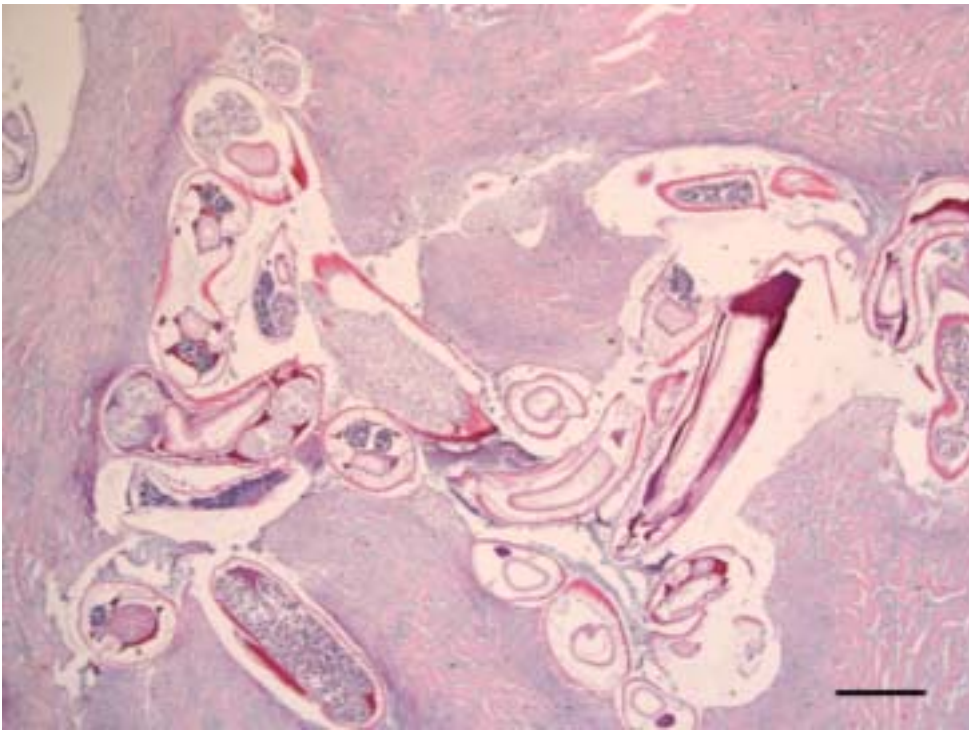


Fig. 20 The inflammatory infiltration was centered around sections of adult nematode, HE stain

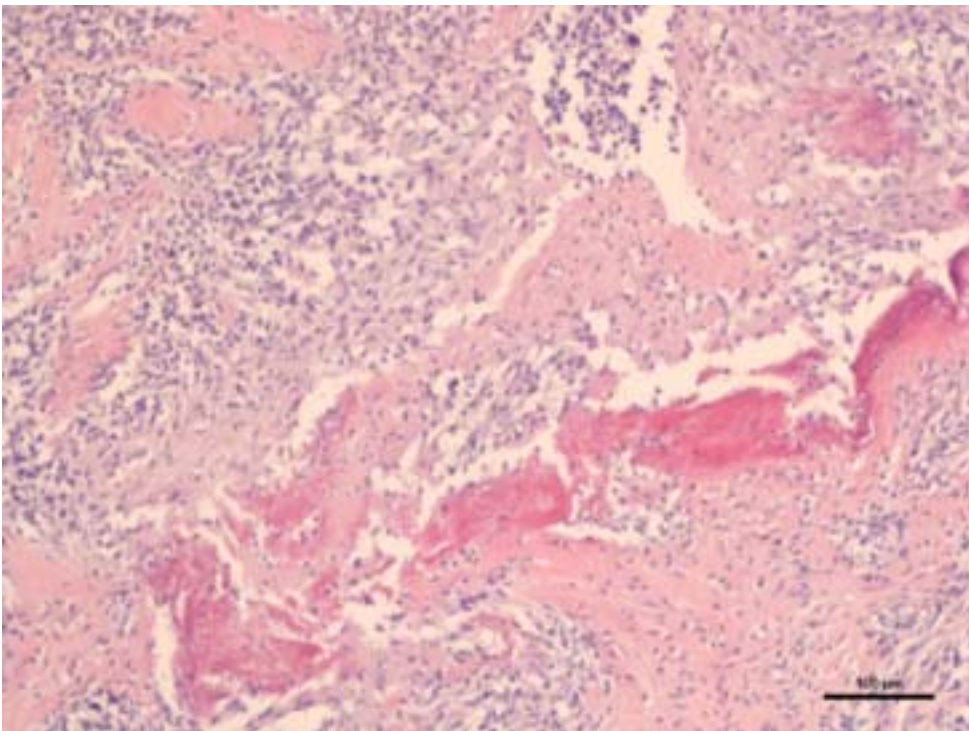


Fig. 21 Necrotic areas with eosinophilic amorphous material probably around dead degenerated nematodes, HE stain



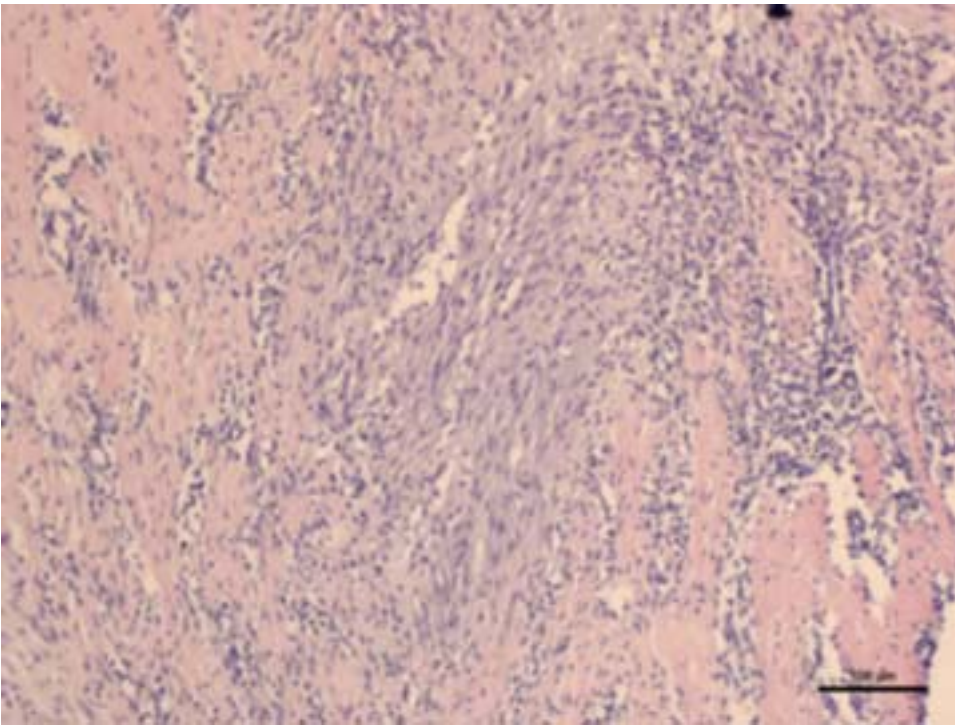


Fig. 22 Well differentiated mature collagen stroma with cords and whorls of spindle cells showing moderate atypia, HE stain

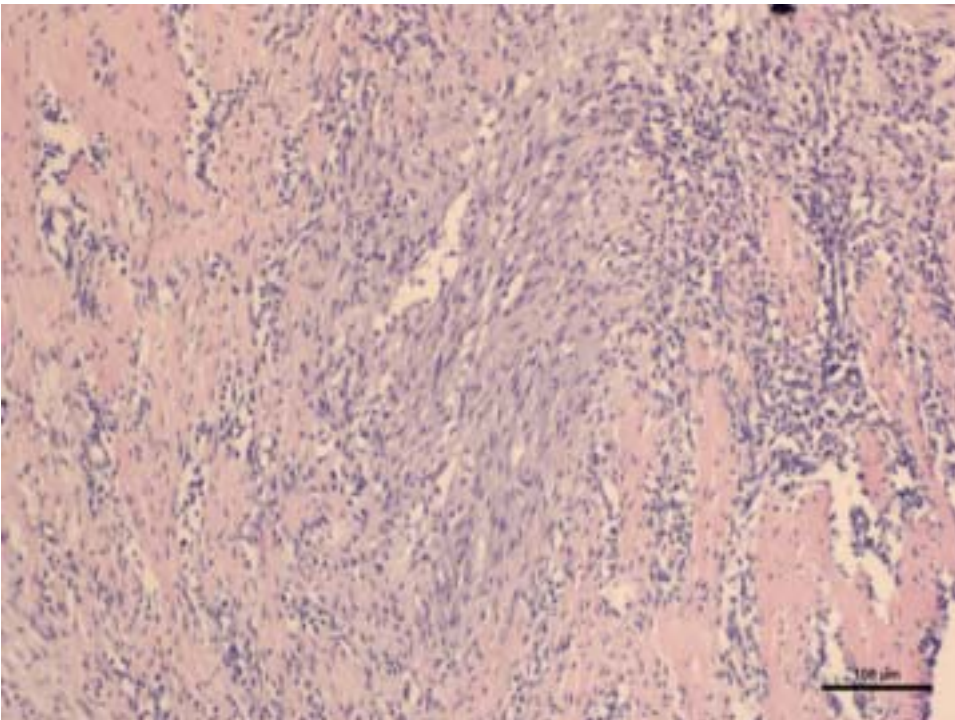


Fig. 23 Well differentiated mature collagen stroma with cords and whorls of spindle cells showing moderate atypia, HE stain

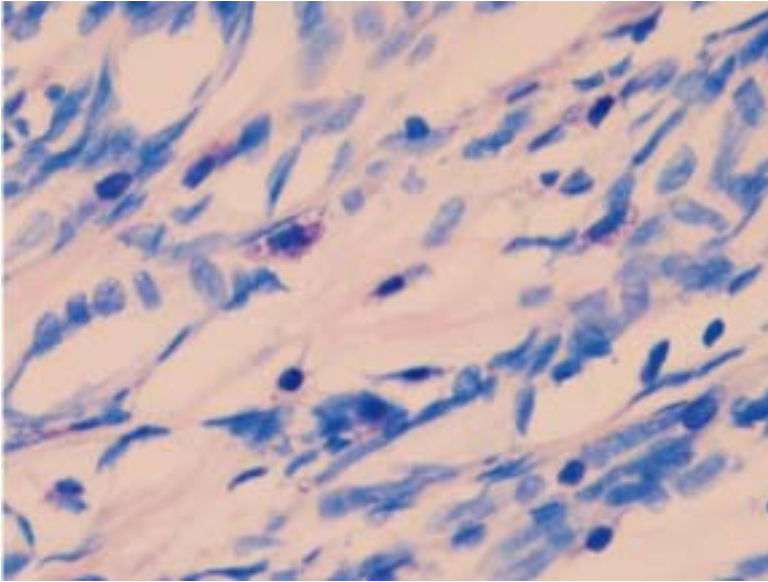


Fig. 24 Scattered spindled mast cells were present.

#### **Further investigations:**

The nematode was exposed to liquid nitrogen and then subjected to the genomic DNA extraction using a commercial kit (DNEasy Tissue kit, QIAgen, Gmbh, Germany). For PCR-coupled sequencing, a 689bp-long region of the *cox1* gene was PCR-amplified using the Spirurida-universal primer set NTF (5'-TGATTGGTGGTTTTGGTAA-3') – NTR (5'-ATAAGTACGAGTATCAATATC-3'). For each DNA extract, the PCR reaction mix was prepared in 50  $\mu$ l with 5  $\mu$ l of template, 100 picomoles of each primer, 25  $\mu$ l of Ready Mix REDTaq (Sigma, St. Louis, MO) and rinsed with distilled water provided by the same manufacturer. PCRs were performed in a thermal cycler (2700, Applied Biosystems, Foster City, CA) using the following cycling protocol: 7 min at 94°C, 40 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, followed by a final extension at 72°C for 10 min. The PCR product was purified and then directly sequenced. Sequences were determined in both orientations using the same primers individually as for the PCR, and the electropherograms were manually checked and edited to assure overall similarity. The sequences were compared with those of the *cox1* gene of other common spirurids available in the GenBank™ using the Nucleotide-Nucleotide Basic Local Alignment Search Tool.

These results are consistent with a worm belonging to *Cylicospirura* genus.

#### **Conclusion:**

To our knowledge, this is the first report of *Cylicospirura* infection of domestic cat in Europe. The life cycle of this parasite is almost unknown. It's thought that the life cycle involves an intermediate host that would be an arthropod, probably a beetle, or a vertebrate paratenic host<sup>6</sup>.

*Cylicospirura*, which is closely related to *Spirocerca* and less strictly to *Physaloptera*, was observed in domestic cats in South Africa<sup>3</sup> and Algeria<sup>6</sup> and in wild cats in Tasmanian Midlands<sup>7</sup>. Other reports involve wild felids from North and South America<sup>1,4,5,6</sup>, India<sup>7</sup>. There are only two reports of this organism in Europe involving herbivores from zoological gardens in Spain<sup>2</sup> and UK<sup>6</sup>. Although *Cylicospirura* has been never associated with gastric wall neoplasia, in contrast to other related spiruridea like *Spirocerca* and *Cyathospirura*<sup>6</sup>,



the extremely reactive mesenchymal cells detected cytologically could raise the suspicion of a non-inflammatory (neoplastic) mass. Nevertheless, histology excluded the diagnosis of neoplasia. Rather, histological analysis evidenced a severe inflammation and reactive fibrous tissue, and the lesion was classified as a nodular chronic mixed type (lymphoplasmacytic, histiocytic and eosinophilic) gastritis with intralesional nematodes. In conclusion, *Cylicospirura* infection should be included among the possible differential diagnoses in the case of nodular gastric lesions with cytological features of severe mixed inflammation associated with reactive fibroplasias.

## **Bibliography:**

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