Pleural fluid accumulation in a kitten

Contributors: Judit Csöndes[1], Viktória Kunos[1], Zoltán Lajos[2], Balázs Bende[3], Bálint Tóth[1], Nándor Balogh[1]

[1]Praxislab Ltd., Hungary

[2]Duo-Bakt Microbiological Laboratory, Hungary

[3]Animal Hospital of Budapest, Hungary

E-mail: csondesjudit@praxislab.hu

Signalment: 9-week-old, female intact, European short-haired cat, "Roxy"

Specimen: Pleural fluid, Wright-Giemsa stain

History: The patient was hospitalized due to one-month-long course of dyspnoea and coughing. Previous treatment included amoxicilline with clavulanic acid, deworming tablet (pirantel-ambonate with praziquantel) and fipronil, (S)-metoprene, eprinomectine, praziquantel containing spot on solution. Despite the initial clinical improvement the general status of the kitten deteriorated recurrently.

Clinical findings: At the time of hospital admission the patient had decreased appetite, suffered from respiratory distress and had no fever. Thoracic X-ray revealed pulmonary infiltration. In-clinic performed haematology and clinical chemistry analysis revealed mild monocytosis and slightly elevated serum globulin level. Result of point-of-care FeLV and FIV tests were negative. Supportive therapy and enrofloxacin treatment was introduced. On the following day fluid accumulation was detected in the pleural cavity and thoracocentesis was performed.

Additional findings: EDTA blood was analysed by ADVIA 120 haematology analyser. Except slightly elevated reticulocyte count (fit to her young age) all cell counts were within the reference interval (**Table 1.**). Microscopic evaluation of the blood film revealed no abnormalities. Moderate hyperglobulinaemia, slightly elevated serum amyloid-A level and mild azotaemia were determined (**Table 2.**).

Table 1. Haematology findings

Parameters	Result	Reference interval
Red blood cell count	7,54	5.00 - 10.00 10^{12}/L
Haemoglobin	109	90 – 150 gram/L
Haematocrit	34,5	26.0 - 47.0 %
MCV	46	42 – 57 fL
MCH	14,4	13.0 - 17.5 pg
MCHC	315	280 – 360 gram/L
Thrombocyte count	281	150 – 450 10^9/L
White blood cell count	10,0	6.0 - 15.0 10^9/L
Neutrophil abs.	4,8	3.1 - 12.5 10^9/L
Lymphocyte abs.	3,99	1.50 - 7.50 10^9/L
Monocyte abs.	0,39	0.15 - 1.10 10^9/L
Eosinophil abs.	0,82	0.06 - 2.21 10^9/L
Basophil abs.	0,00	- 0.08 10^9/L
Large Unstained Cells abs.	0,02	- 0.36 10^9/L
Reticulocyte count abs.	**60,7**	<15 10^9/L

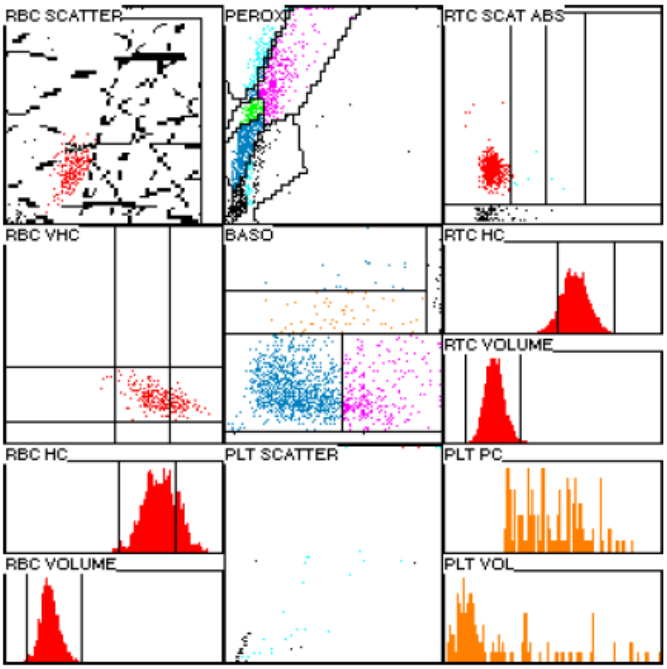
Table 2. Clinical chemistry findings

Parameters	Results	Reference interval
Total protein	**90**	60 – 80 gram/L
Albumin	26,4	25.0 - 45.0 gram/L
Globulin	**63,6**	25.0 - 45.0 gram/L
ALT	56	5 – 60 U/L
Urea	**12,2**	2.5 - 9.9 mmol/L
Creatinine	66	20 – 177 umol/L
Serum amyloid-A	**18,0**	- 4.0 mg/L

Small amount of pleural fluid sample arrived in EDTA tube. The admitted fluid was slightly opaque and had reddish discoloration. **Total protein** content was **56 gram/L** and **total**

nucleated cell count measured both in baso and perox chanels was **8.9 x 10^9/L**. Pleural fluid triglyceride content was 0.24 mmol/L and cholesterol level was 1.0 mmol/L.

Scattergram (ADVIA 120) of the pleural fluid sample:



Questions:

1. **How would you classify the pleural fluid?**

Based on total protein and TNCC of the sample it is an exudate.

2. **Based on clinical picture and laboratory findings which differential diagnoses should take into consideration?**

Laboratory findings point to systemic inflammation and pleuritis. Septic (bacterial) pleuritis should be excluded by microbiological examination even if no detectable bacteria are seen on cytology. In case of septic pleuritis antibiotic sensitivity tests should be performed. The most common bacteria isolated from septic pleural/peritoneal fluids are: Pasteurella spp., Actinomyces spp., Nocardia spp., E. coli, Bacteriodes spp., Peptostreptoccus anaerobius, etc. (Epstein, 2014).

3. **What is your cytological diagnosis and interpretation for the pleural fluid?**

Cytological description: native and cytospin prepared slides were evaluated. Among numerous red blood cells macrophages and non-degenerative neutrophil granulocytes were presented. Presence of several larvae (length: 350-400 μm) at various stages of development and few large circular structures (diameter of 80 - 140 μm) with multiple basophilic nuclei and foamy cytoplasm (parasitic eggs) were also detected. Intra- or extracellular bacteria were not visible.

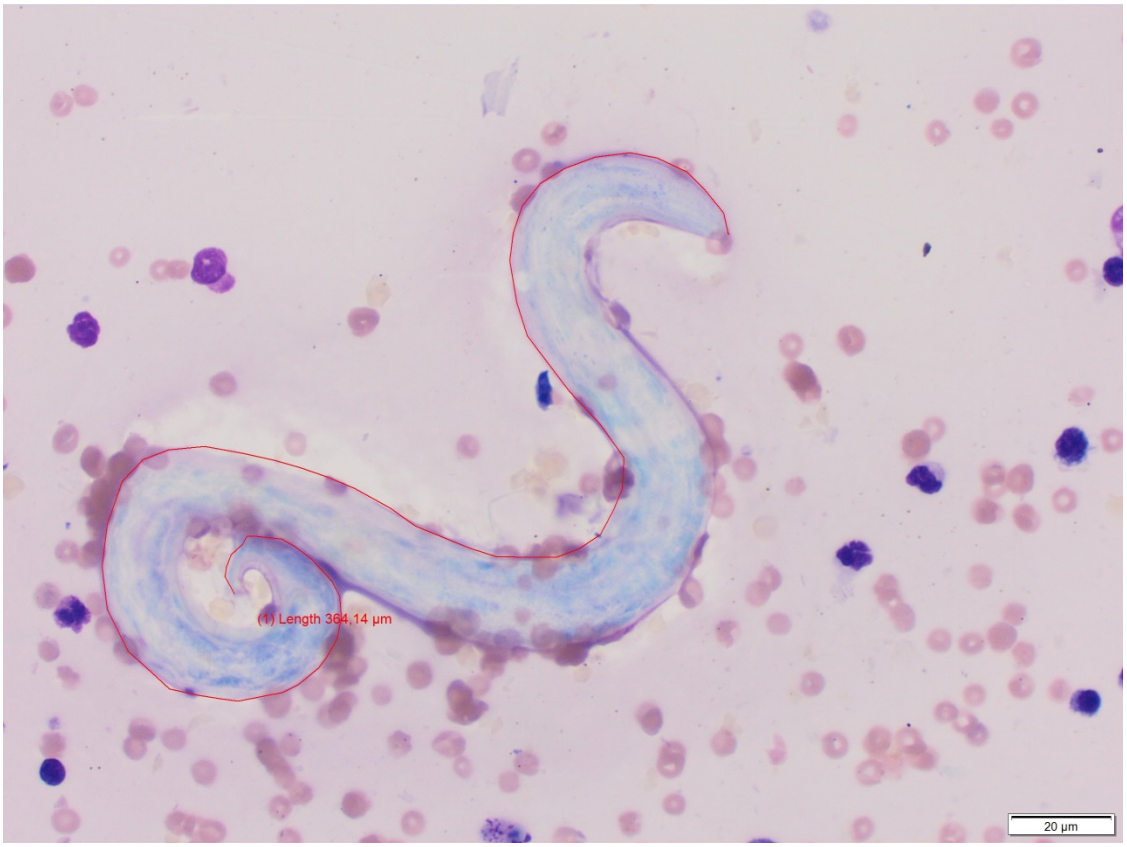


Figure 4. Pleural fluid; cytospin preparation slide (50x, oil immersion Wright-Giemsa)

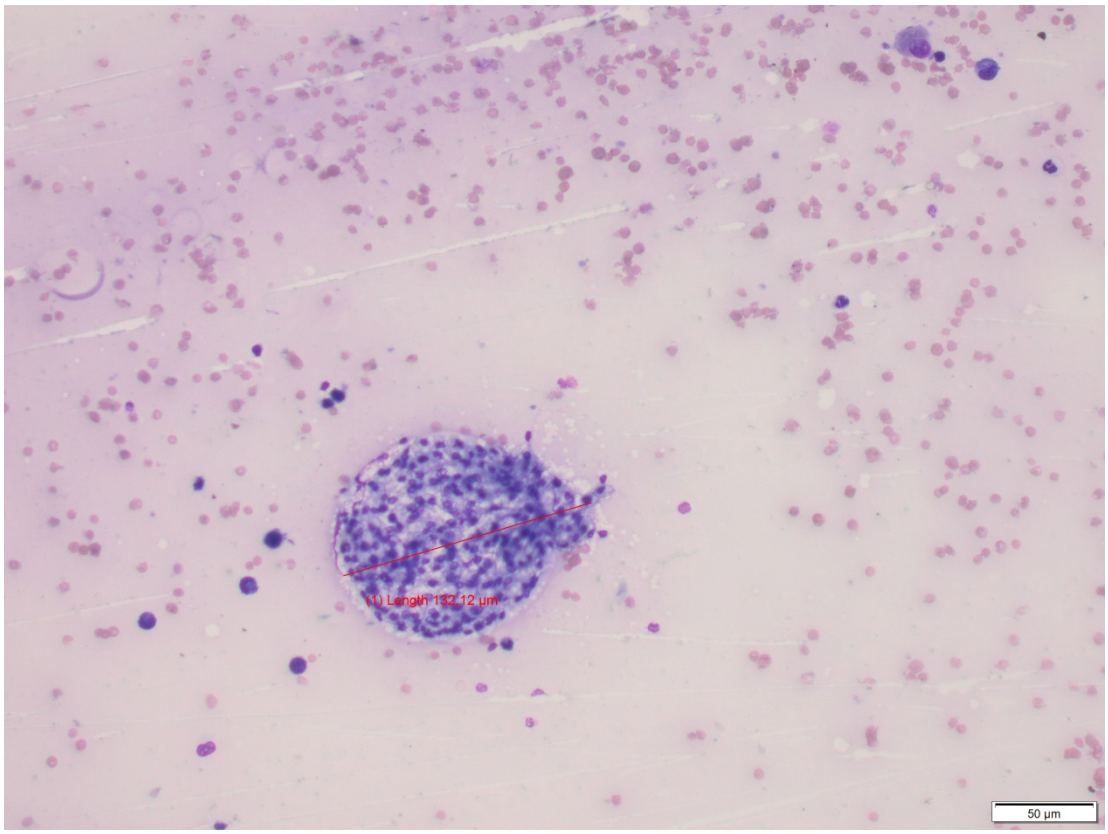


Figure 5. Pleural fluid; cytospin preparation slide (20x, Wright-Giemsa)

Cytological interpretation: Parasitic pleuritis due to lungworm infection, secondary mixed cell inflammation.

Comment: Infection via uterine or milk is highly suspected due to the young age of the patient. Accidental puncture of a subpleural nodule containing eggs and larvaes during thoracocentesis might lead to presence of parasites in the pleural fluid.

4. **What additional tests are recommended?**

Identification of the larval nematodes (Baermann migration method)

Faecal sample was collected and larval isolation by Baermann technique was performed in Duo-Bakt Veterinary Laboratory. First-stage nematode larvae were detected in large number in the faeces. Morphology and size of the first-stage larvae corresponded to *A. abstrusus* with sinus wave-shaped tail and a dorsal subterminal spike (Elsheikha et al, 2016).

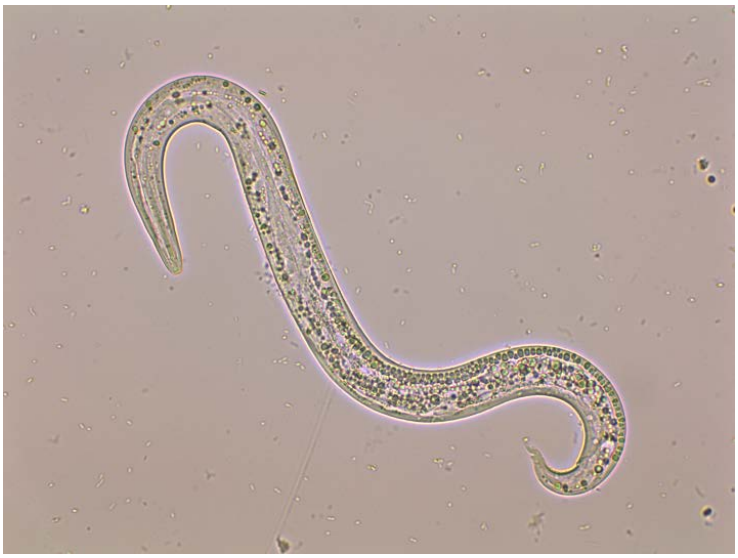


Figure 6. L1 form of *A. abstrusus* detected in the patient's fecal sample by Baermann technique

However PCR technique for *A. abstrusus* DNA detection and distinction from other feline lungworms has been developed, copromicroscopic examination still remains the mainstay of the diagnosis (Elsheikha et al, 2016).
IFAT and ELISA is also available for detection of *A. abstrusus*-specific antibodies (Elsheikha et al, 2016), but the usefulness in that particular case (interference of maternal antibodies or immune tolerance) is not clear.
On the contrary, mixed infections with other lungworms have been registered in cats e.g. *Oslerus rostratus, Troglostrongylus brevior, Troglostrongylus subcrenatus, Angiostrongylus chabaudi, Capillaria aerophila* (Pennisi et al, 2015; Elsheikha et al, 2016, Pennisi et al, 2015). PCR were not performed neither from the pleural fluid nor from the faecal sample.

Microbiological culture of the pleural fluid to exclude/confirm concurrent bacterial infection

Microbiological examination of pleural fluid was not performed. Simultaneous *A. abstrusus* and *Salmonella typhimurium infection* was described by Barrs et al (1999) in a 14- week-old kitten. Although the patient had been treated with different types of antibiotics (effective against Salmonella spp., as well) before collection of faeces and

pleural fluid for analysis, coinfection with *Salmonella spp*. in this case could not be excluded.

5. Should eosinophilia always expected in parasitic disease?

Eosinophilic inflammation is triggered by certain cytokines (e.g. IL-4, IL - 5, IL-13, etc.) and Th2 cells sensitized by parasite antigens or allergens. Eosinophilia associated with parasitism is more likely to occur when parasites are located in tissues rather than in the intestinal lumen (Young – Meadows, 2010). Eosinophilia was reported as one of the most common laboratory alterations in case reports with *A. abstrusus* infection (Pennisi et al, 2015; Elsheikha et al, 2016). In this particular case the background of the absence of eosinophilia or eosinophilic infiltration of pleural fluid is unknown.

Discussion

Lungworm-related pleural fluid accumulation present only in few percentage of pleural effusion (Beatty – Barrs, 2010; König et al, 2018, Ruiz et al, 2018). Feline pulmonary aelurostrongylosis is caused by the nematode *Aelurostrongylus abstrusus* the most common lungworm of the cat (Pennisi et al, 2015; Elsheikha et al, 2016; Moskvina, 2018). Cats usually infected by ingestion of intermediate (snails, slugs) or paratenic (rodents, reptiles, amphibians and bird) host containing L3 larvae. L3 larvae migrate via the intestinal wall and mesenteric lymph nodes to the lung, where they undergo the third and fourth moults and become matured worms (Moskvina, 2018). Vertical transmission of the larvae via placenta or milk is a known way of infection (Tamponi et al, 2014). Adult lungworms locate in bronchioles and alveolar ducts, where the new generation (L1) also develop. The L1 larvae migrate through the small and large airways to the trachea and pharynx, where they are expectorated or passed through the gastrointestinal tract and discharged in the faeces. Peak of the larval shedding is expected during the period 60–120 days post infection (Moskvina, 2018). The prepatent period lasts ca. 35–48 days (Elsheikha et al, 2016).

Pyogranulomatous inflammatory reaction is typically induced by the presence of adults and larvae (Elsheikha et al, 2016; Moskvina, 2018). Feline pulmonary aelurostrongylosis can be subclinical, accompanied by mild, chronic symptoms or it can be a life threatening disease. The clinical symptoms and damage caused by *A. abstrusus* depend on several factors such as parasitic burden, age and immune status of the feline host, presence of other diseases and concurrent infection (Miller et al, 1984; Barrs et al, 1999; Philbey et al, 2014; Pennisi et al,

2015). Detection of the first stage larvae in faecal and respiratory (bronchial mucus or pleural fluid) samples is diagnostic (Miller et al, 1984; Barrs et al, 1999; Pennisi et al, 2015; Elsheikha et al, 2016). Adult *A. abstrusus* rarely detected as they are embedded in the lung parenchyma (Elsheikha et al, 2016).

Leucocytosis, eosinophilia, anaemia and hypoalbuminaemia were reported as most common laboratory alterations in case reports (Pennisi et al, 2015; Elsheikha et al, 2016).

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