Finn Pathologists *The* Veterinary Pathologists

Diagnostic Tests Available for Feline Infectious Peritonitis

Effective treatment options for Feline Infectious Peritonitis (FIP) are now available, but obtaining a definitive diagnosis ante-mortem can be challenging. This fact sheet aims to explain the various diagnostic tests available to vets in practice, in the context of clinical presentation, signalment and other laboratory test results which may also be pertinent to diagnosis in tricky cases. FIP is a complex disease and this fact sheet is not intended to provide an exhaustive guide to the virus or the pathogenesis, but suggested further reading is given at the end of the sheet for people wanting additional information on the topic. Advice on treatment is beyond the scope of this article.

1. Is FIP a potential differential diagnosis?

Patients with FIP can present with a wide array of symptoms, which may also change over time. These can depend in part on whether the effusive or non-effusive form of the disease is present, and/or which body systems are affected. There are several "decision trees" and "diagnosis flowcharts" available to help clinicians through this process (see further reading and information section below).

History:

Few cats remain persistently infected with coronavirus. Therefore the clinical history should include whether there is any chance of recent exposure to feline coronavirus (FCoV) infection, for example via a breeder or rescue shelter, or multi-cat household, usually within the last 18 months. There is often a history of stress within the weeks or months prior to clinical presentation (for example adoption, boarding cattery, vaccination, neutering). A high percentage of affected cats are pedigree and although cats of any age can be affected, there is some age-related resistance suspected and the typical case will be less than 2-3 years old.

Clinical presentation:

Both forms:

- Persistent **pyrexia** of more than 4-days duration; may fluctuate and is typically non-responsive to antibiotics or NSAIDs.
- **Lethargy, anorexia, weight loss** or failure to grow if younger cat
- **Jaundice** (more common in effusive form)
- **Lymphadenomegaly**

Effusive form:

- **Effusions:** may be present in one or more of the following cavities:
- Abdominal, pleural, pericardial, scrotal
- Often acute and progressive
- May present as abdominal enlargement / dyspnoea, tachypnoea.

Non-effusive form:

- **Neurological signs;** may be focal, multifocal or diffuse (e.g. ataxia, fits, nystagmus)
- **Ocular signs** including uveitis, keratic precipitates, aqueous flare, retinal vessel cuffing
- **Renomegaly**
- **Abdominal mass** – often the mesenteric lymph nodes
- Pneumonia
- Dermatological signs; non-pruritic nodules or papules

2. Is there any other supporting evidence from blood tests?

Haematology:

Haematological changes are non-specific to FIP but may include –

- **Lymphopenia**, with neutrophilia and a left shift
- Mild to moderate, normochromic, normocytic **anaemia** (normally but not exclusively non-regenerative)

Serum Biochemistry:

Changes in serum biochemistry are also varied and non-specific to FIP but may include –

- **Hyperglobulinaemia**, often with hypoalbuminaemia or low-normal serum albumin
- **Albumin: globulin ratio low** (suggested cut-offs A:G ratio <0.4 makes FIP a possibility, while A:G rati of >0.8 makes FIP very unlikely).
- **Hyperbilirubinaemia;** especially effusive form, often without marked increase in ALT, ALP or GGT, and in the absence of severe anaemia.
- **Persistently increased levels of acute phase proteins** such as α1-acid glycoprotein (AGP) or serum amyloid A (SAA).

FCoV serology – serum antibody tests for FCoV, performed on a blood sample – a positive FCoV test indicates that the cat has been exposed to and infected with FCoV and has seroconverted, which takes 2-3 weeks from initial infection. ***Please note that the titre in an individual animal is of limited use in distinguishing cats with FIP from those without.*** Many clinically healthy cats have positive, often high FCoV titres, while 10% of cats with FIP are seronegative – therefore interpretation can be difficult. Please note that there is a risk of false negative results in advanced/end stage disease and particularly if FCoV antibody tests are performed on effusion samples.

3. Is there any effusion present for analysis?

Analysis of any effusion includes -

- **Appearance:** straw-coloured, clear, not malodorous, viscous/sticky, and occasionally chylous
- **Protein level: high** – total protein concentration >35g/l (>50% globulins)
- **A:G ratio <0.8**
- **AGP levels raised** (similar to serum)
- **Cytology:** low cellularity. Neutrophils and macrophages (not bacteria, malignant cells or predominantly lymphocytes)
- **Rivalta test:** positive (see below)
- **Immunostaining for FCoV antigen and RT-PCR for FCoV RNA** can also be performed on effusion samples (see later)

Rivalta's test

This test can be performed on an effusion sample to determine whether the sample is a transudate or an exudate. FIP effusions are often described as modified transudates based on cell count but as exudates based on their protein concentration. A positive result with the Rivalta test means the sample is an exudate, but this is not specific for FIP (other examples of diseases resulting in a positive result might also include a septic peritonitis or lymphoma). The test requires 8ml of distilled water and one drop of 98% acetic acid mixed together in a universal pot and one drop of effusion sample carefully placed onto the surface. A positive test result is indicated when the drop of effusion stays attached to the surface of the liquid, retains its shape or floats slowly to the bottom, while a negative result occurs when the test drop disappears and the solution remains clear. Please bear in mind that interpretation can be subjective and problematic at times, and that a positive result is not specific for FIP.

Details on performing and interpreting the Rivalta's test can be found at www.catvirus.com and from the European Advisory Board on Cat Diseases consensus on FIP (http://www.abcdcatsvets.org/feline-infectious-peritonitis/).

4. RT-PCR for FCoV

This is a semi-quantitative assay which looks for RNA from FCoV, but it is not specific for FIP-associated FCoV. The test can be performed on tissues (not formalin-fixed), effusion, blood, CSF or aqueous humour from suspected FIP cases.

Tissue samples from cats with FIP are significantly more likely to be FCoV RT-PCR positive and have significantly higher viral loads than tissue samples from non-FIP cases, however cats without FIP can still be positive for FCoV by this technique. This is because cats without FIP can still potentially have FCoV present within their tissues (i.e. systemic spread of benign coronavirus). FCoV loads tend to correlate with histopathological findings suggestive of FIP.

The presence of high levels of FCoV RNA in tissue, blood, effusion, CSF and/or aqueous humour appears to be highly supportive of a diagnosis of FIP, but cannot necessarily be regarded as a definitive diagnosis in its own right (see Tasker, 2018).

FCoV RT-PCR performed on other samples, including effusion samples, plasma/serum, CSF and aqueous humour has been described, with varying degrees of sensitivity/specificity.

FCoV RT-PCR on faecal samples can be performed but is primarily a tool used to identify cats that are shedding virus within a given household, and not generally used for the diagnosis of FIP.

Analysis for FCoV S gene mutations

Following on from a positive result for FCoV using RT-PCR, it is sometimes possible to detect whether a particular mutation is present within the viral population, for example within the S gene. This is done by targeting very specific parts of the viral genome. This test is not always successful however, for example if only low levels of the virus are present it may not be possible to amplify. The S gene mutation has also been identified in tissues from non-FIP cats with systemic FCoV infection, and non-mutated forms are sometimes detected in cases with FIP, therefore detection of these mutations is also not entirely diagnostic for FIP, and the usefulness of mutation analysis over and above the RT-PCR detecting FCoV is uncertain.

5. Are there tissue samples / lesions for histopathology?

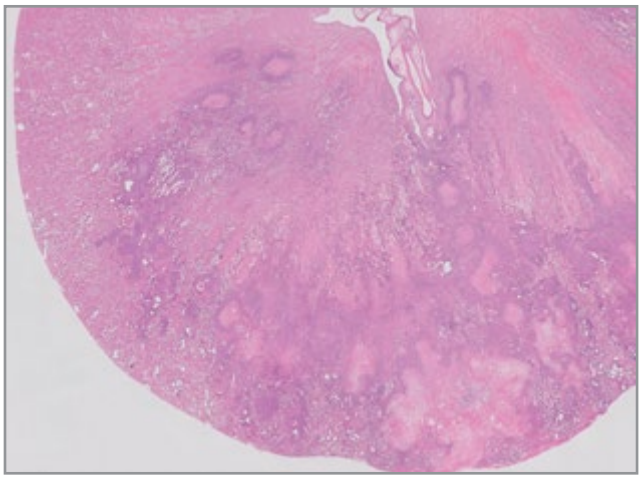
Histopathology

Samples for histopathology may be available, typically from affected tissues such as liver, mesenteric lymph nodes or kidney. This may be ante mortem, or post mortem to try and confirm a suspicion of FIP. Lesions can be very focal in nature, and an absence of typical histopathological changes does not entirely rule out FIP, however submitting biopsies for histopathology also allows the pathologist to look for other potential differential diagnoses.

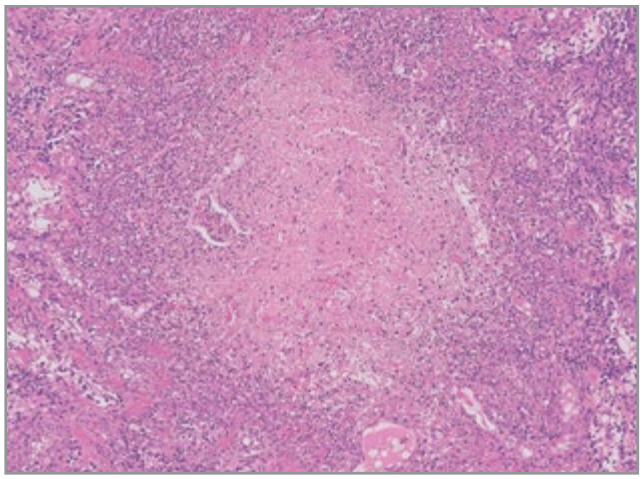
Lesions, when present, tend to be fairly characteristic for FIP and to arise in certain locations. For example, a patchy, often necrotising granulomatous to pyogranulomatous lymphadenitis without other obvious infectious agents, inflammation within the kidney often surrounding the subcapsular veins, multifocal necrotising granulomatous hepatitis with capsular deposits of fibrin, aggregates of perivascular inflammatory cells, often dominated by plasma cells and targeting venules; while none of these are entirely specific to FIP, they are reasonably characteristic and FIP should be on the differential list.

Immunostaining for FCoV Antigen

This is a technique which uses labelled antibodies to detect the presence of FCoV antigen, within the macrophages, within the lesions detected by histopathology (when performed on tissues, this technique is known as immunohistochemistry or IHC). It utilises the same tissue sample as that used for histopathology, so no additional sampling is required. **Similar techniques can be used on cells present within samples of effusion (when it is known as immunocytochemistry or ICC).** The labelled antibody is then detected via enzymatic reactions which produce a colour change.



1. Low power view of a kidney, HE-stained, demonstrating histopathological changes suspicious for FIP. Multifocal to locally coalescing areas of necrotising, granulomatous to pyogranulomatous inflammation, scattered throughout the cortex and medulla



2. Higher power view (100x), kidney, HE-stained, showing one focal area of necrosis, surrounded by macrophages, lesser neutrophils and with lymphocytes and plasma cells towards the periphery.

One benefit of this technique is that it is very specific to FCoV, and another is that it allows for the co-localisation of the FCoV antigen within the lesions, and within the macrophages themselves. Such a positive result for FCoV antigen via immunostaining is said to confirm the diagnosis of FIP (and is currently considered the **"gold standard"** test) however a negative result does not exclude FIP unfortunately. This test can be requested retrospectively if you have already submitted samples. However, if you plan to run ICC on an effusion from the outset, a cell pellet should be made from the fluid by centrifuging the sample in an Eppendorf tube and removing the supernatant. Formalin is then added on top of the pellet. Sensitivity of this test can be increased by repeatedly spinning aliquots of fluid before adding the formalin. Remember to package up any cytology slides separately to sample pots that contain formalin, to avoid artefacts from the formalin fumes.

Further reading and information:

https://catvets.com/guidelines/practice-guidelines/fip-guidelines

www.catvirus.com website of Dr. Diane Addie

http://www.abcdcatsvets.org/feline-infectious-peritonitis/ from the European Advisory Board on Cat Diseases

1. Tasker S. **Diagnosis of Feline Infectious Peritonitis. Update on evidence supporting available tests.** *Journal of Feline Medicine and Surgery* 2018; 20: 228-243.

2. Felten S and Hartmann K. **Diagnosis of Feline Infectious Peritonitis: A Review of the Current Literature.** *Viruses* 2019; 11: 1068

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