REACHING FOR A DIAGNOSIS OF FIP
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THE COMPLEX RELATIONSHIP BETWEEN FELINE CORONAVIRUS AND FELINE INFECTIOUS PERITONITIS (FIP)
Feline coronavirus (FCoV) infection is very common in cats; around 40% of the domestic cat population has been FCoV infected, and this figure increases to 90% in multi-cat households. Natural infections with FCoV are often transient and asymptomatic or result in mild gastrointestinal disease. However, occasionally (1-5% of infected cats), FCoV infection results in FIP. Previously asymptomatic FCoV infection was believed to be confined to the intestinal tract, but we now know that healthy FCoV infected cats can have systemic FCoV infection, albeit with lower FCoV viral loads than cats with FIP. No cure for FIP exists and it is an extremely distressing disease to deal with, both for cat owners and vets. Occasionally, and possibly with increasing frequency recently, outbreaks of FIP (e.g. >10% of cats are affected) in multi-cat households or shelters are reported.

WHAT FACTORS CONTRIBUTE TO THE DEVELOPMENT OF FIP?
Viral factors are likely to be important. The spike (S) protein of FCoV is a transmembrane protein that mediates host cell entry and it contains a fusion peptide that enables fusion of the FCoV envelope with the host cell membrane; mutations in the S protein fusion peptide can result in amino acid changes that mediate tropism changes in FCoV. Recent studies have identified mutations in the FCoV S protein fusion peptide that were thought to be markers of FIP. However more recently we have shown that these mutations are more likely to be markers of systemic FCoV infection, which can occur in both FIP and non-FIP cats, rather than FIP per se. However these mutations are still important as it is probably via these, and/or perhaps other mutations, that the FCoV acquires its macrophage tropism to allow it to spread systemically and contribute to the development of FIP. However other factors are likely to be important in determining whether FIP results. Host factors will play a part e.g. the immune response of the cat may be able to prevent the development of FIP, and breed and genetics may play a role. Environmental factors, such as the level of stress and overcrowding in a household, may also influence the outcome of FCoV infection and whether FIP develops in an individual cat.

REACHING FOR A DIAGNOSIS OF FIP
Sadly it is not easy to nail a diagnosis of FIP! A single minimally invasive FIP test that can definitively diagnose all FIP cases simply does not exist. Definitive diagnosis traditionally relies on histopathological examination of tissues ± immunological staining of FCoV antigen. Immunological staining of FCoV antigen in effusion samples is also probably adequate for definitive diagnosis in effusions with features characteristic of FIP. Evaluation of the reliability of such tests is sometimes difficult in publications as the criteria used to definitively diagnose FIP vary e.g. histopathology or immunological staining of effusions or FIP diagnostic algorithms.

Many differential diagnoses exist for FIP but major ones to consider are lymphocytic cholangitis (cats are usually relatively well with elevated liver cholestatic markers), toxoplasmosis (uncommon but history of hunting/raw meat ingestion, can show neurological signs ± uveitis) and mycobacterial infections (cats often relatively well with skin, lymph node and lung signs predominating).

Background information & clinical signs
Before performing diagnostic tests, one must remember that FIP is most common in young (<3 years, especially <2 years, but also a smaller peak in cats >10 years) male cats. Some breeds in some countries may be predisposed (e.g. Burmese, Australian Mist, British Shorthair & Cornish Rex in Australia) but generalised breed predispositions are not thought to exist. A recent history of stress (adoption, being in shelter, neutering, upper respiratory tract disease, vaccination etc.) may be apparent. Typical clinical sign include lethargy, anorexia, weight loss, pyrexia and jaundice. Effusive or wet FIP (<80% cases) is associated with abdominal and/or pleural and/or pericardial effusions and is often quite acute in nature. Dry or non-effusive FIP is characterized by neurological [ataxia, nystagmus, seizures], ocular [uveitis] and skin [small papules] signs and is more chronic, although overlap between the wet and dry forms of FIP are seen. Focal (involving lymph node or intestine) FIP occur occurs. Recently, pyrexia was far more common in cats with effusions and uncommon in cats with neurological signs of FIP. Clinical signs can progress so repeated clinical examinations are important to detect newly apparent signs.

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Routine haematology
Haematology can support a diagnosis of FIP but changes are non-specific. Lymphopenia is particularly common (55-77% of cases; although a recent study found only 49.5% of FIP cases to be lymphopenic\textsuperscript{15} and wet FIP cases (56.1%) were found to be more likely to be lymphopenic than dry (26.8%) cases), with neutrophilia (39-57%), a left shift, and mild-moderate normocytic, normochromic anaemia (37-54%; worsening, and increased prevalence, of anaemia are also seen during FIP disease progression) also reported\textsuperscript{15,16,19,20}. An association between FIP and microcytosis (with or without anaemia) was recently reported\textsuperscript{5}. Severe immune-mediated haemolytic anaemia can occur with FIP\textsuperscript{16}, but this is uncommon.

Serum biochemistry
FIP can be associated with hyperproteinaemia (up to 60% cases, especially in dry FIP cases, although lower prevalences (17.5%) have been reported recently\textsuperscript{15} due to hyperglobulinaemia (89% cases), usually with a low or low-normal serum albumin (64.5%); consequently the albumin: globulin (A:G) ratio is low (<0.4 means FIP very likely, >0.8 makes FIP very unlikely; reference range 0.45-1.2))\textsuperscript{16,19,20}. A study\textsuperscript{21} in a population of cats with a low prevalence of FIP (akin to the situation we usually encounter in practice) found that an A:G ratio >0.6 was useful to rule out FIP, but that lower values were not helpful in ruling in FIP.

α1-acid glycoprotein (AGP), an acute phase protein, is elevated in many inflammatory and non-inflammatory diseases (>0.48 mg/ml), but AGP levels in FIP are often markedly elevated (>1.5 mg/ml), so the magnitude of increase may be helpful in aiding diagnosis of FIP\textsuperscript{22-24}. Hyperbilirubinaemia can occur (21-63% cases), especially in wet FIP, often without marked elevations in ALT, ALP, GGT, which can help increase suspicion of FIP. Worsening and increased prevalence of hyperbilirubinaemia are reported during FIP disease progression\textsuperscript{19}.

FCoV serology
Serum FCoV antibody tests are usually enzyme-linked immunosorbent assays (ELISAs), indirect immunofluorescence antibody (IFA) or rapid immunomigration tests\textsuperscript{25}. Most tests use FCoV-infected swine or cat cells as a substrate and titres are read in distinct multiples of serum dilutions. A positive FCoV antibody test indicates that the cat has been infected with FCoV and has seroconverted (takes 2-3 weeks). Although FIP cases tend to have higher FCoV antibody titres than cats without FIP, there is much overlap, so the value in an individual cat is limited\textsuperscript{26}. Many clinically healthy cats (esp. those in multicat households) have positive titres (which can be very high), whilst ~10% of cats with FIP are actually seronegative (sometimes due to the presence of virus in the sample binding antibody and rendering it unavailable to the serological test\textsuperscript{27}).

Analysis of effusion samples
This is very helpful in the diagnosis of FIP; so obtaining samples should be prioritised e.g. repeated ultrasonography. FIP effusions are usually clear, viscous and straw-yellow. They are usually protein rich (thick proteinaceous backgrounds are often described on cytology) with total protein concentration of >35 g/l and >50% globulins. They usually have similar low A:G ratios and raised AGP concentrations to those in serum. FIP effusions are poorly cellular (usually 5x10^3/l cells) typically with non-degenerate neutrophils and macrophages. FIP effusions are exudates based on their protein concentration but modified transudates based on cell counts. Immunostaining for FCoV antigen and reverse-transcriptase (RT-) polymerase chain reaction (PCR) for FCoV on effusion samples is also possible.

Reverse-transcriptase (RT-) polymerase chain reaction (PCR)
RT-PCR for FCoV has been used to amplify FCoV RNA in blood, effusion, faecal (to detect FCoV shedders c.f. FIP diagnosis) or tissue samples.

Several studies have used FCoV RT-PCR assays in tissues and effusions from both FIP and non-FIP cats. We found that tissue samples collected from cats with FIP have significantly higher FCoV loads by quantitative RT-PCR\textsuperscript{13} than non-FIP cat tissues samples. Indeed FCoV loads tend to correlate with pathological findings\textsuperscript{28}. Although it is recognised that the non-invasive collection of such samples is difficult, it may be that RT-PCR performed on tissue samples collected by ultrasound guidance e.g. tru-cut biopsy, becomes a useful additional supportive (c.f. definitive) diagnostic test as it is quicker to perform than histopathology. Recent work, published as an abstract\textsuperscript{29}, suggests that ultrasound-guided fine needle aspirates (FNAs) may also be good targets for PCR. Effusions in wet cases may be easier to target/sample and are known to often contain FCoV RNA\textsuperscript{28}, recent studies amplified FCoV RNA in most (72-89%) FIP effusion samples but not in any non-FIP effusion samples\textsuperscript{30,32}; showing good sensitivity and excellent specificity, although the excellent specificity is likely to be due to the fact that none of the non-FIP cats in the studies were FCoV infected. However a positive FCoV RT-PCR result in an effusion is useful as an aid to the diagnosis of FIP.
Studies\(^{30,32}\) have also performed FCoV RT-PCR on plasma or serum samples from FIP and non-FIP cats; very few (9-15.4\%) of the FIP cases gave positive results, and none of the non-FIP cases were positive. It is known that the level of FCoV RNA in the blood of FIP cases is usually undetectable\(^{28}\) and these findings suggest that blood/plasma/serum samples are not appropriate to use for FCoV RT-PCR. Peripheral blood mononuclear cells (PBMCs) may be a better target for PCR, as shown in a recent study\(^{32}\), but sensitivity was still very poor at 28.6\%.

A recent paper\(^{33}\) described the use of FCoV RT-PCR on CSF samples and found it to have 100\% specificity for the diagnosis of FIP but a sensitivity of only 41.2\%. However the cases in this study did not all have neurological signs (CSF was collected at post-mortem examination from a range of cats c.f. in neurological cases in which CSF was being collected for diagnostic purposes ante-mortem) and so the population tested may not reflect those that would have CSF samples taken during diagnostic investigations; the sensitivity rose to 85.7\% when only cats with neurological and ophthalmological signs were considered. Thus a positive RT-PCR FCoV result on CSF may also be helpful in the diagnosis of FIP.

Therefore, although the finding of FCoV in samples by RT-PCR, as shown above, can be helpful/supportive in the diagnosis of FIP, it should not be used to definitively diagnose the disease alone.

**PCRs targeting the S protein mutation**

Research has described specific mutations in the FCoV S protein as being markers of FCoVs associated with FIP\(^{11}\), meaning that the presence of such mutations could be used to definitively diagnose FIP. The Chang study deduced this by comparing the sequences of FCoVs found in the tissues of FIP cats with those found in the faeces of healthy non-FIP cats. However we hypothesized that these mutations could reflect the tropism of the virus (systemic vs intestinal) rather than the disease (FIP vs non-FIP), especially knowing that non-FIP cats can have systemic FCoV infection. In our recent research\(^{13}\) we compared FCoV sequences in the tissues of FIP cats and those in the tissues of non-FIP cats, thus evaluating systemic FCoV infection in both non-FIP and FIP cases, rather than comparing tissues in FIP cases with faeces in non-FIP cases. We found that the S protein mutations in the FIP tissues are also present in most of the tissues of non-FIP cats that have systemic FCoV infection.

Although these cats (i.e. non-FIP cats with systemic FCoV infection) are uncommon, this does mean that any PCR targeting these S protein mutations cannot be used to definitively diagnose FIP as these mutations are likely to be markers of systemic FCoV infection rather than of FIP per se. Studies\(^{10,31}\) looking at S protein mutation in effusions have found that the majority of FCoVs in the effusions of FIP cats do indeed have the S protein mutations described by Chang\(^{11}\). In the study by Longstaff et al\(^{13}\), 12/17 FIP effusion samples had S protein mutations, whilst 1 did not have a mutation and 4 could not be sequenced due to the low levels of FCoV present.

In the study by Felten et al\(^{30}\) 32/36 FIP effusion samples had S protein mutations, whilst 3 did not have mutations and 1 could not be sequenced. It is of note from these data that it is sometimes not possible to define sequences in FCoV positive samples due to low levels of FCoV, or possibly alternative sequences, being present, limiting the number of samples that will yield results for S protein mutation analysis. This limitation, together with the finding of mutations in non-FIP cases (even though generally not many non-FIP cats are FCoV RT-PCR positive) seriously questions the benefits of looking for mutations (e.g. by sequencing) subsequent to finding the presence of FCoV in FIP cats by RT-PCR.

**Histopathological examination of tissues**

Samples of tissue e.g. liver, kidney or mesenteric lymph nodes, collected ante-mortem (by ultrasound-guided percutaneous tru-cut biopsy, laparoscopy or laparotomy) or at post-mortem examination, can be evaluated for characteristic histopathological changes of FIP. Histopathological changes are considered reliable for diagnosis. However, a lack of pathological lesions is more difficult to interpret as small samples e.g. tru-cut biopsies may miss such lesions due to their multifocal distribution, or due to non-affected organs being sampled\(^{34}\).

Additionally a recent study\(^{22}\) documented 5/8 FIP cases did not have histopathology changes consistent with FIP, even though large representative biopsies were taken, and diagnosis in these cases was based on positive FCoV immunostaining. So in some cases histopathological examination may not be as definitive as hoped and FCoV immunostaining may help in such cases.

**Immunological staining of FCoV antigen**

This is performed using immunohistochemistry (IHC) on formalin-fixed tissues or immunocytochemistry (ICC) on cytology (typically effusion) samples. These techniques exploit the binding of antibodies to FCoV antigens/proteins in tissues or cells; detection of this reaction can be by enzymatic reactions producing a colour change or by fluorescence (immunofluorescence).
Positive immunological staining of tissues is said to confirm a diagnosis of FIP (i.e. it is very specific) but a negative result does not exclude FIP as FCoV antigens may be variably distributed within lesions\cite{4} and thus not detected in some FIP cases. This somewhat contradicts the suggestion by some that immunostaining is mandatory to confirm/exclude FIP in doubtful cases\cite{5}.

Immunostaining of effusion samples has shown variable sensitivity (ranging from 57 to 100\%)\cite{35-40} since this technique relies on staining FCoV within cells (macrophages) in the effusion, and if the effusion is cell-poor, or if the FCoV antigen is being masked by FCoV antibodies in the effusion, a false negative result may be obtained. Immunostaining was thought to be very specific although recent studies have questioned this as 2/7 (heart failure and cholangiocarcinoma cases) non-FIP effusions were positive by IF in one study\cite{38} and 8/29 (including 2 cats with heart failure and 2 cats with neoplasia) non-FIP effusions were positive by ICC in another\cite{35}. However the reported poorer specificity may be due to the double staining in one study (staining for both FCoV antigen and macrophages [via MHC II staining] was used), and the suboptimal storage of slides in the other, which could cause non-specific staining and false positive results. Successful ICC in CSF in neurological FIP has been reported\cite{34}, and a very recent study evaluated ICC in CSF samples taken either ante-mortem (in cases with neurological signs) or post-mortem (in cases without neurological signs). This study found 17/21 FIP cases to be ICC positive but also 3/20 non-FIP cats\cite{42}, limiting the specificity. FNAs could also be a target for ICC but sensitivity may be poor due to difficulties in targeting lesions.

CONCLUSIONS
Many features of a cat’s history (e.g. fluctuating non-responsive pyrexia), clinical examination (e.g. effusions, uveitis) and laboratory testing (e.g. A:G ratio, AGP, positive PT-PCR) can increase our suspicion of a diagnosis of FIP, making it a likely diagnosis ante-mortem. The detection of FCoV S protein mutations by RT-PCR does not appear to offer additional information for diagnosis. Histopathology and immunostaining may be needed to confirm a definitive diagnosis before euthanasia, although these samples can be difficult to obtain non-invasively but immunostaining of characteristic FIP effusions is helpful.

NB. Treatment of FIP is out of the scope of these notes but very promising results for the treatment of experimental FIP has recently been reported with a coronavirus protease inhibitor\cite{43}.

REFERENCES


