FELINE INFECTIOUS PERITONITIS:

HOW CAN WE GET A DIAGNOSIS?

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Feline infectious peritonitis (FIP) is usually regarded as an incurable disease and an important cause of death in young cats caused by feline coronavirus (FCoV). FCoV infection is endemic amongst cats worldwide. In the UK, around 40% of the domestic cat population has been infected with FCoV and in multi-cat households this figure increases to almost 90% *(Addie 2000, Addie and Jarrett 1992, Hartmann 2005, Sparkes 1992)*. FIP usually arises sporadically and unpredictably, with only a small percentage of cats developing FIP within the first three years of entering a seropositive household. Rarely FIP can arise as an 'outbreak' in a group of cats over a short period of time *(Pedersen 2009, Potkay and others 1974)*. FIP is extremely distressing to deal with, for both cat owners and veterinary surgeons, because of the difficulties in achieving an ante mortem diagnosis, the fatal nature of the disease, and the difficulties of control of FCoV infection.

What Causes FIP?

During natural FCoV infection the virus replicates within enterocytes, particularly of the colon and to a lesser extent the small intestine *(Kipar and others 2010)*.

Concurrently viral RNA is variably detectable in mesenteric lymph nodes, liver, lungs and other organs, typically within specialised resident macrophages in the absence of pathology, providing potential sources for recurrent viraemia and persistent infection. Infections are usually asymptomatic or result in transient mild gastrointestinal disease (e.g. diarrhoea). Viral particles are shed in the faeces and subsequently ingested by a susceptible cat. Risk factors for the development of the disease are multifactorial *(see Fig. 1)*, but a detailed discussion of these risk factors and their management are beyond the scope of this article.

In a small number of individual cats the infecting FCoV becomes capable of replicating extensively within monocytes/macrophages leading to pathological changes that culminate in vasculitis and granuloma development in organs *(Kipar and others 2005)*. In the early stages of disease the clinical signs may be vaguesigns consistent with a systemic inflammatory response, such as lethargy, pyrexia and weight loss, are often present. Subsequently the vasculitis can result in the peritoneal pleural and pericardial effusions seen in the 'wet' form of the disease. In contrast the 'dry' form of the disease is characterised by the organ system most affected by the granuloma formation e.g. neurological dysfunction with central nervous system involvement, uveitis with ocular involvement. It has been proposed that the molecular

switch permitting the replication in monocytes / macrophages, and the subsequent development of FIP, arises from nucleotide mutation(s) in less pathogenic FCoVs in individual infected cats; known as the "internal mutation" hypothesis *(Pedersen 2009)*.

An alternative "virulent/avirulent" hypothesis had been proposed, which stated that distinct populations of enteric and FIP FCoV strains are circulating in cat populations, and that these are independently acquired *(Brown and others 2009)*. Recently whole genome sequencing data identified a genetic mutation, common to the >90% FIP tissue-derived FCoVs, and present in none of the asymptomatic faeces-derived FCoVs, *(Chang and others 2012)*.

This genome mutation provides a very useful potential future target for FIP diagnostics but does not completely confirm the "internal mutation" hypothesis and exclude the possibility of other explanations in other situations. This is because the FCoV genome mutation rate is rapid, meaning that this genome mutation should be generated many times over during the course of a typical FCoV infection in a cat. However FIP only arises sporadically in FCoV-infected cats, suggesting that factors other than the described genetic mutation also play a role in the development of FIP. Host factors are likely to play a role in this.

SMALL STABLE GROUPS

OVERCROWDING

HYGIENE, CLEAN KITTENS, SPOTLESS LITTER TRAYS

ENVIRONMENTAL CONTAMINATION

CONTROL OTHER DISEASES

DELAY REHOMING

STRESS

SEPARATE ISOLATION AND KITTENING UNITS

SHEDDING

MODULATE IMMUNE RESPONSE

AVOID SAME PAIR MATINGS?
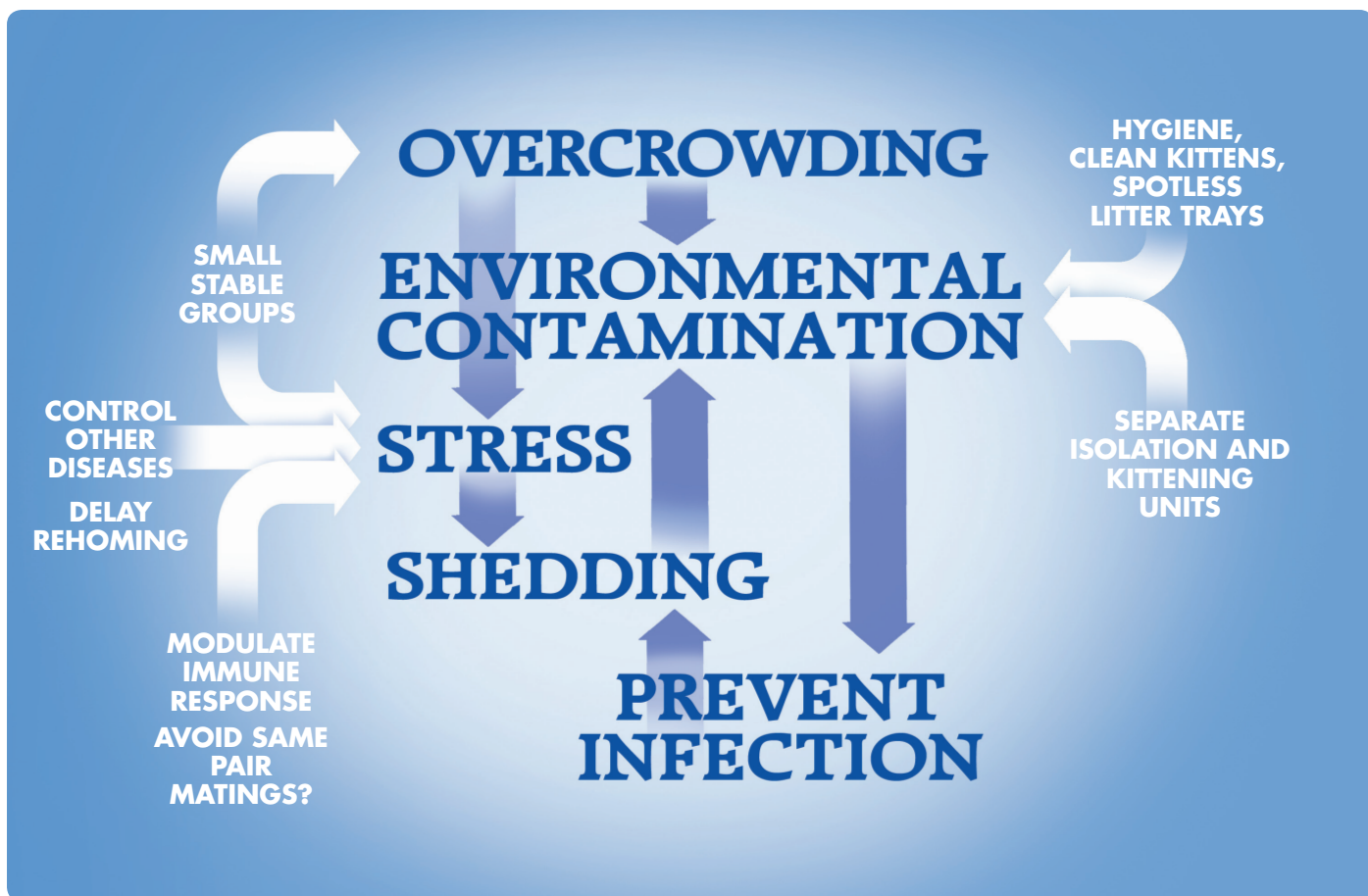
PREVENT INFECTION

Fig. 1: Risk factors involved in Feline Infectious Peritonits development and their management.

Diagnosing FIP

FIP can be difficult to definitively diagnose despite a high degree of clinical suspicion based on history, clinical signs and routine laboratory tests.

History & Clinical Signs

The wide range of clinical signs makes FIP a differential in many different clinical cases. However, history and clinical signs can be used to increase the index of suspicion.

- FIP is most common in young cats (<3 years), but a smaller peak also occurs in older cats (>10 years).
- Pedigree cats and cats from multicat households are at increased risk.
- A recent history of stress (rehoming, neutering, introduction of new cats, vaccination) may be apparent.
- Typical clinical signs of FIP: lethargy, anorexia, weight loss, pyrexia, jaundice, ascites *(see Fig. 2)* and/or pleural effusion and/or pericardial effusion, neurological signs and/or ocular changes etc.

NB: FIP is a progressive disease: clinical signs change over time so it is important to repeat clinical (including ophthalmic and neurological) examinations.

Blood Tests

Haematology and serum biochemistry can support a diagnosis of FIP, and although changes are largely non-specific they can used to increase the index of suspicion.

Haematology

- Lymphopenia (55-77% of cases).
- Neutrophilia (39-55% of cases).
- Mild to moderate normocytic, normochromic anaemia (37-54% of cases).

Serum Biochemistry

- Hyperproteinaemia (up to 60% of cases).
  - hyperglobulinaemia.
  - low or low-normal serum albumin.
  - albumin: globulin (A:G) ratio.
    low (< 0.4) = FIP very likely.
    high (> 0.8) = FIP very unlikely.
  - Hyperbilirubinaemia (21-36% of cases; especially in effusive cases; magnitude increases as the disease progresses).
  - Liver enzymes (ALT, ALP & GGT) often normal or only mildly or moderately elevated.

Additional serum testing

- Protein electrophoresis
  - increased $\alpha 2$- globulins (mostly haptoglobin).
  - increased γ-globulins
- Raised $\alpha 1$-acid glycoprotein (>0.48 mg/ml is abnormal but levels in FIP cases are often markedly elevated at >1.5 mg/ml)

Fig. 2: British Shorthair with 'wet' FIP showing abdominal distension consistent with ascites.

FCoV Serology

Commercial testing of serum FCoV antibodies typically use enzyme-linked immunosorbent assays (ELISAs) or indirect immunofluroescence antibody (IFA) tests. They only test for the presence of antibodies against any type of CoV and cannot differentiate antibodies induced by FIP-causing FCoVs from those not associated with disease. Methodology and antibody titre results can differ between different laboratories (so one cannot directly compare results). A positive FCoV antibody test **only** indicates that the cat has been infected with an FCoV and has seroconverted. Seroconversion takes 2-3 weeks. Although cases of FIP tend to have higher antibody titres than non-FIP cases, the degree of overlap makes interpretation in an individual cat difficult. Indeed, most seropositive cats will never develop FIP, and around 10% of cats with FIP are seronegative.

> Effusion samples (usually peritoneal or pleural) are very helpful in the diagnosis of FIP. They may be classified as exudates based on their high protein concentration (>35 g/l) but are more of a modified transudate based on their low cell counts (usually <10 x10^9 cells/l).

Body Cavity Effusions

Identification and analysis of effusions can be very useful in the diagnosis of FIP. Ascites is the most commonly encountered body cavity effusion; however, pleural effusion and / or pericardial effusion may be present in the presence or absence of ascites. Repeated imaging (especially ultrasonography) can be useful to detect subtle effusions and direct fluid sampling. Characteristics of FIP effusions include:

- They are usually clear, viscous and straw-yellow in colour.
- Typically they have a total protein concentration of >35 g/l and a predominance (>50%) of globulins.
- Similar biochemical changes to those found in the serum exist in effusions: i.e. low A:G ratios, increased α2 globulins and γ-globulins, and markedly elevated α1-acid glycoprotein levels.
- They are often (but not always) poorly cellular. Cell counts are usually <10, (but occasionally counts higher than 25 x10^9/l have been reported). The cell types most frequently are non-degenerate neutrophils, macrophages and lymphocytes.

NB: Lymphocytic cholangitis, malignancy (e.g. lymphoma) and bacterial peritonitis can produce abdominal effusions of a similar nature to FIP; remember that cytology (neoplastic cells and large numbers of [septic] neutrophils respectively) may help differentiate the latter two diagnoses, whilst lymphocytic cholangitis will be accompanied by at least moderate increases in liver enzymes (esp. ALP and GGT).

Reverse-transcriptase (RT-) polymerase chain reaction (PCR) for detecting FCoV

RT-PCR can detect viral FCoV RNA in blood, effusions, faeces (to detect FCoV shedders) or tissue samples. Current PCR assays detect any FCoV and are not specific for those associated with FIP. The use of RT-PCR to detect FCoV in blood samples showed promise in some studies, although the level of FCoV in the blood of cats affected with FIP can be very low. RT-PCR on effusion or tissue samples is potentially more helpful. Recent studies suggest that FCoV RNA can be amplified by RT-PCR from the vast majority of FIP effusion samples tested, but not from non-FIP effusions *(Held and others 2011, Tsai and others 2011)*. Work at the University of Bristol has found similar RT-PCR results using effusion samples, and also of tissue samples, although non-invasive collection of tissue samples is obviously more difficult. In the future RT-PCR performed on tissue samples collected by minimally invasive techniques e.g. Tru-Cut biopsy, may become a useful diagnostic test as it is quicker to perform than histopathology. However, further studies are required to assess the sensitivity and specificity of RT-PCR, as cats with intestinal FCoV infection in the absence of FIP can also be viraemic, whilst those with FIP can have low blood copy numbers and the tissues biopsied may not contain granulomatous lesions. To date there are no commercial RT-PCR tests for the detection of the FCoV genome mutation associated with the FIP-phenotype *(Chang and others 2012),* but this shows promise for future diagnostic tests for FIP.

Histopathological examination of tissues

Routine histopathology

Historically a definitive diagnosis of FIP relied on histopathological examination of affected tissues and the identification of characteristic changes (pyogranulomatous parenchymal foci, perivascular mono-nuclear infiltrates, fibrinous polyserositis). Samples of tissue, typically from mesenteric lymph nodes, liver, kidney and spleen or less commonly from the thorax (these are harder to obtain), can be collected ante mortem (by ultrasound-guided percutaneous Tru-Cut biopsy, laparoscopy or laparotomy) or at post-mortem. Histopathology has been used as the "gold standard" diagnostic test for the diagnosis of FIP *(Hartmann and others, 2003)*. However, routine histopathology is not 100% sensitive: lesions may be missed due to their multifocal distribution e.g. if small samples are taken, or if non-affected organs being sampled *(Giordano and others 2005)*. Immunostaining for FCoV antigen (see below) can be used to further confirm a diagnosis of FIP, and can be used in cases that have an absence of classical histopathology changes *(Giori and others 2011)*.

Immunological staining of FCoV antigen

Immunohistopathology or immuno-cytology staining of formalin-fixed tissues or effusion cytology samples, respectively, has been used to identify FCoV antigen associated with pathology in tissues or in the cells of an effusion. Positive immunological staining of tissues is said to confirm a diagnosis of FIP (i.e. it is very specific), although a negative result does not exclude FIP as FCoV antigens may be variably distributed within lesions *(Giordano and others 2005)*. Immunostaining of effusion samples has also shown variable sensitivity: a false negative result may be obtained if the effusion is cell-poor (i.e. few macrophages in the sample), or if the FCoV antigen is complexed by FCoV antibodies in the effusion. An abstract at a recent conference *(Held and others 2011)* reported that two of 50 cats without FIP had positive immunostaining on their effusions. However, immunostaining is a useful adjunct test in the diagnosis of FIP. It is available from the Veterinary Laboratory Services, School of Veterinary Science, University of Liverpool, United Kingdom.

Conclusions

Many features of a cat's history, clinical signs and laboratory testing can increase our suspicion of a diagnosis of FIP, potentially to the point that a presumptive diagnosis of FIP can be made, particularly in the face of owner financial constraints or clinical deterioration. A definitive diagnosis can be made in the majority of cats with using histopathology and immunostaining. However, no test is 100% sensitive or specific and it is important not to interpret any clinico-pathology results in isolation. RT-PCR shows promise as an additional non-invasive test for the diagnosis of FIP but further work is required to fully determine its sensitivity and specificity. Detection of the FCoV genome mutation associated with the FIP-phenotype may have the potential to increase the specificity of RT-PCR in the future.

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