



Feline Coronavirus & Feline Infectious Peritonitis

ABCD¹ Guideline with literature review

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Key Points

- Feline coronavirus (FCoV) is a ubiquitous virus of domestic and large cats.
- Most FCoV-infected cats either stay healthy or show only mild enteritis.
- Only a small proportion of FCoV-infected cats goes on to develop feline infectious peritonitis (FIP).
- FCoV transmission is faecal-oral via litter trays and fomites.
- FCoV infection of monocytes is the key event in FIP pathogenesis.
- Most likely, internal mutations of FCoV (to mutants with a switch of cell tropism arising in an individual cat) are the reason for the development of highly pathogenic FIP-inducing FCoV (internal mutation theory).
- Coronaviral genomes possess a high level of genetic variation due to the error rate of RNA polymerase leading to different types of mutations.
- FIP disproportionately affects pedigree cats under two years old.
- Sampling the effusion, when present, is the most useful diagnostic step.
- The definitive diagnosis of FIP relies on consistent histopathological changes in affected tissues and this, together with FCoV antigen immunostaining, is considered the gold standard for diagnosis.
- Faecal RT-PCR is not useful for diagnosis of FIP but for identification of FCoV shedders within a cat colony.
- A positive FCoV antibody test is not confirmatory of FIP (it is not a „FIP-test“) but absence of FCoV antibodies makes FIP less likely.
- Without treatment with new potentially curative anti-coronaviral drugs (not yet widely available), FIP has a very poor prognosis.
- ABCD FIP diagnostic trees (please see the ABCD Tool “FIP diagnostic trees“) provide information on how to reach a ‘likely’ diagnosis of FIP in cats to increase confidence in the use of antivirals.
- The ABCD considers the FIP vaccine to be non-core and it is not recommended in FCoV antibody-positive cats. However, FCoV antibody-negative kittens could potentially benefit from vaccination.

Agent properties

Virus classification

Some key aspects of virus properties are shown in Figs. 1 and 2. Feline Coronavirus (FCoV) is a large, spherical, enveloped virus particle and is classified in the order *Nidovirales*; family *Coronaviridae*; genus *Alphacoronavirus*; species *Alphacoronavirus 1*, which also includes canine enteric coronavirus (CCoV), transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCoV) (De Groot et al., 2012). The newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is very distinct and different to FCoV, belonging to a different genus; SARS-CoV-2 is a member of the genus *Betacoronavirus* (Haake et al., 2020) and separate guidelines on SARS-CoV-2 in cats have been published (Hosie et al., 2021) together with updated information available in the ABCD Guideline on SARS-CoV-2 and cats.

Virus genome and structure

Being an enveloped virus, FCoV is readily inactivated by most disinfectants, steam and washing at 60°C. It has been suggested it preserves its infectivity for days to a few weeks, depending on environmental conditions (Scott, 1988).

The 5' two-thirds of the positive sense coronavirus (CoV) genome consist of two overlapping open reading frames (ORFs 1a and 1b) that encode non-structural polyprotein (pp) 1 (pp1a and pp1b) (Fig. 1). The polyproteins are cleaved into individual non-structural proteins (nsps), including RNA-dependent RNA polymerase, that plays a role in viral replication. ORF 1a also encodes for viral proteases including the viral 3C-like protease being a potential target for antiviral therapy (see treatment section). The other third of the genome consists of ORFs encoding structural proteins, spike [S], matrix [M], nucleocapsid [N] and envelope [E] (see Fig. 2) and some non-structural accessory proteins 3a, 3b, 3c, 7a and 7b (see Fig. 1) (Terada et al., 2014). The non-structural proteins are involved in the replication of the virus, and modification of the host immune response, but are not incorporated into the mature virus particle.

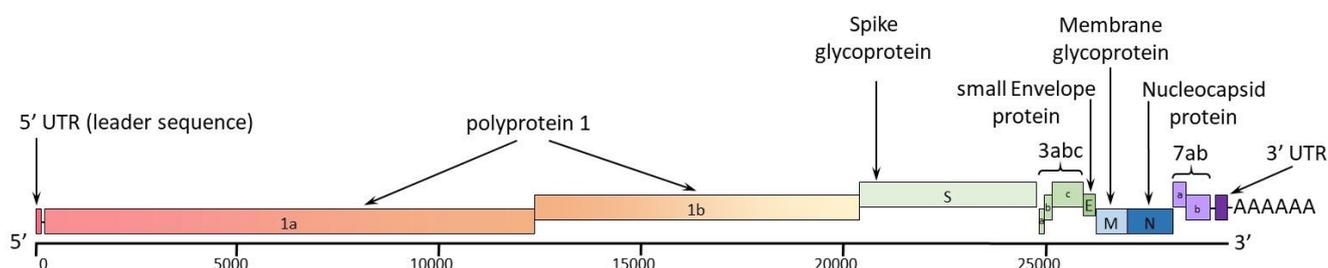


Fig. 1. FCoV genome; *Courtesy of Emi Barker, Langford Vets, University of Bristol (Barker and Tasker 2020b)*. FCoV is a single stranded RNA virus. The FCoV genome of 27–32kb encodes a replicase polyprotein, four structural proteins (spike [S], membrane [M], nucleocapsid [N] and envelope [E]) and non-structural accessory proteins 3 a,b,c and 7a,b. UTR indicates untranslated region.

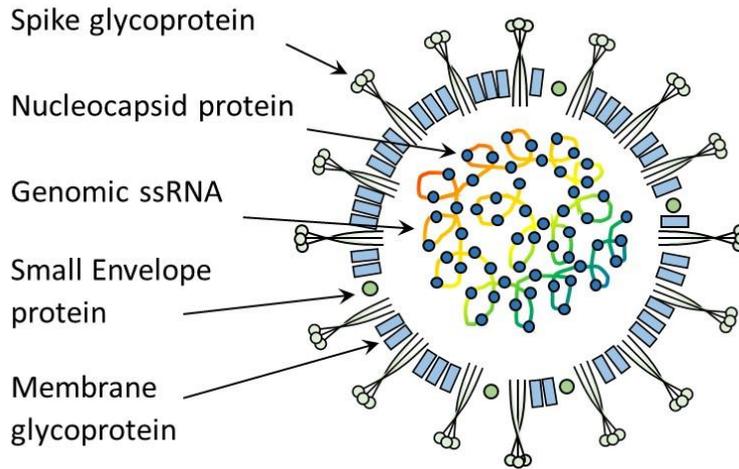


Fig. 2. FCoV structure; *Courtesy of Emi Barker, Langford Vets, University of Bristol (Barker and Tasker 2020b).* Schematic diagram showing single stranded (ss) RNA and the structural proteins: spike, envelope, membrane and nucleocapsid proteins.

Type I and type II FCoVs

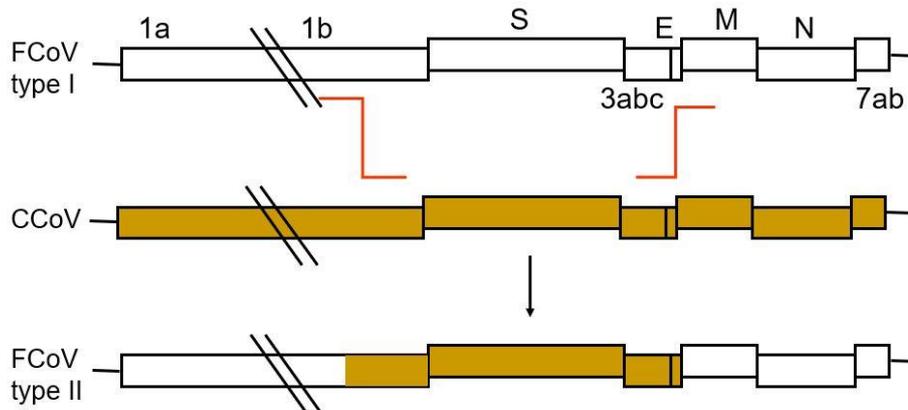


Fig 3. Origin of Feline coronavirus (FCoV) type II; *Courtesy of Peter Rottier, University of Utrecht.* Schematic diagram showing how type II FCoV arises from recombination of FCoV type I (shown in white) with CCoV (shown in brown).

Type II FCoV strains arise from recombination with CCoV (Fig. 3), usually including the spike of CCoV and varying amounts of adjacent ORF 3 genes (Herrewegh et al., 1998; Le Poder et al., 2013; Terada et al., 2014). The RNA-dependent RNA polymerase makes a full-length negative-strand RNA copy of the genome as well as a nested set of smaller subgenomic RNAs (RNAs) with a common 3' end. These negative strand RNAs serve as templates for new positive sense genomes and positive sense subgenomic mRNAs. The subgenomic mRNAs have a nested-set structure with sequences starting at the 3' terminus and extending to various distances toward

the 5' end. If a real-time reverse-transcriptase (RT)-PCR assay is designed to amplify 3' subgenomic mRNAs, this can influence the quantitative results for apparent FCoV load (see reverse-transcriptase (RT)-PCR section in diagnosis). In general, only the 5' most ORF of each subgenomic mRNA is used for encoding of the proteins even though the subgenomic mRNAs have more than one coding sequence (except of the smallest one).

FCoV genome mutations

Coronaviral genomes possess a high level of genetic variation due to the error rate of RNA polymerase leading to different types of mutations including insertions, deletions, and introduction of stop codons as well as recombinations. The hypothesis is that genetic variation and subsequent selection also facilitates switching of cell tropism within an FCoV-infected cat that develops feline infectious peritonitis (FIP).

FCoVs are assigned to two pathotypes or biotypes generally referred to as feline enteric coronavirus (FECV), which mainly replicates in the enteric epithelium, and feline infectious peritonitis virus (FIPV), which results in a mostly lethal infection with efficient replication in monocytes or macrophages (Barker and Tasker 2020b). Since it is known that all FCoV can be found and replicate systemically (Meli et al., 2004; Kipar et al., 2006; Fish et al., 2018) (even in cats without FIP), we prefer to call both biotypes “FCoV” but distinguish them as a “less virulent FCoV” and an “FIP-associated FCoV”. Therefore, these terms will be used when possible in this guideline to underline the real differences in biological behaviour between the FCoV.

Although the genes involved in the FCoV virulence shift are still unknown, mutations in different genes have been postulated to be associated with the switch of the less virulent FCoV into the virulent FIP-associated FCoV, including the spike gene and accessory genes 3c and 7b (Pedersen et al., 2012) (see Fig. 2). The spike proteins are the main determinant of entry into host cells (Belouzard et al., 2012), as they possess both receptor binding and fusion functions (Millet and Whittaker 2015). Two alternative amino acid differences in the putative fusion peptide of the S protein (called M1058L and S1060A – nomenclature based on position and nature of amino acid change i.e. methionine to leucine at position 1058 and serine to alanine at position 1060) have been detected that together distinguished FIP-associated from less virulent FCoV in 95.8% of cases in one study (Chang et al., 2012) and 93% of cases in another (Decaro et al., 2021).

Another mutation was detected in the cleavage site between the receptor binding (S1) and the fusion domain (S2) of the spike protein. While all less virulent FCoV had a conserved furin cleavage site, in most FIP-associated FCoV at least one substitution was found (Licitra et al., 2013). Other mutations in the S1/S2 cleavage site have been described (Healey et al., 2022). Mutations in the heptad repeat 1 region of the S gene are also said to be associated with FIP (Bank-Wolf et al., 2014; Lewis et al., 2015).

The ORF 3 gene encodes for a protein for which the function is still unknown. Interestingly, mutations leading to a truncated protein were detected in approximately two-thirds of the 3c genes of FCoV found in cats with FIP (Pedersen et al., 2009; Chang et al., 2010a; Hsieh et al., 2013), while the ORF 3 gene was intact in all FCoV in faecal samples. This suggests that an intact 3c is an absolute requirement for infection of the gut epithelial cells (Chang et al., 2010a; Pedersen et al., 2012), but is not necessary for replication in monocytes. FIP-associated FCoV with an intact 3c will replicate in the gut but this virus does not seem to be transmitted to other cats (Pedersen et al., 2012).

There is a general consensus that the less virulent FCoV converts to the FIP-associated FCoV in the individual cat by modifications that include a cell tropism change from enterocytes to monocytes/macrophages (Pedersen

et al., 2009; Chang et al., 2012; Barker et al., 2013). This so-called internal mutation theory is supported by several studies showing a close genetic relationship between the FIP-associated FCoV and FCoV from faecal samples of cats living in the same environment (Chang et al., 2011; Decaro et al., 2021), representing a much closer relationship than to FCoV collected from cats of other environments. The internal mutation theory was questioned at one point based on the results of a single study that indicated that “FECV” and “FIPV” were two distinct types of FCoV circulating independently in the population (Brown et al., 2009). However, in that study, samples were derived from a population of shelter cats, a population in which introduction of different genetically unrelated FCoV can be expected because of their different geographic origin (Pedersen 2014b). The internal mutation theory has now been widely accepted amongst researchers.

In addition to distinguishing the two pathotypes, the less virulent FCoV and the FIP-associated FCoV, there is another classification based on differences in antigenic and genomic properties in type I and type II FCoV. Both, type I and type II FCoV can occur as less virulent FCoV and as FIP-associated FCoV (Pedersen 2014b). Type I FCoV is most prevalent worldwide (Addie et al., 2003; Lin et al., 2009; Soma et al., 2013; Terada et al., 2014; Wang et al., 2014; Decaro et al., 2021, Lin et al., 2022). Type II FCoV results from double recombination between type I FCoV and CCoV (Herrewegh et al., 1998; Terada et al., 2014) within the feline cell, which permits entry of both FCoV and CCoV (Tusell et al., 2007; Terada et al., 2014). Recombination events have occurred many times across the world, with individual type II FCoV containing CCoV spike genes and variable amounts of 3abc, and envelope genes, but not the nucleocapsid gene, which remains of FCoV origin (Herrewegh et al., 1998; Terada et al., 2014) (see Fig. 3). Most research has focused on type II FCoV strains since they, unlike type I FCoV, can be readily propagated *in vitro* (Pedersen et al., 1984), facilitating experimental studies, despite most field infections being type I FCoV. Experimental studies have tried to develop culture methods for type 1 FCoVs using both permanent feline intestinal epithelial cell cultures of ileocyte and colonocyte origin (Desmarests et al., 2013) and colonic organoid preparations (Tekes et al., 2020), but neither are currently routinely available for use.

Epidemiology

Transmission of FCoV

FCoV is a contagious virus, and transmission is often indirect, such as contact with objects (e.g. via litter trays, scoops, brushes, vacuum cleaners, shoes), handling at cat shows, shelters or in a veterinary practice. Faeces are the main source of FCoV, with litter boxes representing the principal source of infection in groups of cats. Cats are most likely to be infected orally following contact with FCoV in faeces. Thus, the major route of transmission is faecal-oral.

A case report (Andre et al., 2020), documenting FIP-associated rhinitis, suggested that the respiratory tract might be a place of entry for transmission for FCoV, but further studies are required. Since virus is found only rarely in the saliva of healthy cats, close contact or sharing feeding bowls are not major routes of infection (Addie and Jarrett 2001). Transplacental transmission has been described from a queen that developed FIP during pregnancy (Pastoret and Henroteaux 1978), but this phenomenon is extremely rare (Addie and Jarrett 1990). A study (Stranieri et al., 2020a) evaluated testicular tissue and semen in tom cats for FCoV by RT-PCR to evaluate the risk of venereal transmission of FCoV. FCoV RNA was amplified from around 15% (6 of 39) of testicles in the study and none of the 17 semen samples tested, suggesting venereal transmission was unlikely. Transmission of FCoV via blood transfusion has not been reported.

In FCoV infected breeding catteries, kittens commonly become infected at a young age within a few weeks of age (Lutz et al., 2002) (see also “Pathogenesis and Immunity”).

After natural infection, cats begin to shed virus in the faeces within one week (Meli et al., 2004) and continue to shed for weeks, months, and a few even for life (persistent) (Addie and Jarrett 2001; Addie et al., 2003; Meli et al., 2004; Pedersen et al., 2008). Shedding is usually intermittent and recurrent (although a few cats appear to recover and no longer shed after an initial period of shedding), but some cats have persistent shedding (Addie et al., 2003; Pedersen et al., 2008), which may be influenced by the dose of virus received at inoculation (Vogel et al., 2010). Faecal excretion reaches high levels (Addie and Jarrett 2001; Addie et al., 2003; Pedersen et al., 2008; Vogel et al., 2010). The higher the FCoV antibody titre, the greater is the chance of the cat shedding FCoV (Addie and Jarrett 2001; Lutz et al., 2002; Pedersen et al., 2008; Addie et al., 2015; Felten et al., 2020)), as well as the greater the frequency of faecal FCoV shedding and FCoV load present (Felten et al., 2020). Due to the suspected short duration of any immunity following infection, failure to separate non-shedding cats from virus shedders could favour spread and persistence of FCoV in a household, which could account for the high antibody prevalence in the multi-cat environment.

Horizontal transmission of FIP, in contrast to FCoV, is not believed to occur very frequently, if at all (see Pathogenesis and Immunity, and Disease Management sections).

Although FCoV and CCoV are closely related, contact with dogs does not appear to be a major predisposing factor for CoV infection of cats (Le Poder et al., 2013). However, (Benetka et al., 2006) found feline/canine CoV recombinant viruses in cats of a rescue shelter which housed both cats and dogs. In the M protein gene these strains were more closely related to FCoV-like CCoV than to FCoV, suggesting that infection with CCoV and subsequent recombinations with FCoV had occurred within this environment.

Prevalence of FCoV

With the exception of a few islands of isolated feline populations (e.g. the Falkland Islands) (Horzinek and Osterhaus 1979; Levy et al., 2008; Addie et al., 2012), FCoV infection has been reported worldwide. FCoV, and therefore FIP, is particularly common where conditions are crowded (Sharif et al., 2009; Felten et al., 2020), while the prevalence is lower in individually housed, stray or feral cats (Addie and Jarrett 1992; Herrewegh et al., 1995; Addie 2000; Cave et al., 2004; Bell et al., 2006b; Taharaguchi et al., 2012), although some feral cat studies have found very high prevalences (Posch et al., 2001); this is probably related to the population tested and their interactions and litter substrate habits. Wild felids, especially those in zoos, can also be FCoV-infected (Kennedy et al., 2002). FCoV-infected cheetahs are even predisposed to develop FIP (Evermann et al., 1988).

FCoV is highly contagious, and in households where it is present, prevalence of antibodies indicating exposure is often close to 100% (Felten et al., 2020). Cats who spent over 60 days in UK shelters were five times more likely to have antibodies (Cave et al., 2004).

In a Japanese study including 17,392 cats, the antibody prevalence was 66.7% in purebred cats, and 31.2% in domestic cats (Taharaguchi et al., 2012). Prevalence increased greatly in purebreds by three months of age, while it did not fluctuate greatly in non-pedigree breeds with aging, indicating that cattery environments can contribute to FCoV epidemics. Purebred cats from northern regions of Japan were commonly antibody-positive (76.6% in Hokkaido, 80.0% in Tohoku), indicating that cattery cats in cold climates might be more closely confined. Among purebred cats in Japan, the breeds American shorthair, Himalayan, Oriental, Persian, and

Siamese showed low antibody prevalence, while the breeds American curl, Maine coon, Norwegian forest cat, ragdoll and Scottish fold had high antibody prevalence (Taharaguchi et al., 2012).

The prevalence of FCoV in various countries is given in table 1.

Table 1: Prevalence of FCoV in various countries

Country	What detected	Number of cats	Prevalence	Reference
Australia (Sydney)	Antibodies	49 feral cats 306 owned cats	0 % 34 %	(Bell et al., 2006a)
Austria	Antibodies	157 cats without FIP: 17 single cats 31 multi-cat household 99 shelter cats 10 feral cats	71 % 59 % 84 % 68 % 90 %	(Posch et al., 2001)
Falkland Islands	Antibodies	10 feral cats 95 pet cats	0 % 0 %	(Addie et al., 2012)
Germany	Antibodies FCoV RNA in faeces	82 cats from 19 breeding catteries	78% 71%	(Felten et al., 2020)
Japan	Antibodies	17,392	66.7 % in purebred cats 31.2 % in domestic cats	(Taharaguchi et al., 2012)
Korea	Antibodies	212 total 107 pet cats 105 shelter cats 129 healthy cats 83 sick cats	13.7 % 14.0 % 13.3 % 10 % 19.3 %	(An et al., 2011)
Korea	FCoV RNA in faeces	212	6.6 %	(An et al., 2011)
Malaysia	FCoV RNA in faeces	24 cats in a Persian cattery 20 cats in a rescue cattery	96 % 70 %	(Sharif et al., 2009)
Sweden	Antibodies	209	17 % domestic 65 % pedigree	(Ström Holst et al., 2006)

Country	What detected	Number of cats	Prevalence	Reference
Switzerland	Antibodies	466 DHS and DLH cats 143 purebred cats	49 % 78 %	(Fehr et al., 1997)
Taiwan	Antibodies	760 healthy cats	28.2 %	(Wang et al., 2014)
Turkey (Bursa province)	Antibodies	100	21 %	(Pratelli et al., 2009)

Prevalence and risk factors for FIP

The prevalence of FIP within a cat population as a whole was 0.52% (60 of 11,535) of all the cats examined at the North Carolina State University College of Veterinary Medicine (1986 – 2002), which is high considering the fact that US university teaching hospitals are tertiary referral centres (Pesteanu-Somogyi et al., 2006). In multi-cat environments, such as at breeders and shelters, up to 12% of FCoV-infected cats can succumb to FIP (Addie and Jarrett 1995). The incidence of FIP in a household or cattery increases with the number of cats (Kass and Dent 1995).

While cats of any age or breed can develop FIP, FIP disproportionately affects pedigree cats under two years old (Rohrbach et al., 2001; Norris et al., 2005; Pesteanu-Somogyi et al., 2006; Tsai et al., 2011; Worthing et al., 2012; Soma et al., 2013; Riemer et al., 2016). In Australia, 71% of cats with FIP were purebred and 55% less than two years old (Norris et al., 2005). In a North Carolina study, 67% of cats with FIP were less than two years, and pedigree cats were also over-represented: FIP was present in nearly 1.3% of the pedigree cats compared to 0.35% in mixed breed cats, and breed predisposition was statistically significant in the Abyssinian Bengal, Birman, Himalayan, Ragdoll and Rex breeds (Pesteanu-Somogyi et al., 2006). In a study in Australia, domestic crossbred, Persian and Himalayan cats were significantly under-represented in the FIP cohort, while several other breeds were over-represented, including British Shorthair, Devon Rex and Abyssinian (Worthing et al., 2012).

Percentage of effusions that were positive by FCoV reverse-transcriptase (RT)-PCR varied with the cat's breed and age in a study in Japan (Soma et al., 2013) and with age in a study in China (Line et al., 2022). In these studies FIP was not confirmed as a diagnosis and RT-PCR used on effusions to indicate FIP. In cats up to one year, 95% of effusions of pedigree cats were RT-PCR positive, whereas FCoV RNA was only found in 79% of the effusions of domestic cats, and up to the age of five years, effusions of purebred cats were more likely to be FCoV RT-PCR-positive than were those from domestic cats.

Some authors have noted a predisposition for FIP in male over female cats (Rohrbach et al., 2001; Benetka et al., 2004; Norris et al., 2005; Worthing et al., 2012; Riemer et al., 2016), while others found no sex predisposition (Pedersen 1976). Pedigrees of cats that die of FIP can often be traced back to the stud cat, rather than the queen (Foley and Pedersen 1996).

Pathogenesis

As noted above, the major route of FCoV infection is faecal-oral. Following ingestion of the virus, such as by grooming paws contaminated during litter tray use or from eating fomite-contaminated food, the virus first enters and replicates within the epithelial cells of the small intestinal villi. Type II FCoV uses the feline aminopeptidase-N receptor (fAPN) present on the intestinal villi and the monocyte (Tusell et al., 2007; Tekes et al., 2010). The receptor for type I FCoV remains unknown (Dye et al., 2007; Tekes et al., 2010).

FCoV shedding occurs in the faeces from two to three days post-infection (Meli et al., 2004; Kipar et al., 2010). This infection is not usually associated with clinical signs, but sometimes is accompanied by enteritis (Sabshin et al., 2012). Occasionally, very severe, indeed fatal, coronavirus enteritis has been reported (Kipar et al., 1998). As described earlier, virus shedding of type I FCoV in faeces follows two patterns. (1) Most transiently infected cats shed virus for two to three months (Addie and Jarrett 2001) - immunity is short-lived because these cats can be re-infected by the same or a different strain of FCoV, within a few weeks (Addie et al., 2003), showing recurrent infections. (2) Around 13% of cats infected with type I FCoV become persistently infected carrier cats. However, in contrast, cats experimentally infected with type II FCoV shed virus for around two weeks (Stoddart et al., 1988) and no carrier cat has been reported. Only a small proportion of FCoV-infected cats goes on to develop FIP (Pedersen 1987; Kipar et al., 2005).

From two weeks post infection, the virus is found in the colon (Kipar et al., 2010). In persistently infected asymptomatic carrier cats, the ileo-caecocolic junction is the main site of viral replication (Herrewegh et al., 1997).

The mesenteric lymph nodes (MLNs), as the most likely first site of FCoV spread from the intestine regardless of subsequent viraemia, have been evaluated for mediators of the innate immune response, and evidence for toll-like receptor involvement has been found in the response to FCoV infection (Malbon et al., 2019).

Efficient FCoV replication in activated monocytes and macrophages is a key event in FIP pathogenesis (Malbon et al., 2020a): whether or not the cat will go on to mount a successful immune response and eliminate the virus; whether the cat will mount a semi-successful immune response, remaining clinically well, but shedding FCoV in the faeces for months to years; or whether the cat will mount a deleterious immune response, resulting in a widespread pyogranulomatous vasculitis and ultimately premature death without effective antiviral treatment. The outcome of infection of the monocytes and macrophages is partially dependent on the cell; however, virulent strains do replicate more efficiently within permissive monocytes and macrophages (Dewerchin et al., 2005). Monocytes from an outbred population of cats varied in their ability to sustain FCoV replication regardless of whether the strain of FCoV was deemed very virulent or relatively avirulent, with the monocytes of some cats not sustaining replication of either FIP-associated FCoV or less virulent non-FIP-associated FCoV (Dewerchin et al., 2005). What happens in monocytes and macrophages following FCoV infection is quite extraordinary: usually an infected cell will display viral antigens in association with feline leucocyte antigen (the feline version of the major histocompatibility complex) on its surface to enable antibody-mediated, or cell-mediated, lysis. but in cats with FIP, infected macrophages lacked surface expression of viral antigens (Cornelissen et al., 2007).

FCoV viraemia, when it occurs, is short-lived, peaking about seven to 14 days post-infection and declining thereafter (Kipar et al., 2010; Mustafa-Kamal et al., 2019); thus, by the time clinical signs of FIP appear, viraemia cannot always be detected as RT-PCR on blood samples to detect FCoV RNA has usually been negative in cats

with FIP. However, this pattern has not been observed in recent treatment studies, which found that a high percentage of cats with FIP had detectable FCoV in their blood by RT-PCR at diagnosis (Katayama et al., 2021; Krentz et al., 2021) (see later section on diagnosis for further discussion), suggesting that FCoV RNA detection in blood could be revisited as a potential diagnostic tool for FIP.

The virulence of the virus, the viral load and the cat's immune response determine whether or not FIP will develop. Thus, both viral genetics and host immunity are likely to play a role in the development of FIP (Addie and Jarrett 1995; Dewerchin et al., 2005; Rottier et al., 2005; Hsieh and Chueh 2014; Pedersen et al., 2014; Mustafa-Kamal et al., 2019; Malbon et al., 2020b). Resistance in terms of the ability to fight off FCoV infection increases between six and 12 months of age (Pedersen 2014b).

In those cats in which FCoV is able to replicate freely within the monocytes, infected monocytes attach to the walls of small and medium sized veins, releasing matrix metalloproteinase-9 (MMP-9) which destroys the collagen of the basal lamina of affected vessels. This event permits extravasation of the monocytes, where they differentiate into macrophages. Breakdown of the endothelial tight junctions allows plasma to leak out of the vessels (Kipar et al., 2005). It is believed that death of virus-laden macrophages (apoptosis) plays a key role in FCoV dissemination (Watanabe et al., 2018). In more acute forms of FIP, many blood vessels are affected, and this leakage becomes apparent clinically as an effusion in the abdominal, thoracic and/or pericardial cavities. In more chronic forms of FIP, fewer blood vessels are affected, but the perivascular pyogranulomata can become quite large, even easy to mistake for a tumour on gross examination, at exploratory laparotomy or necropsy. The FCoV-infected macrophages release cytokines such as tumour necrosis factor alpha (TNF-alpha) (Takano et al., 2007b): TNF-alpha upregulates fAPN (Takano et al., 2007b), causes lymphopenia (Takano et al., 2007a) and inhibits neutrophil apoptosis (Takano et al., 2009). The role of TNF-alpha is important in the development of FIP, such that anti-TNF-alpha antibodies have been used as a possible therapy (Doki et al., 2013; Doki et al., 2020b).

As described above, FIP arises in a small percentage of FCoV-infected cats *in vivo*, following FCoV infection, and horizontal transmission of FIP, via a FIP-associated FCoV strain, is not believed to occur very frequently, if at all. Several experimental and field observations support the assumption that cats do not become infected with FIP-associated FCoV via the natural route. First, FIP-associated FCoV strains from different cats of the same household show mostly unique genetic characteristics suggesting that these viruses developed independently in individual cats (Chang et al., 2012; Barker et al., 2013; Licitra et al., 2013). Only a very small percentage of cats with FIP may shed FIP-associated FCoV, most likely because these mutated viruses cannot replicate in enterocytes (Pedersen et al., 2009; Pedersen et al., 2012; Wang et al., 2013; Porter et al., 2014). Furthermore, faecal samples of cats with FIP do not cause disease after oral inoculation (Pedersen et al., 2012). Also, in multi-cat households, FIP cases are often limited to a single (or few) cat and additional cases might not occur for several years. However, a few cases have been reported in which a higher number of cats (>10%) developed FIP in multi-cat environments (Graham et al., 2012; Barker et al., 2013; Wang et al., 2013). In these so-called mini-outbreaks other factors than direct horizontal transmission likely play a role (see chapter "Control of FCoV and FIP in specific situations").

Immunity

FIP is associated with severe suppression of natural killer cells and regulatory T cells, central players in the innate and adaptive cell-mediated immunity (CMI), respectively (Vermeulen et al., 2013). Until the study on FCoV replication in monocytes by (Dewerchin et al., 2005), the outcome of FCoV infection had been mainly attributed to virulence factors (mutations, deletions) in the virus (Pedersen 2014b), although host factors obviously play a role in pathogenesis. One of the most investigated cytokines important in FCoV infection has been interferon gamma (IFN- γ), which is an important modulator of CMI. The expression of IFN- γ mRNA by leucocytes in the circulation or in tissues has been investigated in many studies using RT-PCR and immunohistochemistry (Gunn-Moore et al., 1998; Dean et al., 2003; Kiss et al., 2004; Berg et al., 2005; Gelain et al., 2006). Some studies (Gunn-Moore et al., 1998; Kiss et al., 2004; Gelain et al., 2006) found high IFN- γ mRNA expression in the peripheral blood leucocytes of clinically normal cats with FCoV infection, but low expression in cats with FIP. In contrast, IFN- γ mRNA is abundant within FIP lesions (Berg et al., 2005). (Giordano and Paltrinieri 2009) concluded in their paper that although cats resistant to FIP have strong CMI, which can be measured by high serum IFN- γ production, CMI is also likely to be involved in the pathogenesis of FIP, albeit at a tissue level, as evidenced by high IFN- γ concentration of the FIP effusions. These findings could be the basis of further studies into the mechanisms through which IFN- γ production could prevent the onset of FIP. The importance of CMI in the resistance to FIP was further investigated in an experimental study (Mustaffa-Kamal et al., 2019) in which the antiviral T cell responses were measured during primary and secondary exposure to FIP-associated FCoV. Definitive adaptive T cell responses predictive of disease outcome were not detected during the early phase of primary infection with FIP-associated FCoV but recovery antiviral T cell responses were seen later in primary infection for a subset of cats showing slow progression to FIP or resistance to FIP compared to those showing fast progression to FIP. The emergence of antiviral T cell responses after secondary exposure (rechallenge) to FIP-associated FCoV in cats that were resistant to FIP after primary infection also suggested a role of CMI in the later control of infection with FIP-associated FCoV and disease progression.

(Hsieh and Chueh 2014) investigated whether single nucleotide polymorphisms (SNP) in the feline IFN- γ gene (fIFNG) were associated with the outcome of FCoV infection. Some "FIP-resistant" and "FIP-susceptible" alleles have been suggested, and a subsequent study found an increased frequency of documented fIFNG SNPs in pedigree cats, but small numbers limited statistical analysis (Kedward-Dixon et al., 2020). A larger study (Barker et al., 2020) published on the prevalence of fIFNG SNPs in non-pedigree cats did find a statistical association between presence or absence of FIP and genotype; however, the presence of the 'protective' genotype in 16% of the cats with FIP and its absence in 66% of the cats without FIP limits its use in individual cats or to guide breeding.

The role of humoral immunity in protecting against FIP is ambiguous. Maternally derived antibodies have been suggested to provide protection until about five to six weeks of age (Addie and Jarrett 1992) until they decline and become undetectable by six to eight weeks of age. However, infection at two weeks of age has also been detected rarely (Lutz et al., 2002), questioning protection by maternally derived antibodies. On the other hand, cats with active enteric FCoV infections have strong systemic IgG and mucosal secretory IgA responses that wane after FCoV clearance, with no evidence of a mucosal IFN- γ T cell response, suggesting that humoral responses can control infection (Pearson et al., 2019).

Antibody production to FCoV takes 10-28 days post-infection (Meli et al., 2004; Vogel et al., 2010). Clearance of natural infections has been associated with antibodies directed against the FCoV S protein (Gonon et al., 1999).

Conversely, in experimental infections, antibodies directed against the S protein can be detrimental (Vennema et al., 1990). In cats with pre-existing antibodies, 'antibody dependent enhancement' (ADE) has been observed experimentally, resulting in a more rapid disease course and earlier death. This enhancement was observed irrespective of whether cats had acquired antibodies through passive or active immunization using some experimental vaccine studies (Weiss and Scott 1981; Vennema et al., 1990; De Groot and Horzinek 1995). However, in field studies cats developed FIP on first exposure to FCoV (and thus, had not had pre-existing antibodies) and cats experienced repeated infections by FCoV without developing FIP, leading to the conclusion that ADE is a laboratory phenomenon which is not important in the real world (Addie et al., 1995; Addie et al., 2003). Additionally, an experimental study (Mustaffa-Kamal et al., 2019) documented that nine of ten cats that had not developed FIP following primary infection with a FIP-associated FCoV strain resisted development of disease following rechallenge.

Clinical signs

Clinical signs associated with FCoV infection

FCoV infection does not usually cause any clinical signs in cats following infection. Occasionally it is accompanied by enteritis (Sabshin et al., 2012) with clinical signs of diarrhoea and/or vomiting. Occasionally, very severe, indeed fatal, coronavirus enteritis has been reported (Kipar et al., 1998).

Clinical signs associated with FIP

General clinical signs of FIP

The clinical picture of FIP varies considerably, reflecting the variability in the distribution of the vasculitis and granulomatous lesions. The vasculopathy can result in ('wet') effusions whilst the granuloma formation results in ('dry') mass lesions. A form which includes the development of effusions is regarded as being most common (Sparkes et al., 1991; Tsai et al., 2011; Riemer et al., 2016): 78% of 224 cases of FIP had effusions (Riemer et al., 2016). The distinction between so-called 'effusive' and 'non-effusive' forms of FIP is important for diagnostic purposes; however, there is considerable overlap between the two forms, and indeed FIP cases with effusions also have pyogranulomatous lesions visible at *post-mortem* examination and, similarly, many cats with a non-effusive form will eventually develop effusions. Clinical signs of FIP can change over time, therefore repeated physical examinations are important to detect newly apparent clinical signs; for example, an effusion can develop or ocular changes can become visible on ophthalmoscopic examination.

Non-specific clinical signs can occur in both cats with effusion or without effusion and include lethargy, anorexia and weight loss (or failure to gain weight/stunted growth in kittens), although occasionally some cats remain bright and retain good body condition. A fever, that can be fluctuating and that is moderate and typically <40 °C (but sometimes can be higher) and that is refractory to many drugs and non-responsive to antibiotics, is commonly present. One study describing referral cats with a history of fever found that FIP was the most common diagnosis made, highlighting its importance as a differential diagnosis for fever even at referral level (Spencer et al., 2017). Another study (Riemer et al., 2016), which described the clinical features of FIP, documented fever in 55.8% of FIP cases. Fever was shown to be more common in cats with effusion than in cats without effusion (Riemer et al., 2016).

FIP can be associated with effusion formation in one or more body cavities. Abdominal effusions leading to a clinical presentation of ascites, sometimes with abdominal distension (Fig. 4), are the most common effusions seen with FIP (Riemer et al., 2016).



Fig. 4. Ascites in a young sphinx cat presenting with effusive FIP; *Courtesy of Hannah Dewerchin, Ghent University*

A pleural effusion can be present concurrently to abdominal effusion. In some cats, the effusion is restricted to the thorax; cats with pleural effusion can present with dyspnoea (Pedersen 2009; Beatty and Barrs 2010; Spencer et al., 2017). In a retrospective study including 306 cats diagnosed with pleural effusion of established aetiology, FIP was only diagnosed in 8.5% of cats, while cardiac disease was the most common aetiology (35.3%), followed by neoplasia (30.7%), pyothorax (8.8%) and chylothorax (4.6%). Cats with FIP were significantly younger than those with cardiac disease and neoplasia, and cats with cardiac disease had a significantly lower body temperature, higher serum alanine aminotransferase and alkaline phosphatase activity, and lower protein concentrations and nucleated cell counts in the effusion than cats with FIP (Konig et al., 2019). Pericardial effusions (Fischer et al., 2012b; Baek et al., 2017), with or without effusions in other body cavities, are also occasionally reported. Rarely, effusion in the scrotum is present in intact male cats due to a serositis involving the tunica vaginalis of the testes, leading to scrotal enlargement. When effusions form in FIP, the disease progression is often quite acute in nature, progressing within a few days or weeks and severely limiting survival (Ritz et al., 2007).

When effusions are not present in FIP cases, FIP is often more difficult to diagnose, as fever, anorexia, lethargy and weight loss (or failure to gain weight in kittens) can be the only signs, particularly in the early stages of disease. It also tends to be more chronic than FIP associated with effusions, progressing over a few weeks to months. Additional signs of non-effusive FIP depend on the organs affected by the granulomatous lesions and can include the central nervous system (CNS), eyes and/or abdominal organs (such as the liver, abdominal lymph nodes, kidney, pancreas, spleen and/or gastrointestinal tract) (Norris et al., 2005), but such signs can also

be seen in cats with effusions, so they are not restricted to non-effusive FIP. As well as granulomatous lesions, the kidneys can sometimes show immune-mediated glomerulonephritis when affected by FIP; this is caused by immune complexes through the excessive production of non-neutralizing antibodies in FIP (Hartmann et al., 2020).

Renomegaly, but also occasionally a reduction in kidney size, can occur. A pyogranulomatous pneumonia is occasionally seen (Trulove et al., 1992; Macdonald et al., 2003) causing respiratory signs. Abdominal lymphadenomegaly can be present. In one retrospective study of suspected cases of FIP (Yin et al., 2021), 41.3% of cats had a palpable abdominal mass on palpation, believed to be either mesenteric lymphadenomegaly or an intestinal mass. Jaundice can occur (Fig. 5), more commonly in cats with effusions, but the degree of hyperbilirubinaemia is often not high enough to result in clinical jaundice (Pedersen 2009; Riemer et al., 2016).



Fig. 5. Icterus can occur in cases with FIP, particularly in cats with effusive FIP. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK.*

Clinical signs of FIP associated with the intestinal tract

FIP can also manifest in the intestinal tract and/or regional lymph nodes (sometimes called “focal FIP”) presenting typically as a palpable abdominal mass due to primary involvement of the mesenteric lymph nodes and/or intestinal tract. It can be particularly challenging to diagnose as the lesions can be hard to initially differentiate from neoplasia (Kipar et al., 1999), toxoplasmosis (Cohen et al., 2016) or mycobacterial infection (O’Halloran and Gunn-Moore 2017). Diarrhoea is sometimes reported (Yin et al., 2021). FIP involving the intestinal tract can manifest as a protein-losing enteropathy, leading to low total protein and globulin values, in contrast to the usual presentation of FIP. Often these cats present with mesenteric lymph node enlargement due to necrogranulomatous lymphadenitis (Kipar et al., 1999; Hugo and Heading 2015), or solitary mural intestinal lesions of the colon or ileo-caecocolic junction with associated regional lymphadenopathy (Harvey et al., 1996). Cats with intestinal FIP usually have a history of vomiting and diarrhoea or constipation.

Dermatological clinical signs of FIP

Dermatological signs are occasionally reported in FIP and can manifest as multiple non-pruritic or pruritic nodules or papules (Cannon et al., 2005; Declercq et al., 2008; Bauer et al., 2013; Redford and Al-Dissi 2019), due to pyogranulomatous-necrotising dermal phlebitis/vasculitis. Skin fragility syndrome has also been reported

(Trotman et al., 2007). Priapism has been reported as a result of granulomatous changes in tissues surrounding the penis (Rota et al., 2008).

Neurological clinical signs of FIP

Neurological FIP can result in clinical signs associated with focal, multifocal or diffuse changes in the brain, spinal cord and meninges. Up to 30% of cats with FIP show neurological signs (Kline et al., 1994; Foley et al., 1998; Foley and Leutenegger 2001; Negrin et al., 2007; Kent 2009; Negrin et al., 2010; Ives et al., 2013; Doenges et al., 2016). Sometimes cats with FIP present with only neurological disease (Rissi 2018). Three clinical syndromes were identified in a retrospective study of neurological FIP (Crawford et al., 2017); of 24 cats, three had a T3-L3 myelopathy, seven had central vestibular syndrome and 14 had multifocal CNS disease. Commonly reported signs include ataxia (with varying degrees of tetra- or paraparesis; Figs. 6 and 7), hyperaesthesia, nystagmus, seizures (Timmann et al., 2008), behavioural and mental state changes and cranial nerve deficits. Central vestibular clinical signs can include head tilt, vestibular ataxia, nystagmus, obtunded appearance and postural reaction deficits. Interestingly, a retrospective study (Grapes et al., 2021), that reviewed cats presenting with vestibular disease, did not identify any discrete clinical characteristics that would help differentiate cats with vestibular disease due to FIP from other causes. This was a surprise given that FIP primarily affects younger cats and is often associated with concurrent non-neurological signs. The absence of clinical characteristics specifically associated with FIP may have been because the study included a number of younger cats with other diagnoses (middle ear polyps, thiamine deficiency, intracranial empyaema and otitis media/interna) and cats with intracranial empyaema can have non-neurological systemic signs. Fever was shown to be less common in cats with neurological FIP compared to those without neurological signs (Riemer et al., 2016). A retrospective study (Mella et al., 2020) of cats referred for investigation of spinal disease found FIP to be the cause in 18 of the 221 cats in the study; concurrent systemic abnormalities and abnormal findings on clinical examination were significantly associated with a diagnosis of FIP, but these features were also associated with a diagnosis of spinal lymphoma (16 cats) and empyaema (3 cats).



Fig. 6. Ataxia can occur in cats with neurological FIP. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol UK*



Fig. 7. Ataxia (wide-based stance) and obtundation in a cat with neurological FIP. *Courtesy of Diane Addie, www.catvirus.com and Allan May, University of Glasgow*

Ocular clinical signs of FIP

FIP was the most commonly diagnosed cause of uveitis after idiopathic uveitis in one study of 120 cats with uveitis in the US (15.8% had FIP) (Jinks et al., 2016) and another of 92 cats with uveitis in the UK (16.3% had FIP) (Wegg et al., 2021). A study describing the ocular lesions in 15 cats with FIP found effusions in 13 cats and no effusion in only two cats (Ziolkowska et al., 2017) although other authors have found a low prevalence of effusions in cats with FIP-associated uveitis (Wegg et al., 2021). Ocular manifestations of FIP comprise anterior and/or posterior uveitis (Foley et al., 1998; Norris et al., 2005; Doenges et al., 2016; Jinks et al., 2016) (Fig. 8) and can be unilateral or bilateral in nature (Wegg et al., 2021). Important differential diagnoses include toxoplasmosis (Ali et al., 2021), lymphoma, FIV and FeLV infection (Jinks et al., 2016; Wegg et al., 2021). Clinical signs include changes in iris colour, dyscoria or anisocoria secondary to iritis, sudden loss of vision and hyphaema (Figs. 8 and 9). Keratic precipitates can appear as 'mutton fat' deposits on the ventral corneal endothelium (Fig. 10). The iris can show swelling and a nodular surface, and aqueous flare can be detected. On ophthalmoscopic examination, chorioretinitis, fluffy perivascular cuffing (representing retinal vasculitis), dull perivascular puffy areas of pyogranulomatous chorioretinitis, linear retinal detachment, vitreous flare and fluid blistering under the retina can be seen.



Fig. 8. FIP-associated anterior uveitis can manifest variably such as with the presence of hyphaema. *Courtesy of Maria Bonino and Erica Carter*



Fig. 9. FIP-associated anterior uveitis can manifest variably such as with the presence of hyphaema. *Courtesy of Albert Lloret, Universitat Autònoma Barcelona*



Fig. 10. FIP-associated anterior uveitis can manifest variably such as with the presence of inflammatory keratic precipitates. *Courtesy of Eric Déan*

Other clinical signs of FIP

FIP-associated rhinitis (Andre et al., 2020) has been described in a young cat that presented with some upper respiratory signs as well as other more typical signs of FIP; extensive respiratory panel testing on upper respiratory tract swabs in this cat revealed only a low positive for *Mycoplasma felis* whilst histopathological examination of lung (and liver and intestine) and nasal samples (including FCoV antigen immunohistochemistry on the nasal samples) confirmed a diagnosis of FIP. Another report described three cats with FIP that had presented with mild upper respiratory signs before showing other more typical signs of FIP (pyrexia, icterus, lethargy, anorexia, effusions) within the following 10 days (Healey et al., 2022). Myocarditis associated with FIP has also been described in a cat without effusion (Ernandes et al., 2019); this particular case presented with fever, weight loss and diarrhoea before developing dyspnoea and then neurological and ocular signs of FIP. Histopathology of various organs, including cardiac tissue, was consistent with FIP and FCoV antigen immunohistochemistry of the heart was also positive.

Diagnosis

Now that effective treatment is available for FIP (see section on Treatment), with antiviral agents such as GS-441524, some clinicians are using a rapid and sustained positive response to antiviral treatment as a means of supporting a diagnosis of FIP. A balance between the costs of diagnostic testing and treatment also needs to be made. Ideally, clinicians should be confident that FIP is a very likely diagnosis before starting antivirals, due to the cost and length of treatment required. The ABCD diagnostic trees provide information on how to reach a 'likely' diagnosis of FIP in cats to increase confidence in the use of antivirals (see the ABCD Tool "FIP diagnostic trees"). If a sustained response to antivirals is not maintained in a treated cat, the clinician should re-evaluate the original diagnosis of FIP.

Diagnosis of FCoV Infection

FCoV infection is not associated with clinical signs in the majority of cats; only a minority develop FIP. Confirmation of FCoV infection in healthy cats is not often indicated unless control of FCoV infection is being attempted by the household owner, either to generally try and reduce the risk of FIP developing in the household or because a case of FIP has been diagnosed in the household. More details on diagnosis of FCoV infection by faecal RT-PCR and FCoV antibody testing can be found later in the sections on 'General approach to FCoV & FIP control' and 'Management of apparently healthy FCoV-infected cats'.

FCoV infection is sometimes accompanied by enteritis (Sabshin et al., 2012). If FCoV infection is suspected in young cats with vomiting and/or diarrhoea, diagnosis of FCoV infection can be achieved via RT-PCR on faecal samples or rectal swabs. However, many other causes of enteritis exist in cats, which should be considered before making a definitive diagnosis of FCoV-associated enteritis.

Diagnosis of FIP

This section will focus on the diagnosis of FIP in sick cats showing clinical signs that could be suggestive of FIP. A cat cannot develop FIP unless it has been infected with FCoV and demonstration of FCoV in affected tissues and effusions is helpful during diagnostic investigations of FIP. Please also use the ABCD Tool "FIP Diagnostic trees" on the ABCD website.

Signalment and background for FIP

When approaching a case in which FIP is considered a differential diagnosis, one must remember that FIP is more common in young cats [especially under two years old (Riemer et al., 2016)] and that male (Rohrbach et al., 2001; Norris et al., 2005; Worthing et al., 2012; Riemer et al., 2016; Yin et al., 2021) cats are at slightly higher risk of disease. Additionally, most cats that develop FIP have been housed in multi-cat households previously. Although certain breeds have been shown to be predisposed to FIP in certain countries (Pesteanu-Somogyi et al., 2006; Worthing et al., 2012), it is believed that this is due to genetic risk factors being present in those breeds in those countries rather than existing worldwide generalised breed predispositions (Riemer et al., 2016). A recent history of stress (e.g., adoption, being in a shelter, neutering, upper respiratory tract disease, vaccination) is commonly apparent (Rohrer et al., 1993; Riemer et al., 2016) and can contribute to the development of FIP in a FCoV-infected cat.

Approach to the diagnosis of FIP

In cats with FIP that have an effusion, sampling the effusion is the single most useful diagnostic step in the diagnosis of effusive FIP; this is because tests on effusions often have a higher diagnostic value, in comparison to tests on blood (Hartmann et al., 2003) and samples are often relatively easy to obtain. If the effusion is not large in volume, imaging can be used (Pedersen 2014a) to confirm, identify and localize smaller volumes. Ultrasonography is generally regarded as being more sensitive than radiography for the detection of small volumes of fluid in the thorax and abdomen, but this depends on where pockets of fluid reside. Repeated ultrasonography to identify any small volume effusion is recommended and, similarly, ultrasonography can be used to guide sampling of small pockets of fluid (Tasker 2018). Once an effusion is sampled, the first thing to do is to take note of its appearance: if it is frank blood, or if it can be discerned as urine, FIP is very unlikely. Additionally, purulent exudates are usually not caused by FIP. The presence of chyle will usually indicate other diseases, such as heart failure, lymphoma or a ruptured thoracic duct, but cats with FIP with pure chylous effusion have been reported (Savary et al., 2001). FIP effusions are usually clear, viscous/sticky and straw-yellow in colour (Fig. 11).



Fig. 11. Abdominal effusion sample collected from a cat with FIP showing typical yellow-straw fluid; Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK

Diagnosing FIP if no effusion is present, however, can be very challenging due to the sheer number of possible clinical signs and the non-specificity of most of them (e.g., anorexia, lethargy, weight loss, pyrexia). Aetiological definitive diagnosis of FIP cases that do not have effusions by biopsy collection *ante-mortem* can be very difficult due to, for example, problems accessing affected tissues, contra-indications (such as the need for general anaesthesia) for the invasiveness of taking biopsies from a sick cat and/or costs involved in tissue collection. Cases with neurological or ocular signs can be approached via possible sampling of cerebrospinal fluid (CSF) or aqueous humour. Currently, there is no non-invasive, confirmatory test available for cats with FIP that don't have effusions, although in some cases valuable information can be gained through analysis of fine needle aspirate (FNA) samples collected from affected organs, if accessible, as described later.

The following information on the diagnosis of FIP will consider the merits and drawbacks (and sometimes sensitivity and specificity) of tests available for the diagnosis of FIP, and FCoV infection if relevant. Although each individual test will be described, it should be remembered that when a cat with suspected FIP is being investigated, a veterinarian will be interpreting several test results at the same time, as well as taking into account the signalment and background of the cat. Such interpretation is important in helping determine how likely FIP is as a diagnosis, in the absence of a definitive diagnosis. The advantage of integrating multiple test results during interpretation has been shown in a published study on the diagnosis of FIP (Stranieri et al., 2018) and in an abstract discussing a machine learning approach to the FIP diagnosis (Dunbar et al., 2018).

Laboratory changes in FIP

Routine haematology in FIP

Routine haematological changes are not specific for FIP but common abnormalities seen include lymphopenia (seen commonly and maybe more in cats with effusions than in cats without), neutrophilia, a left shift and a mild to moderate normocytic, normochromic anaemia (Sparkes et al., 1991; Rohrer 1992; Sparkes et al., 1994; Norris et al., 2005; Tsai et al., 2011; Riemer et al., 2016; Yin et al., 2021; Addie et al., 2022). An association between FIP and microcytosis (with or without anaemia) was reported (Riemer et al., 2016). Immune-mediated haemolytic anaemia occasionally occurs (Norris et al., 2005; Riemer et al., 2016). A decreasing red blood cell count is a poor prognostic sign (Tsai et al., 2011).

Serum biochemistry in FIP

Serum biochemistry changes are also non-specific in cats with FIP, but certain abnormalities can be helpful in making one consider FIP as a differential diagnosis.

Hyperglobulinaemia is often reported in FIP and can be accompanied by hypoalbuminaemia or low to normal serum albumin (Rohrer 1992; Riemer et al., 2016). The presence of hypoalbuminaemia alongside hyperglobulinaemia means that hyperproteinaemia does not always occur (Riemer et al., 2016). This combination of changes can cause the albumin to globulin (A:G) ratio to be low, and this ratio can be used to help evaluate how likely FIP is; the A:G ratio has a higher diagnostic value than either total serum protein or globulin concentration (Hartmann et al., 2003). Various A:G ratio cut offs have been suggested; e.g. an A:G ratio of <0.4 makes FIP very likely, whilst an A:G ratio of >0.8 makes FIP very unlikely (Sparkes et al., 1991; Norris et al., 2005; Tsai et al., 2011). One study (Jeffery et al., 2012) using a population of cats with a prevalence of FIP of 4%, reported that a serum A:G ratio of >0.6 was useful in ruling out FIP, but that lower ratios were not helpful in ruling in FIP. Additionally, frequency and extent of hypoalbuminaemia, hyperglobulinaemia and low A:G ratio

reported in cats with FIP have decreased in more recent years (Riemer et al., 2016; Stranieri et al., 2017a), which could be due to veterinarians diagnosing FIP earlier, meaning that cases have not progressed to show these changes. Polyclonal and monoclonal elevated γ -globulins have been reported in cats with FIP (Taylor et al., 2010), although polyclonal elevations are far more common.

High bilirubin levels in the absence of both haemolysis and moderate elevations of liver enzyme activity should raise the suspicion of FIP. Hyperbilirubinaemia occurs in 22-63% of cats with FIP (Sparkes et al., 1991; Norris et al., 2005; Tsai et al., 2011; Riemer et al., 2016), and is especially seen in FIP cases that have effusions (Riemer et al., 2016). High bilirubin values are not always correlated with elevated liver enzymes (Riemer et al., 2016), as hyperbilirubinaemia in cats with FIP is not necessarily a reflection of parenchymal liver disease but can be due to excessive erythrocyte fragility leading to haemolysis with reduced clearing of haemoglobin breakdown products (Pedersen 2014b) or altered bilirubin metabolism due to high TNF-alpha levels leading to reduced bilirubin transport into and out of liver cells. ALT, AST and ALP were normal in 86, 66 and 95%, respectively, of cats with FIP (Riemer et al., 2016). It has been found that the level of bilirubin can rise as FIP progresses, and that rising bilirubin levels (and falling red blood cell counts) are a poor prognostic sign (Tsai et al., 2011).

As described earlier, the kidneys can be affected in FIP via granulomatous lesions or glomerulonephritis (Hartmann et al., 2020); these changes can result in azotaemia although this is more commonly seen in cases without effusions (Riemer et al., 2016).

Acute phase proteins (APPs) are produced in the liver in many inflammatory and non-inflammatory diseases in response to cytokines released from macrophages and monocytes. The major APP in cats is α 1-acid glycoprotein (AGP), which has an immunomodulatory function, and assays are available for its measurement in some laboratories. The reference range for AGP serum concentrations is <0.48 mg/ml (<480 μ g/ml), and a moderately elevated serum AGP concentration of >1.5 mg/ml is frequently reported in cats with FIP (Stranieri et al., 2018; Addie et al., 2022). The magnitude of the increase in serum AGP might be helpful in the diagnosis of FIP (Duthie et al., 1997; Paltrinieri et al., 2007a; Giori et al., 2011; Hazuchova et al., 2017). One report (Paltrinieri et al., 2007a) found that markedly elevated serum AGP concentrations of >3 mg/ml could support a diagnosis of FIP in cats with a low pretest probability of disease (i.e. with a history and clinical findings not typical of FIP), whereas less marked elevations were supportive in cats with a higher pretest probability of disease. However, another, albeit very small, study of cats with FIP actually found that moderately elevated AGP concentrations of >1.5 mg/ml were still able to discriminate between cats with and without FIP (Giori et al., 2011); interestingly, this study comprised unusual cases of FIP in which some aspects of presentation were atypical although a diagnosis of FIP was confirmed in all cases. However, it must be emphasised that AGP is not specific for FIP and can be increased in other diseases. It has been suggested that an AGP of ≤ 1.5 mg/ml could be useful to rule out FIP (Stranieri et al., 2018). However, AGP concentrations have been found to increase moderately and transiently in all the cats in a household before the appearance of cases of FIP in an environment with endemic FCoV infection (Paltrinieri et al., 2007b). It has also been found that AGP is hyposialylated in cats with FIP but not usually in clinically healthy FCoV antibody-positive cats or cats with other diseases (Cecilian et al., 2004; Rossi and Paltrinieri 2009), but testing for sialylation of AGP is not available routinely. Serum amyloid A, another APP, is also markedly increased in cats with FIP (Ritz et al., 2007; Krentz et al., 2021; Yin et al., 2021), but further work is required to evaluate its diagnostic value.

Cytology and biochemistry on effusions

As described under imaging, ultrasonography or radiography can be used to identify or confirm the presence of effusions and to assist in sample collection (Pedersen 2014a), which can be important as having a sample of effusion to analyse is very helpful in the diagnosis of FIP.

FIP effusions are highly proteinaceous, with a total protein concentration that is usually >35 g/l, consistent with that of an exudate. An early study (Shelly et al., 1988) describing the characteristics of effusions of 12 cats with FIP reported total protein concentrations of 32-99 g/l (median 59 g/l). In contrast, the cell counts of effusions due to FIP are often relatively low, usually <5 x10⁹/l cells (which would be more consistent with a modified transudate); sometimes cell counts are higher, for example up to 20 x10⁹/l cells. Cytology is typically pyogranulomatous in nature with macrophages, non-degenerate neutrophils and few lymphocytes. Thick eosinophilic (pink-red) proteinaceous backgrounds are often described on cytology too (Yin et al., 2021). If cytology reveals a septic neutrophilia (typically with degenerate neutrophils containing bacteria), neoplastic cells or a marked lymphocyte population, FIP is highly unlikely (Paltrinieri et al., 1999).

Typical effusions of cats with FIP have low A:G ratios; an A:G ratio of <0.4, has a high positive predictive value, whereas a value of >0.8 has a high negative predictive value (Shelly et al., 1988; Riemer et al., 2016). One study found that elevated effusion AGP concentrations (of >1.55 mg/ml) were more useful (sensitivity and specificity of 93%) in differentiating effusions of cats with FIP from those of cats without FIP when compared with AGP levels in serum or other acute phase proteins (Hazuchova et al., 2017); however, the diagnosis of FIP in the cats in this study was not always confirmed.

Rivalta's point-of-care test on effusions

Rivalta's test is a crude point-of-care assay that was originally developed to differentiate a transudate from an exudate in humans. However, it is important to note that a positive result is not specific for FIP, and positive results have been reported in cats without FIP but also in those with septic peritonitis and lymphoma (Fischer et al., 2012a). The positive predictive value was 58.4% in a study of cats who presented with effusion, in which the prevalence of FIP was 34.6% (Fischer et al., 2012a). If positive, effusion cytology can be helpful to discriminate between these causes (Paltrinieri et al., 1999). However, the test had a high negative predictive value of 93.4% for exclusion of FIP (Fischer et al., 2012a). Thus, a negative Rivalta's test is useful as it can be used to rule out FIP quickly and cheaply at point-of-care. A positive result needs confirmation of FIP with other tests.

To perform the Rivalta's test, 8 ml of distilled water at room temperature and one drop of 98% acetic acid (white vinegar can be used instead) (Fischer et al., 2013) are mixed in a test tube, and then one drop of effusion is carefully placed or layered onto the surface of the solution. A positive Rivalta's test is indicated by the drop staying attached to the surface of the liquid, retaining its shape with a connection to the surface, or floating slowly to the bottom of the tube as a drop or jellyfish-like (Fig. 12). A negative test is indicated by the drop disappearing and the solution remaining clear. However, interpretation of results can be problematic due to subjectivity and difficulties in deciding whether a result is positive or negative (Fischer et al., 2013). A video showing how to perform the test can be accessed at <http://www.youtube.com/watch?v=XmOk2veunqA>



Fig. 12. This image shows a positive Rivalta's test; a drop of abdominal effusion has been placed onto the surface of a mixture of 8 ml of distilled water and one drop of 98% acetic acid (or household white vinegar) and it has retained its shape with a connection to the surface. This is not a very specific test for FIP but can be performed in-house; a positive test increases the likelihood of FIP, while a negative test makes FIP very unlikely. *Courtesy of Diane Addie, www.catvirus.com*

CSF analysis

CSF is commonly collected from cats with neurological signs, although care should be taken with cisternal CSF sampling as the risk of brain herniation is high (Negrin et al., 2007; Penderis 2009; Rissi 2018; Hoey et al., 2020); and thus, ideally advanced imaging, such as CT or MRI, should be performed beforehand to assess the potential risk of herniation.

CSF samples from cats with FIP can show elevated protein concentrations (of >0.3 g/l [>30 mg/dl] in cisternal samples, and >0.46 g/l [>46 mg/dl] in lumbar samples with reference ranges of ≤ 0.3 g/l and ≤ 0.46 g/l for cisternal and lumbar CSF samples, respectively); occasionally marked elevations of protein occur (>2 g/l [200 mg/dl]). Additionally, CSF samples of cats with FIP often have an increased cell count ($>0.008 \times 10^9/l$ [>8 cells/ μ l]) in either lumbar and/or cisternal samples; reference range $\leq 0.008 \times 10^9/l$ [≤ 8 cells/ μ l]); occasionally this pleocytosis is extremely marked in cats with FIP (cell counts of $>1 \times 10^9/l$ [>1000 cells/ μ l]). Cytological examination of the CSF can show the pleocytosis to be predominantly neutrophilic, mononuclear, mixed or pyogranulomatous (Singh et al., 2005; Crawford et al., 2017; Felten et al., 2021). Some cats with neurological FIP have unremarkable CSF analysis results (Foley et al., 1998; Boettcher et al., 2007).

Aqueous humour analysis

Information on aqueous humour sampling techniques is described in detail in the literature (Linn-Pearl et al., 2015), but referral or consultation with an ophthalmologist for those unfamiliar with the collection technique is recommended. Aqueous humour samples from cats with FIP show cytological features similar to what is found in CSF samples, i.e. mixed inflammation with neutrophils with or without macrophages.

Diagnostic imaging

Routine imaging: Ultrasonographic and radiographic findings

Ultrasonography or radiography can be used to identify or confirm the presence of effusions and to assist in sample collection (Pedersen, 2014a). A review of abdominal ultrasonographic findings in 16 cats with FIP (Lewis and O'Brien, 2010) showed the presence of peritoneal fluid in seven cases and retroperitoneal fluid was found in one cat. Abdominal lymphadenopathy was documented in nine cats. The liver was of normal echogenicity in 11 cats and variably hypoechoic or hyperechoic in the remainder. Five cats had hypoechoic subcapsular rims in one or both kidneys. The spleen was of normal echogenicity in most cats and hypoechoic in two. A retrospective ultrasonographic study (Ferreira et al., 2020) focused on the significance of the medullary rim sign (MRS) in the kidneys of cats; of 661 cats that had undergone abdominal ultrasonography, 23 cats were diagnosed with FIP; 15 had MRS and eight did not, and this corresponded to a significant association between the presence of MRS and FIP. A diagnosis of FIP was made by the clinician but further details not given. A thick marked intensity type of MRS was most seen with FIP. It is clear that there are no specific ultrasonographic or radiographic findings in FIP. Imaging can also be of use to direct sampling of abnormal tissues, e.g. FNA for cytology can be collected to reveal non-septic pyogranulomatous inflammation, or ultrasound-guided needle core (e.g. Tru-Cut) biopsies (TCB) can be collected and submitted for histopathology. Pneumonia due to FIP that is occasionally reported can be associated with radiographic changes.

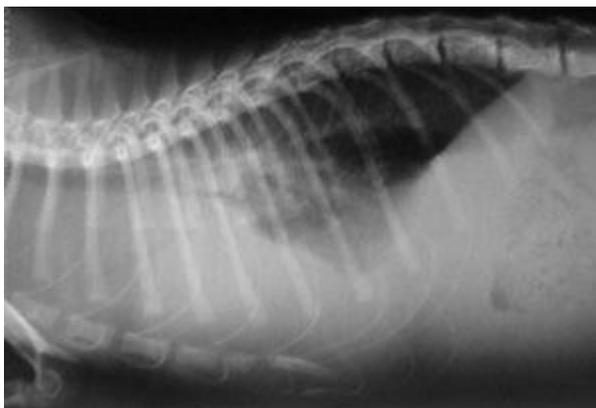


Fig. 13. Right lateral thoracic radiograph showing the presence of effusion; *Courtesy of Albert Lloret, Universitat Autònoma Barcelona*



Fig. 14. Ultrasonography of a young cat with FIP showing pericardial and pleural effusion; ultrasonography can be used to guide sampling of the effusion. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK*



Fig. 15. Ultrasonography of a young cat with FIP showing abdominal effusion; ultrasonography can be used to guide sampling of the effusion. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK*



Fig. 16. Ultrasonography of a cat with FIP and renomegaly with a loss of normal renal architecture; ultrasonography might be useful to guide FNA or TCB sampling of organs by targeting abnormal tissue. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK*

Advanced imaging: Magnetic resonance imaging (MRI) and computerised tomography (CT)

When a cat is showing neurological signs, imaging of the brain by MRI, if available, can be useful to demonstrate neurological abnormalities due to FIP. Obstructive hydrocephalus, syringomyelia, foramen magnum herniation and marked contrast enhancement of the meninges, third ventricle, mesencephalic aqueduct and brainstem have been reported in cats with FIP (Foley et al., 1998; Negrin et al., 2007; Penderis 2009; Crawford et al., 2017). Some cats only show abnormalities after administration of contrast (Foley et al., 1998; Negrin et al., 2007), and some cats have normal MRI, even after contrast administration, despite the presence of meningoencephalitis (Negrin et al., 2007) A description of CT findings in cats with neurological FIP has not been published, and although hydrocephalus and/or syringohydromyelia can sometimes be detected by CT, MRI is likely to be more sensitive in the detection of subtle intraparenchymal lesions (Negrin et al., 2009).

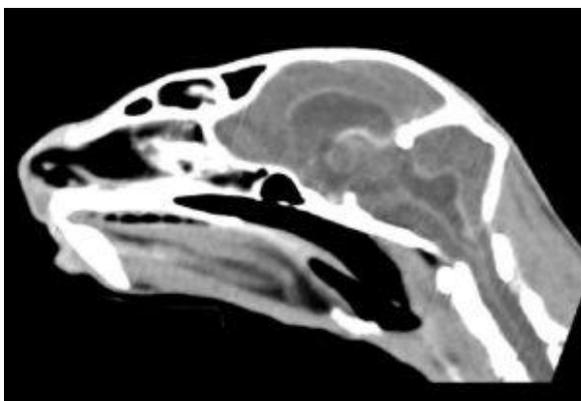


Fig. 17. CT of the head of a cat with neurological signs due to FIP post-contrast – reconstructed to show a sagittal midline view. Enhancement of the ventricles is apparent. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK*

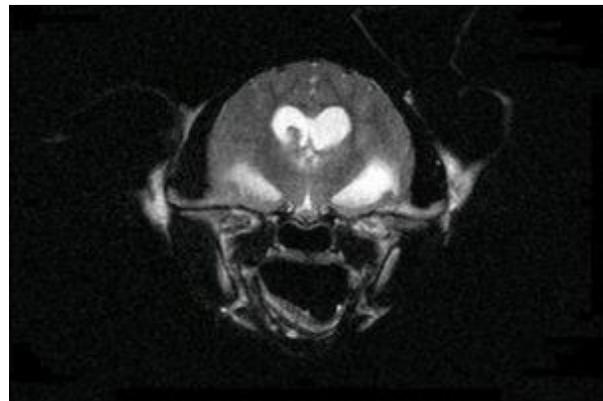


Fig. 18. T2W transverse MRI of a cat with neurological signs due to FIP, showing enlargement of ventricles; the signal void in the right ventricle is likely due to an enlarged choroid plexus. The brain also appears swollen with a lack of visible sulci. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK*

Direct detection of the infectious agent

Detection of FCoV antigen

Histopathological examination of tissues with FCoV antigen immunostaining

The definitive diagnosis of FIP relies on consistent histopathological changes in affected tissues and this, together with FCoV antigen immunostaining, is considered the gold standard for diagnosis.

Immunostaining exploits the binding of antibodies to host cell-associated FCoV antigens, which are subsequently visualised by enzymatic reactions producing a colour change in a process called immunohistochemistry (IHC). However, care must be taken to ensure that adequate controls are in place since non-specific staining can occur, leading to false positive results (see below).

The “classical” FIP histopathological lesion is a blood vessel surrounded by an inflammatory lesion dominated by monocytes/macrophages intermingled with a few neutrophils and lymphocytes (Kipar et al., 2005), which are mainly CD4+ (Paltrinieri et al., 1998). Occasionally, monocytes can be seen attached to endothelial cells or emigrating from the vessel (Kipar et al., 2005). Periventricular encephalitis and leptomeningitis are commonly seen in neurological FIP (Mesquita et al., 2016; Rissi 2018). A useful study (Stranieri et al., 2020b) documented the following patterns as being consistent with FIP lesions:

- Pyogranulomas on one or more serosal surfaces;
- Granulomas with or without necrotic areas;
- Lymphocytic and plasmacytic infiltrates in specific sites (e.g., band-like infiltrate in serosal surfaces, perivascular infiltrate in meninges and CNS);
- Granulomatous to necrotizing vasculitis and fibrinous serositis.

Histopathology alone is sometimes used to definitively diagnose FIP (Felten et al., 2017a). In one study analysing 93 tissues from 14 cats with FIP (Stranieri et al., 2020b), histopathological lesions consistent with FIP were found most commonly in the lung (76.9% of samples) then kidneys (64.3%), mesenteric lymph nodes (61.5%), liver (57.1%) and spleen (57.1%). Differential diagnoses for pyogranulomatous inflammation include other infectious diseases (e.g., infections with mycobacteria, actinomyces, nocardia, rhodococcus, pseudomonas {Milliron, 2020 #674}, bartonella, fungal), as well as rarer idiopathic sterile pyogranulomatous disease cases which can present with mass lesions, such as in the lymph nodes (e.g. mesenteric, submandibular) (Giuliano et al., 2020) or skin.

However, in addition to histopathological changes, a definitive diagnosis of FIP should rely on the demonstration of positive immunostaining for FCoV antigen within appropriate cells (particularly macrophages) within the histopathological lesions, such as by IHC (Kipar et al., 1998; Kipar and Meli 2014; Stranieri et al., 2020b). Positive FCoV antigen IHC is highly specific and reliable (Tammer et al., 1995; Rissi 2018; Stranieri et al., 2020b) as long as it is performed with appropriate controls and reagents that prevent non-specific binding of the FCoV antibody to the tissues, as otherwise false positive results occur, although visualization of the pattern of FCoV antigen staining by a pathologist should discern non-specific staining. However, a negative result does not

exclude FIP as FCoV antigens can be variably distributed within lesions (Giordano et al., 2005; Emmeler et al., 2020; Stranieri et al., 2020b) and might not be detected in all histopathological sections prepared from FIP-associated tissues changes (Kipar and Meli 2014). If unexpected negative IHC results are obtained, it is worth requesting additional sections of biopsies to be cut and examined by the pathologist (Tasker 2018; Stranieri et al., 2020b).

Samples of affected tissues (e.g., liver, kidney, spleen, mesenteric lymph nodes) can be collected at necropsy or *in vivo* by laparotomy, laparoscopy or ultrasound-guided TCB. The samples most likely to be useful are those that are affected by the disease process, and inference of this would hopefully be gained by the results of the diagnostic testing (e.g., imaging results, pyogranulomatous inflammation on FNA cytology) as well as clinical signs.

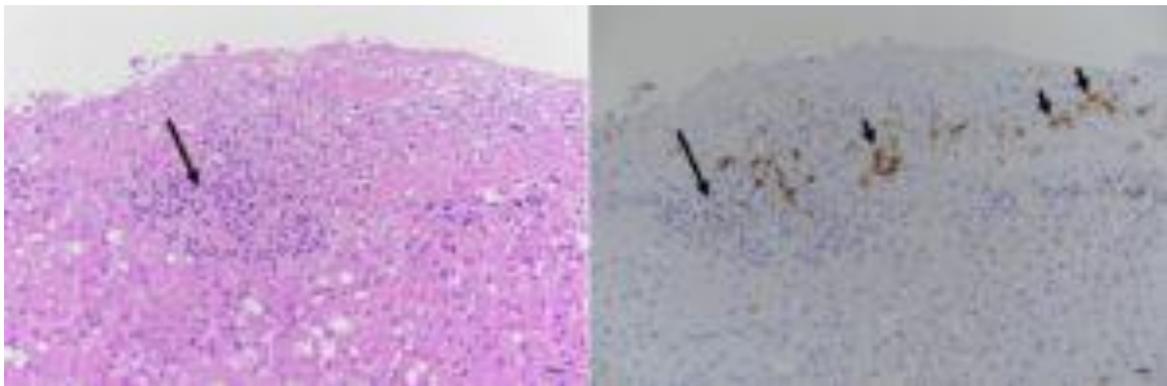


Fig. 19. Histopathology and positive FCoV antigen immunostaining in a cat with FIP: liver, fibrinous perihepatitis with embedded FCoV-infected macrophages (arrowheads) and focal granulomatous infiltrate (arrow) with FCoV-positive macrophages. *Courtesy of Anja Kipar, University of Zurich*

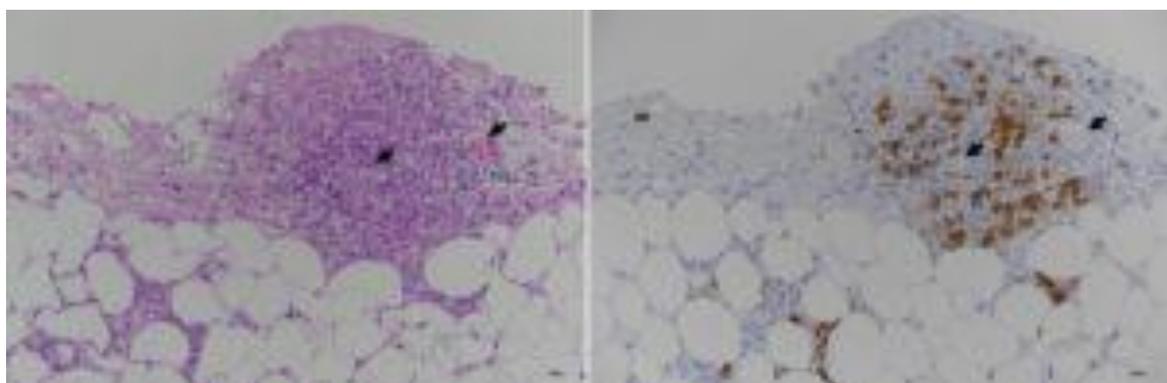


Fig. 20. Histopathology and positive FCoV antigen immunostaining in a cat with FIP: Mesentery with focal granulomatous infiltrate with embedded small veins (arrowheads) and abundant FCoV-positive macrophages. *Courtesy of Anja Kipar, University of Zurich*

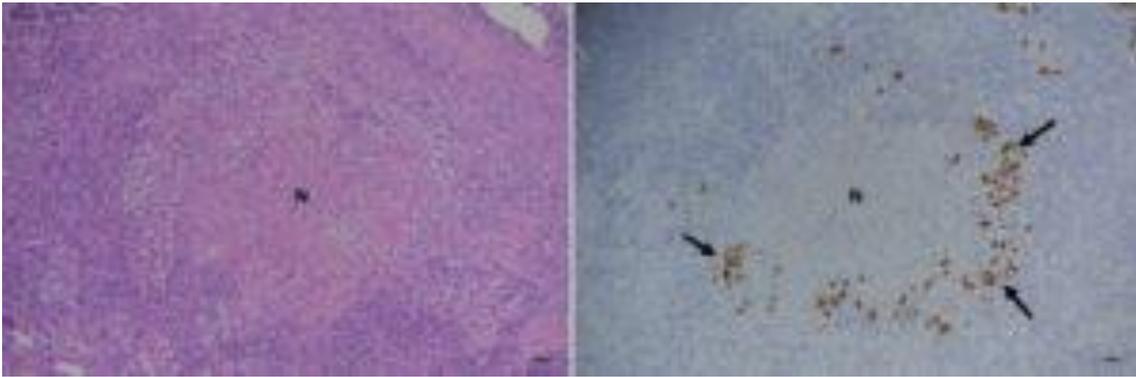


Fig. 21. Histopathology and positive FCoV antigen immunostaining in a cat with FIP: Mesenteric lymph node with focal granulomatous infiltrate with extensive central necrosis (N) and abundant FCoV-infected macrophages (arrowheads) in the surrounded infiltrate. *Courtesy of Anja Kipar, University of Zurich*

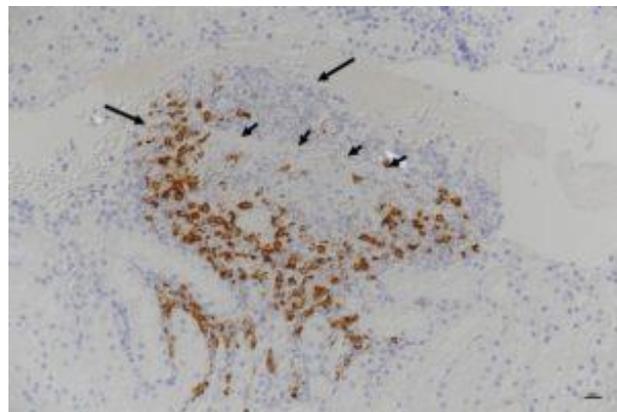


Fig. 22. Histopathology and positive FCoV antigen immunostaining in a cat with FIP: Kidney, stellate vein in subcapsular cortex with granulomatous (peri)phlebitis. Focally, the granulomatous infiltration has destroyed the vascular basement membrane (left arrow), protrudes into the lumen of the vein (wall-bound thrombus; right arrow) and is present in surrounding tissue, containing abundant FCoV-infected macrophages (arrows). Short arrows outline the remnants of the basement membrane. *Courtesy of Anja Kipar, University of Zurich*

If cats are euthanised due to suspected FIP, it is important to try to collect samples for IHC staining at post-mortem examination to confirm the disease if possible. Gross findings sometimes are suggestive of FIP (Tasker and Dowgray 2018) (Figs. 23 and 24), but lesions might not be obvious. Indeed, it is known that histopathological changes consistent with FIP can be seen in tissues that have not shown macroscopic changes at post-mortem examination (Stranieri et al., 2020b). Large pyogranulomatous lesions can be mistaken for tumours (Fig. 24). Histopathological examination, with FCoV antigen staining (see above) should ideally be performed to confirm the diagnosis of FIP.



Fig. 23. Gross appearance of fibrinous plaque-like inflammation present on the surface of the spleen in a case of FIP with an abdominal effusion that underwent post-mortem examination. *Courtesy of Séverine Tasker, Bristol Veterinary School, University of Bristol, UK.*



Fig. 24a & b. Gross appearance of the kidneys from two cats with FIP, showing renomegaly with pyogranulomas visible on the renal surface on post-mortem examination. The right image shows how these pyogranulomas can be centred on blood vessels. These lesions could be mistaken for tumours on gross post-mortem, which is why histopathology and ideally IHC staining is necessary.

Cytology with FCoV antigen immunostaining on effusions, FNAs, CSF and aqueous humour

FCoV immunostaining can be performed on cytology samples using immunocytochemistry (ICC) or immunofluorescence (IF), where host cell-associated FCoV antigens are detected with FCoV-specific antibodies conjugated with enzymes or fluorescent markers. The presence of FCoV antigens can then be demonstrated by either enzymatic reactions producing a colour change (see Figs. 25 and 26) or by visualization of fluorescence using a UV microscope, respectively. ICC can be a useful test to be performed on effusions, FNAs, CSF and aqueous humour samples (Linn-Pearl et al., 2015).

FCoV immunostaining of effusion samples has shown variable sensitivity, ranging from 57 to 100% (Parodi et al., 1993; Hirschberger et al., 1995; Paltrinieri et al., 1999; Hartmann et al., 2003; Litster et al., 2013; Felten et al., 2017b). Since this technique relies on staining FCoV within macrophages in the effusion, false negative results can occur (Hellemans et al., 2020) and can result if the effusion is cell-poor and/or the FCoV antigen is masked by FCoV antibodies in the effusion. Immunostaining was thought to be very specific, although two of seven non-FIP effusions (one of the two cats had heart failure, the other one cholangiocarcinoma) were positive by IF in one study (Litster et al., 2013), and eight (three cats with heart failure and five cats with neoplasia) of 29 non-FIP effusions were positive by ICC in another (Felten et al., 2017b), questioning the specificity of ICC. However, the reported poorer specificity might be due to the methodology, and neither of these studies included negative control slides. Indeed, methodology for ICC that includes dual staining for macrophages as well as FCoV antigen potentially increases specificity by avoiding false positive results from the staining of dead cells or debris, and may be available diagnostically in the future (Howell et al., 2020). Some researchers prefer the use of cell pellets from centrifuged effusion samples to prepare formalin-fixed, paraffin embedded samples that can then be treated like a tissue specimen for FCoV antigen IHC (Kipar and Meli 2014) or IF (Hellemans et al., 2020), to improve the reliability of detection of FCoV antigen (Kipar and Meli 2014), although the processing time required for the latter would be longer than for direct cytological immunostaining.

FCoV immunostaining of FNA samples has not yet been described in any large comprehensive studies. Two studies did describe the successful amplification of FCoV RNA from ultrasound-guided FNAs of abnormal tissue in 11 of 11 cats (Freiche et al., 2016), and from mesenteric lymph node FNAs (Dunbar et al., 2019) in 18 of 20 cats with FIP without effusions. This successful sampling and RNA detection suggest that demonstration of FCoV antigen by immunostaining on cytology samples might also be useful in cats with FIP if abnormal tissues are sampled, but this needs further evaluation.

FCoV immunostaining using ICC has been reported as being successful in detecting FCoV in the CSF of a cat with neurological FIP (Ives et al., 2013). One study evaluated ICC in the CSF of cats with and without FIP, that presented with and without neurological signs, collected at *post-mortem* examination (Gruendl et al., 2016); this study found that 17 of 20 cats with FIP gave positive ICC results but of 18 cats without FIP, three also had positive results (one cat with mediastinal lymphoma, one with lymphocytic meningoencephalitis and one with hypertensive angiopathy with brain haemorrhage), limiting the test's specificity. The reasons for the positive ICC results in three cats without FIP are not known but possibilities raised in the study include concurrent presence of FIP alongside the other diseases present (although IHC staining of neurological tissues was also negative), detection of the presence of systemic FCoV antigen in the absence of FIP or non-specific staining and aberrant antibody binding, although these were deemed unlikely. These analyses (Gruendl et al., 2016) excluded those cats that had no cells present in their CSF as ICC could not be performed on these cats. The same group (Felten et al., 2021), performed CSF ICC on two cats with neurological signs that did not have FIP and although one of these was positive, the cytology of the CSF was lymphomonocytic, which would not have been consistent with a diagnosis of FIP. This same study also performed CSF ICC on seven cats with confirmed FIP, three with neurological signs and four without; two of the three cats with neurological FIP were ICC positive whilst three of the four cats with non-neurological FIP were ICC positive. Most of the ICC positive results in the FIP cats in this study showed pyogranulomatous cytology in the CSF, consistent with FIP. Application of ICC to CSF samples collected *ante-mortem* from a larger number of cats with neurological signs due to FIP and other causes would be desirable to further evaluate the usefulness of this technique.

The use of FCoV antigen immunostaining has also been described in aqueous humour samples collected directly following euthanasia from 26 cats with confirmed FIP and 13 cats with other diseases (Felten et al., 2018); most (25 with FIP and 11 with other diseases) of these cats were also included in a subsequent study describing both FCoV RT-PCR and FCoV antigen immunostaining in cats with FIP (31 cats) and cats with other diseases (27 cats) (Sangl et al., 2020). These two studies reported sensitivities of 64.0% and 62.5%, respectively, for aqueous humour FCoV antigen immunostaining, but most of the cats with FIP in these studies did not present with uveitis. The specificities were 81.8% and 80%, with positive results occurring in one control cat with lymphoma and one control with a pulmonary adenocarcinoma (in both cats the aqueous humour cytology was not consistent with FIP). Accompanying cytology, therefore, is important to aid interpretation. However, aqueous humour as a target sample is interesting as it could be collected non-invasively from cats with suspected FIP, although the sample collection technique used in the published studies (Felten et al., 2018; Sangl et al., 2020) might need modification for use *ante-mortem* as described elsewhere (e.g., use of a smaller 27-29 gauge insulin needle) (Linn-Pearl et al., 2015). Further evaluation of ICC on aqueous humour samples collected *ante-mortem* from cats with uveitis due to FIP and other causes are needed to further evaluate the usefulness of ICC in the diagnosis of FIP.

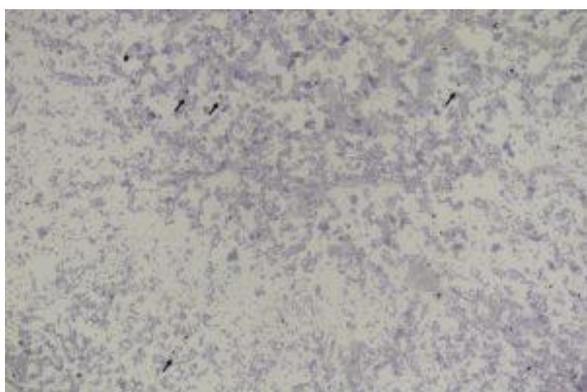


Fig. 25. Immunocytochemistry showing the presence of FCoV antigen in macrophages in an effusion of a cat with FIP. Overview at 40x magnification, cytospin, scattered positive macrophages clearly visible at low power (e.g. arrows) before cells further identifiable. *Courtesy of Alex Malbon and Anja Kipar, University of Zurich*

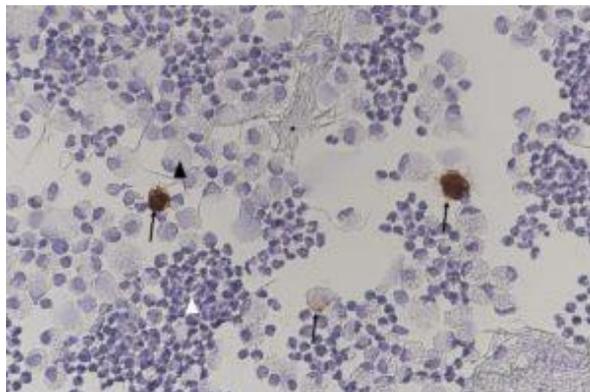


Fig. 26. Immunocytochemistry showing the presence of FCoV antigen in macrophages in an effusion of a cat with FIP. 400x magnification, cytospin, highly cellular – macrophages (black arrowhead) and neutrophils (white arrowhead), fibrin (*). Scattered positive macrophages (arrows); *Courtesy of Alex Malbon and Anja Kipar, University of Zurich*

Comparative usefulness of tissue and FNA sampling for immunostaining

A study evaluated the usefulness of hepatic and renal TCBs, and FNAs, collected from cats with FIP confirmed by histopathology and FCoV immunostaining (Giordano et al., 2005). The sensitivity of TCBs and FNAs from hepatic and renal tissues was poor (64% and 82% for hepatic TCB and FNAs respectively, and 39% and 42% for renal TCB and FNAs) although combining analysis of TCB and FNA results for each of the tissues increased sensitivity (to 86% for liver and 48% for kidney). In this study the cytological and histopathological findings of the FNAs and TCBs were classified according to whether they were consistent with FIP for calculation of specificity and sensitivity. However, specific lesions within the liver and kidneys were not targeted for sampling in this study,

and targeted sampling under ultrasound-guidance might improve sensitivity. One study, comprising 20 cats with FIP, compared RT-PCR results on FNAs and incisional biopsies (IBs) of popliteal and mesenteric lymph nodes, liver, spleen, omentum and kidneys by RT-PCR. Percentages of positive RT-PCR results were similar or even identical for FNA and IB in intra-abdominal organs. Although immunostaining results for FNA and IB were not reported in the study, it still provides important information in relation to the value of sampling by FNA or IB, since obtaining IB is highly invasive and usually cannot be performed without anaesthesia (Emmler et al., 2020).

Detection of FCoV RNA by reverse-transcriptase polymerase chain reaction (RT-PCR)

In general, PCR is a method by which DNA is exponentially amplified using primers to target a specific sequence, enabling sensitive detection down to a very low starting DNA copy number. Post-PCR amplification processing (e.g., sequencing) can be applied as well if needed. PCR only amplifies DNA so, because FCoV is an RNA virus, a pre-PCR step using a viral enzyme, reverse-transcriptase (RT), is required to generate a strand of cDNA using the original FCoV RNA template, in a process known as reverse transcription. A combination of this process and PCR is known as RT-PCR (Barker and Tasker 2020b). The RT-PCR assays available to detect FCoV RNA often amplify both cell-associated subgenomic mRNA (RNA produced in feline cells when the FCoV replicates), as well as cell-associated and virus particle-associated genomic RNA (which correlates to the presence of whole FCoV). Where the PCR primers bind to the FCoV genome determines whether subgenomic mRNA is preferentially amplified (Barker and Tasker 2017; Barker and Tasker 2020b). Those RT-PCR assays that favour amplification of subgenomic mRNA might overestimate the FCoV viral loads present in the sample (Barker and Tasker 2020b). Laboratories should be able to report the analytical sensitivity and specificity of their RT-PCRs and also provide details with the positive and negative controls that they use. As an RNA virus, FCoV shows a high rate of errors during replication and any mutations at the site of primer and/or probe binding can result in loss of RT-PCR assay efficiency, and ultimately sensitivity. RT-PCR conditions can be altered to tolerate such mutations, but this can result in a loss of specificity (Barker and Tasker 2017). Additionally, RT-PCRs designed to target type I FCoV, which represents the majority of field strains found in naturally infected cats, although geographical variation exists (Hohdatsu et al., 1992; Benetka et al., 2004) might not amplify type II FCoV if the primers and probe bind to the region of the FCoV genome which differs between the two (i.e. the spike (S) protein) (Herrewegh et al., 1998; Terada et al., 2014; Decaro et al., 2021).

FCoV RT-PCR has been used to detect FCoV RNA in blood, effusion, tissue (including aspirates), CSF, or aqueous humour samples from suspected cases of FIP, with varying results. Assays used should be quantitative (q in RT-qPCR), and able to report the FCoV load present in the sample, and this information is an important aid to interpretation of results. This is because systemic FCoV infection can occur in healthy cats and cats without FIP (albeit uncommonly) but generally with lower FCoV viral loads than in cats with FIP (Meli et al., 2004; Kipar et al., 2006; Kipar et al., 2010; Desmarests et al., 2016), so a positive RT-PCR result is not specific for FIP, but positive results with a high FCoV load can be used to support a diagnosis.

Running FCoV RNA RT-PCRs can be rapid, although, once the time taken to submit the sample to the laboratory is factored in, reporting of results can still take a few days. This is usually quicker than immunostaining on tissue biopsy samples and often also quicker than immunostaining on effusion samples. More recently rapid molecular techniques (loop mediated isothermal amplification) for detecting FCoV RNA in-house as point-of-care tests have been described (Stranieri et al., 2017b; Gunther et al., 2018), but they suffered from poor sensitivity.

FCoV RT-PCR on blood samples

Samples derived from blood (e.g., whole blood, serum, plasma or peripheral blood mononuclear cells [PBMCs]) can be subjected to RT-PCR for FCoV RNA following RNA extraction. Earlier studies have shown these samples to be rarely positive in cats with FIP; for example, when FCoV RT-PCR was performed on plasma or serum samples from cats with and without FIP (Doenges et al., 2017; Felten et al., 2017a; Felten et al., 2017c), although none of the cats without FIP cases gave positive results, only 0 to 15.4% of the FIP cases were positive for FCoV RNA. Similarly, FCoV RNA was only rarely detected in whole blood of 20 cats with FIP in an experimental study (Pedersen et al., 2015), and although whole blood or PBMCs might be a better target for RT-PCR than serum (Doenges et al., 2017), sensitivity was still poor at 28.6%. However, one study (Stranieri et al., 2018) amplified FCoV RNA by RT-PCR (based on the 3' UTR of the FCoV genome) from pellets derived from whole blood in six of eight (75.0%) cats with FIP but none of eight cats with diseases other than FIP. Additionally, another study (Krentz et al., 2021) documented that 15 of 18 (83.3%) cats with confirmed or highly suspected FIP were positive by RT-PCR for FCoV RNA in blood samples and a study which tested the whole blood of 125 cats with suspected effusive FIP by RT-PCR (Katayama et al., 2021) found 114 (91.2%) to be positive, although a positive RT-PCR result on blood was one of the criteria used to deduce a diagnosis of FIP influencing interpretation. Interestingly the study by Krentz and colleagues (Krentz et al., 2021), which found FCoV RNA in blood samples from 83.3% of cats with FIP, used the same RT-PCR assay [based on the 7b gene of the FCoV genome (Gut et al., 1999)] as the previous study which documented very few positive RT-PCR results on blood samples from 20 cats with FIP (Pedersen et al., 2015). The reason for the discrepancy between these results is not known and needs further investigation; for example, it may be due to sample collection, processing or storage conditions. Specificity of FCoV RT-PCR is also an issue as healthy and ill cats without FIP can have detectable viraemia in the blood, albeit uncommonly. One study (Fish et al., 2018) found that nine of 205 (4.4%) healthy US shelter cats were FCoV RNA RT-PCR positive in buffy coats prepared from blood; one of those had replicating virus in the bloodstream, as demonstrated by a positive FCoV mRNA RT-PCR result, and this 8-week old kitten was likely undergoing viraemia. Neither this kitten, nor seven of the nine FCoV RNA RT-PCR positive cats with follow up developed FIP during the subsequent six months.

These results with FCoV RT-PCR on blood samples make it an interesting avenue to explore as a test to support a diagnosis of FIP in the future as more recent studies suggest that cats with FIP can often be FCoV RT-PCR positive on blood samples.

FCoV RT-PCR on effusions

Effusion samples in cats with FIP often contain FCoV RNA (Pedersen et al., 2015), which can be detected by RT-PCR. Centrifugation of the effusion sample to yield a cell pellet to use for RNA extraction can improve sensitivity (Hellemans et al., 2020). Published studies had amplified FCoV RNA in most (72-100%) effusion samples from cats with FIP (Doenges et al., 2017; Felten et al., 2017c; Longstaff et al., 2017; Stranieri et al., 2018; Katayama et al., 2021) but usually not in any effusions from cats without FIP (Doenges et al., 2017; Felten et al., 2017c; Longstaff et al., 2017). However, subsequent studies have challenged the specificity of this test. One study (Barker et al., 2017) amplified FCoV RNA, albeit at a low level, in abdominal fluid from one of 29 control cats that did not have FIP. Another study (Felten et al., 2017a) amplified FCoV RNA from three (two of these three had low levels of FCoV RNA) of 24 control cats without FIP that had effusions tested. In the latter study, the control cats that generated positive FCoV RT-PCR results had neoplasia (lymphoma and a malignant round cell tumour) or chronic kidney disease (this cat had higher FCoV RNA levels in the effusion). Additionally, one study (Stranieri et al., 2018) amplified FCoV RNA (levels not reported) from the effusion of one cat with an intestinal carcinoma (out

of six control cats with effusions tested). Finally, another study (Hellemans et al., 2020) documented a specificity of 81.2% for RT-PCR on effusions as positive RT-PCR results were obtained in three of 16 samples from cats without FIP; however, confirmation of the absence of FIP in these three cats was only based on a negative IF immunostaining result on effusion samples from the cats, and it might well be that these three cats did indeed have FIP. Overall, it can be assumed that the presence of FCoV RNA, particularly in high levels, in an effusion that also has cytological and biochemical features suggestive of FIP, is highly supportive of a diagnosis of FIP.

FCoV RT-PCR on tissue and FNA samples

When tissue biopsy samples are obtained from cats with suspected FIP, the samples should be submitted for histopathology and IHC, as this allows for a definitive diagnosis of FIP. However, if a delay in analysis is expected, tissue can also be submitted for RT-PCR as finding high levels of FCoV RNA in a sample of an affected organ can be helpful and supportive of a diagnosis of FIP. This is because it is known that tissue samples from cats with FIP are significantly more likely to be FCoV RT-PCR positive (Barker et al., 2017; Stranieri et al., 2018), and have significantly higher FCoV RNA loads by RT-PCR (Porter et al., 2014) than tissue samples from cats without FIP. In one study that included 20 cats with FIP confirmed by immunohistochemistry, 70-90 % of IBs of popliteal and mesenteric lymph nodes, liver, spleen, omentum and kidneys were positive by RT-PCR (Emmler et al., 2020). However, it is important to remember that cats without FIP can be positive for FCoV RNA by RT-PCR in tissues. One extensive study evaluating FCoV RT-PCR in 260 tissue samples from 57 cats with FIP, and 258 tissue samples from 45 cats without FIP (Barker et al., 2017) found that 90.4% of tissue samples from cats with FIP were FCoV RT-PCR positive compared to only 7.8% of tissue samples from cats without FIP.

In cats with FIP, FCoV RNA loads correlate with histopathological findings suggestive of FIP (Pedersen et al., 2015; Barker et al., 2017). Thus, the presence of high levels of FCoV RNA in tissue samples is highly supportive of a diagnosis of FIP. It is usually suggested that tissue samples should not be formalin-fixed before RT-PCR, as formalin can degrade RNA and decrease PCR sensitivity (Tasker 2018), although a study has described the successful use of FCoV RT-PCR in formalin-fixed paraffin-embedded tissues in cats with FIP (Sangli et al., 2019).

FNAs, such as obtained by ultrasound guidance, could be a good alternative to tissue samples for FCoV RT-PCR analysis, with the advantage of relatively easy collection. One study (Freiche et al., 2016) described successful amplification of FCoV RNA from ultrasound-guided FNAs of abnormal tissue in 11 cats with FIP without effusions, suggesting that FNAs could be a useful sampling material for RT-PCR in cats with non-effusive FIP. A controlled study of FCoV RNA detection in FNAs collected from the mesenteric lymph nodes from 20 cats with FIP without effusions reported a sensitivity of 90.0 % and specificity of 96.1 % (Dunbar et al., 2019). In that study FCoV RNA survived normal mailing as the FNAs tested by RT-PCR were sent by regular mail without ice or an RNA preservative (Dunbar et al., 2019). As outlined earlier, a study of 20 cats with FIP compared RT-PCR results on FNAs and IBs of popliteal and mesenteric lymph nodes, liver, spleen, omentum and kidneys by RT-PCR. Percentages of positive RT-PCR results were similar or even identical for FNA and IB in intra-abdominal organs (Emmler et al., 2020).

FCoV RT-PCR on CSF samples

Samples of CSF can be submitted for FCoV RT-PCR. Two studies have described the use of FCoV RT-PCR on CSF samples and found it to have 100% specificity for FIP but a sensitivity of only 41.2% (Doenges et al., 2016) or 30% (Felten et al., 2021) in cats with FIP. Other studies (Foley et al., 1998; Barker et al., 2017) have also shown poor sensitivity. However, not all cats included in all of these studies had neurological signs, as CSF was

collected at *post-mortem* examination independent of presenting signs (Doenges et al., 2016; Barker et al., 2017; Felten et al., 2021), such that the population tested does not necessarily reflect a population that would have had CSF samples collected for diagnostic purposes. Indeed, in one study (Doenges et al., 2016), the sensitivity of RT-PCR rose to 85.7% when only cats with neurological and ophthalmological signs were considered. The same group found similar findings in a larger number of cats (Felten et al., 2021), where the sensitivity of RT-PCR was only 30% when both neurological and non-neurological FIP cases were included, but rose to 83.3% when only cats with neurological FIP were considered. In one study (Soma et al., 2018) it was found that all CSF samples with a CSF FCoV antibody titre of >640 that were tested for FCoV RNA were positive by RT-PCR. This study was limited by the fact that FIP was not confirmed in all cats, but it does suggest at least an association between high CSF FCoV antibody titres and positive CSF FCoV RT-PCR. In summary, FCoV RT-PCR on CSF appears to be a useful additional test in cats with neurological signs, as a positive result highly supports a diagnosis of FIP, but a negative result does not rule out FIP.

FCoV RT-PCR on aqueous humour samples

Positive FCoV RT-PCR results have been reported in cats with FIP (Barker et al., 2017; Emmeler et al., 2020) through samples collected at post-mortem examination, but one study also described positive results in two cats on samples collected ante-mortem (Linn-Pearl et al., 2015). One study (Sangl et al., 2020) has documented positive FCoV RT-PCR on aqueous humour samples from 11 of 31 cats with confirmed FIP and none of 27 control cats without FIP. Again, these samples were collected at post-mortem examination and, interestingly, only four of the 31 cats with FIP had ocular signs of uveitis with only two of these four cats being FCoV RT-PCR aqueous humour positive. Although FCoV RT-PCR had a specificity of 100% in this study, its sensitive was low at 35.5%.

FCoV RT-PCR on faeces

FCoV RT-PCR can be performed on faecal samples or rectal swabs, but this is primarily used to identify cats that are shedding FCoV for the management of infection in a multi-cat household, and it is not used to diagnose FIP, as it is known that many healthy cats without FIP shed FCoV. Two studies found that 75% and 65% of cats with FIP shed virus in their faeces (Addie et al., 1996; Barker et al., 2017). Studies testing faecal samples by RT-qPCR from 50 healthy cats in US shelters (Fish et al., 2018), from 82 healthy cats from German catteries (Felten et al., 2020) and 179 cats from German breeding catteries (all with at least 5 cats per household) (Klein-Richers et al., 2020) found that 56%, 71% and 76.5% of cats, respectively, were positive for FCoV RNA. Although one study (Barker et al., 2017) showed that cats with FIP were more likely to be shedding FCoV in their faeces than cats that were euthanased due to diseases other than FIP, in an individual cat, faecal RT-PCR is not useful for diagnosis of FIP.

Molecular techniques characterizing FCoV spike (S) gene mutations following positive RT-PCR for FCoV RNA

Following detection of FCoV RNA in a sample by RT-PCR, varied molecular techniques can then be used to derive or deduce sequence data for the FCoV detected. Available methods are described in full elsewhere (Barker & Tasker, 2020) but in brief comprise of sequencing methods, such as pyrosequencing and Sanger sequencing of FCoVs, most often used in research, and methods designed to detect and quantify specific FCoV mutation sequences, such as PCR with allelic discrimination, which is available commercially for the diagnosis of FIP in some laboratories. However, such techniques are not always successful at deriving the FCoV sequence

data in FCoV RT-PCR-positive samples as the FCoV levels may be too low to allow sequence analysis (particularly in cats without FIP) and FCoV sequence variability can prevent targeted sequencing techniques from generating sequence data (e.g. some sequence analysis methods only detect mutations in type I FCoV, and not type II FCoV) (Barker et al., 2017; Decaro et al., 2021). As described below, FCoV sequence analysis has focused on regions of the S gene, and techniques employed for analysis include pyrosequencing, sanger sequencing and allelic discrimination (Barker and Tasker 2020b),

RNA sequence analysis usually focuses on the region of the S gene of type I FCoV in which certain mutations were found in the FCoV in tissues from cats with FIP but not in the FCoV in the faeces of healthy cats without FIP (Chang et al., 2012; Decaro et al., 2021). Subsequent studies (Porter et al., 2014; Barker et al., 2017) analysed FCoV of both tissue and faecal samples from cats with FIP and cats without FIP (confirmed as having other diseases by histopathology) by pyrosequencing with and without Sanger sequencing. These studies found that these S gene mutations were also found in the FCoV in tissues from cats without FIP; thus, they appear to be associated with systemic FCoV infection rather than FIP *per se* (Porter et al., 2014; Barker et al., 2017). However not all 'systemic' derived samples in FCoV-infected cats show these mutations. One study was published describing a cat with neurological FIP (Andre et al., 2019) in which histological changes of FIP were found only in the central nervous system (CNS). The FCoV in the CNS had S gene mutations, whereas those found systemically in other organs did not. Additionally, a study of seven cats that remained healthy following experimental infection with FCoV (Lutz et al., 2020) aimed to document the presence or absence of S gene mutations in samples of tissue (primarily colon, liver, thymus) and faeces obtained from these cats; S gene sequences could only be obtained in five (four colonic, one liver) samples from four of the seven cats, but none of these contained the S gene mutations. Thus, although S gene mutations are likely to be important in the development of FIP, as they are present in most FIP-associated FCoV, it is proposed that it is via these and/or other mutations that the FCoV acquires its monocyte/macrophage tropism to allow it to spread systemically outside of the gut and contribute to the development of FIP. Other viral factors are likely to then allow effective and sustained replication in monocytes, and activation of infected monocytes, in cats that develop FIP following systemic FCoV infection (Kipar and Meli 2014).

S gene mutation analysis on tissue samples

An extensive study (Barker et al., 2017), that included 260 tissue samples from 57 cats with FIP, and 258 tissue samples from 45 cats without FIP, calculated that S gene mutation analysis using pyrosequencing with or without Sanger sequencing on tissues, as an additional step to the detection of FCoV RNA alone by RT-qPCR, only slightly increased specificity for the diagnosis of FIP, from 92.6% to 94.6% (equivalent of five tissues), but moderately decreased sensitivity, from 89.8% to 80.9% (equivalent of 20 tissues). The decrease in sensitivity was because of the detection of non-mutated FCoV in cats with FIP (n=4), the presence of type II FCoV in cats with FIP which is not detected by mutation analysis assays that rely on finding the specific S gene mutations seen in type I FCoV by targeted analysis (n=12) and an inability to sequence the FCoV S gene due to only low FCoV copy numbers being present (n=4). The increase in specificity was due to the detection of non-mutated FCoV in cats without FIP (n=2) and an inability to sequence the FCoV S gene due to low FCoV copy numbers (n=3).

Another study (Sangl et al., 2019), that performed S gene mutation analysis using a commercially available allelic discriminative assay on pooled tissue samples (five per cat) from 34 cats with FIP and 30 cats without FIP, reported a much higher specificity of 100% for S gene mutation analysis, as none of the 30 cats without FIP gave positive results on S gene mutation analysis. However, only three cats without FIP were FCoV RT-PCR-positive

in this study, and in none of these was S gene mutation analysis successful, so the specificity calculation was not based on detecting non-mutated FCoV as such. Sensitivity in this study was moderate at 70.6%, as only 24 of the 34 FIP cases had mutations detected.

One study (Emmler et al., 2020) performed S gene mutation analysis using allelic discrimination on FNAs and IBs of popliteal and mesenteric lymph nodes, liver, spleen, omentum, and kidneys in 20 cats with FIP confirmed by immunohistochemistry. FCoV with S gene mutations were present in at least one sample in each cat, but there was variation in which sample was positive. FCoV with mutations in the S gene was most frequently found in effusions (64%), followed by in IBs of the spleen, omentum and kidney (50%), then in mesenteric lymph node IBs and FNAs (45%), and finally in FNAs of spleen and liver and liver IBs (40%). There was a loss in sensitivity in all tissues when compared to RT-PCR for FCoV without mutation analysis.

Another mutation analysis study using sequencing (Stranieri et al., 2018) on tissues (MLN, spleen, small intestine and lung) in 10 cats with confirmed FIP and eight cats with diseases other than FIP reported a sensitivity of 70% (7 of 10 cats with FIP had mutations) and specificity of 88% (1 of eight cats without FIP had a mutation) compared to values of 91% and 50% respectively for RT-PCR alone.

A study (McKay et al., 2020) from Canada, that also used sequencing to deduce FCoV S gene segment sequences, documented that only nine of the 20 (45%) S gene sequences that could be obtained from 69 tissue samples showing typical histopathological findings of FIP possessed the previously documented S gene mutations associated with systemic infection or FIP; a further 15% contained a novel S gene mutation and 40% had no mutations at all in the region sequenced. Sensitivity and specificity were not calculated, but the lack of finding of S gene mutations in tissues from cats with FIP in this study highlighted a possible sensitivity issue with mutation detection. The authors of the study speculated that the FCoV associated with FIP in Western Canada may have additional or alternative virulence sites that were not identified in the 'traditional' region of the S gene targeted by sequencing in the study.

S gene mutation analysis on effusion and other fluid samples

S gene mutation analysis has also been performed on effusions in various studies using different methods (Felten et al., 2017a; Felten et al., 2017c; Longstaff et al., 2017; Stranieri et al., 2018) with variable sensitivities reported of 40.0% (by sequencing) (Stranieri et al., 2018), 60% (by pyrosequencing and sequencing) (Longstaff et al., 2017), 65.3% (by sequencing) (Felten et al., 2017b) and 68.6% (by allelic discrimination) (Felten et al., 2017a). One study (Lin et al., 2022) of cats with suspected FIP found the M1058L S gene mutation in 89 or 94 samples in which mutation analysis was possible. A study (Barker et al., 2017) that evaluated 51 fluid samples (primarily effusions but also included CSF and aqueous humour) from 57 cats with FIP and 47 fluid samples from 45 cats without FIP calculated that S gene mutation analysis (via pyrosequencing and sequencing), in addition to the detection of FCoV alone by RT-qPCR, did not increase specificity (it stayed at 97.9%) for the diagnosis of FIP, but markedly decreased sensitivity from 78.4% to 60%. Another study (Felten et al., 2017a) that carried out the same calculations on effusion samples, described an increase in specificity from 87.5% to 95.8% for S gene mutation analysis over FCoV RT-PCR alone whilst sensitivity decreased from 97.1% to 68.6%. However, only effusions from three cats without FIP were FCoV RT-PCR-positive and in only one of these, S gene mutation analysis was successful (where mutated virus detected), so the improvement in specificity was not based on detecting non-mutated FCoV. Another study (Hellemans et al., 2020) documented a range of S gene mutations by sequencing in the majority of effusion samples from cats with FIP.

Another study (Sangl et al., 2020) using allelic discrimination evaluating aqueous humour samples by FCoV RT-PCR with subsequent mutation analysis concluded that mutation analysis was not helpful for the diagnosis of FIP; in this study, of 11 aqueous humour samples that were FCoV RT-PCR positive in cats with FIP, only four of the 11 samples yielded successful results for mutation analysis (three with mutated virus and one mixed mutated and non-mutated virus detected).

One study (Felten et al., 2021) using allelic discrimination that evaluated CSF samples by FCoV RT-PCR with subsequent mutation analysis also concluded that mutation analysis was not helpful for the diagnosis of FIP; in this study, of nine CSF samples that were FCoV RT-PCR positive in cats with FIP, only three yielded results for mutation analysis (all three were positive for the presence of S gene mutations). In this study the sensitivity of mutation analysis in cats with FIP was only 10% (rising to only 16.7% when only cats with neurological FIP were considered); specificity of mutation analysis could not be calculated as none of the cats without FIP yielded positive FCoV RT-PCR results upon which to subsequently perform mutation analysis.

These data suggest that detection of S gene mutations alone by the different methods cannot be regarded as being confirmative for FIP. If performed it is important to interpret S gene mutation results in association with other factors (signalment, other test results) to determine how likely FIP is in the cat being tested. Generally, spike gene analysis appears to be of little significant benefit over RT-PCR (Barker and Tasker 2020a).

Indirect detection of the infectious agent

FCoV antibody testing

Antibody testing on blood samples

Serum FCoV antibody tests are usually enzyme-linked immunosorbent assays (ELISA), indirect immunofluorescence antibody (IFA) tests or rapid immunomigration tests (Addie et al., 2015). The porcine coronavirus TGEV or FCoV can be used in these tests as antigen substrates, both being able to detect serum FCoV antibodies; indeed, using TGEV as a substrate in one study (Kummrow et al., 2005) showed higher sensitivity in the detection of serum FCoV antibodies than using FCoV as substrate. In most tests, antibody titres are determined in multiples of serum dilutions. A positive FCoV antibody test indicates that the cat has had contact with FCoV (by natural infection or vaccination) and has developed antibodies; this typically occurs around 10-28 days following natural infection (Meli et al., 2004; Vogel et al., 2010). Although cats with FIP tend to have higher FCoV antibody titres than cats without FIP, there is much overlap, with no difference between median FCoV antibody titres in healthy and suspected FIP cases, so the value in an individual cat to distinguish cats with FIP is very limited (Bell et al., 2006a). It has been suggested that a negative serum FCoV antibody result in a suspected FIP case that doesn't have an effusion is more useful to rule out a diagnosis of FIP than in a cat with effusion (Sparkes et al., 1994; Addie et al., 2009; Addie et al., 2015). However, negative results were reported in three of seven cats with non-effusive neurological FIP (Negrin et al., 2007), although in that study the method of FCoV antibody testing was not described. It is important that the FCoV antibody assay used has adequate sensitivity; otherwise, false negative results can occur (Addie et al., 2015). FCoV antibody tests which begin with a dilution of the sample to 1 in 100, or 1 in 400, are commonly insensitive, missing titres lower than the starting dilution (i.e. those <100 or <400). Only tests which have a starting dilution of 1:25 or less are recommended. Opinions on the usefulness of antibody testing in cats suspected to have FIP vary, but there is no 'FIP antibody test'; all that can be measured is antibody against FCoV.

Antibody testing on effusions

FCoV antibody tests can also be performed on effusion samples. However, in one study, some cats with FIP (although the diagnosis was not confirmed in all cases) had unexpectedly low FCoV antibody titres in their effusions (Meli et al., 2013) and an inverse correlation between FCoV RNA load, measured by RT-qPCR, and FCoV antibodies was found in some samples, suggesting that the FCoV can bind antibodies rendering them unavailable as a ligand in the antibody test (Meli et al., 2013). False negative results for FCoV antibodies on effusions can be particularly a problem with rapid immunomigration/immunochromatography tests (Meli et al., 2013; Addie et al., 2015). However, other studies (Lorusso et al., 2019; Hellemans et al., 2020) have found no evidence of an inverse correlation between FCoV RNA loads and antibody titres in effusions from cases with suspected FIP and both of these studies concluded that a combination of both FCoV RT-PCR and antibody testing would be more helpful to support a diagnosis of FIP compared to either test alone (Lorusso et al., 2019; Hellemans et al., 2020).

Antibody testing on CSF samples

FCoV antibody testing has been performed on CSF samples in cats with FIP with varied results. One study (Foley et al., 1998) reported it to be useful in diagnosing FIP, with comparison of serum and CSF FCoV titres suggesting intrathecal FCoV antibody production, although no controls were included in this study. Another study (Boettcher et al., 2007) found a significant correlation between serum and CSF FCoV antibody titres, suggesting that any CSF FCoV antibodies detected were derived from blood, and thus their detection was not additionally useful for the diagnosis of FIP. Another study suggested that a CSF FCoV antibody titre of >640 might be useful for the diagnosis of FIP (Soma et al., 2018), although the diagnosis of FIP was not histopathologically confirmed in the cats in this study.

Approach to treatment of FIP

Treatment (or euthanasia) of cats with suspected FIP should only be considered after every effort has been made to obtain a definitive diagnosis, since the wrong treatment can be detrimental for a cat. Situations have been described in which a cat with toxoplasmosis was misdiagnosed as possibly having FIP and was treated with glucocorticoids with fatal consequences (Cohen et al., 2016).

Historically, no effective causal treatment was available for FIP, so every cat with confirmed FIP died or was euthanized. Occasionally, cats have survived for several months or years after diagnosis (Ishida et al., 2004; Ritz et al., 2007; Pedersen 2014a; Hugo and Heading 2015; Legendre et al., 2017) receiving treatment, for example glucocorticoid or non-steroidal anti-inflammatories, but it is not clear whether prolonged survival was due to the treatment. There are some rare reports of “recovered” cats in the field, but in these cats, a definitive diagnosis has not usually been obtained or reported. Currently, there is no drug licensed in cats for the treatment of FIP but newer antivirals, discussed below, including remdesivir that is licensed for use in humans (for the treatment of COVID-19) have been used successfully to treat FIP in cats (Anon, 2022).

Prognosis of FIP

The prognosis for a cat with FIP without effective antiviral treatment is extremely poor. In a prospective study including 43 cats with FIP with effusion, the median survival time after definitive diagnosis was eight days (Ritz et al., 2007). Another study of cats with FIP reported a median survival time of 21 days after presentation for cats with effusions and 38 days for cats without effusions (Tsai et al., 2011). Indeed, some cats, mainly those without effusions, can live for several months up to years (Ishida et al., 2004; Ritz et al., 2007; Hartmann and Ritz 2008a; Tsai et al., 2011; Pedersen 2014a; Hugo and Heading 2015; Legendre et al., 2017). The disease progression between onset of clinical signs and death is variable but is shorter in younger cats and cats with effusions than in older cats and cats without effusion (Pedersen 2014b). One cat with FIP lived 200 days after a definitive diagnosis (Ritz et al., 2007; Hartmann and Ritz 2008a), another survived 787 days (Hugo and Heading 2015). One Birman cat that had never developed effusion died of FIP at six years of age; based on its history, the cat likely had a “mild form” of FIP for many years (Pedersen 2014b).

Some parameters can predict survival time; poor general condition, low platelet count, low lymphocyte count, low haematocrit, high bilirubin concentration, low sodium concentration, low potassium concentration, high AST activity, and a large volume of effusion indicate a poor prognosis (Ritz et al., 2007; Tsai et al., 2011). Seizures can also be considered a poor prognostic sign, since they occur significantly more frequently in animals with marked extension of inflammatory lesions in the forebrain (Timmann et al., 2008).

Epidemiological considerations in the management of cats following a diagnosis of FIP

Does a cat with FIP pose a threat to other cats in its household?

Often the question arises whether it is dangerous to bring a cat with FIP back into a household with other cats. In-contact cats have likely been exposed to the same FCoV isolate that originally infected the cat that has FIP. Still, the key question remains whether mutated virus associated with the switch from enteric infection to systemic infection and the development of FIP could be transmitted from cat to cat. In order to answer this question, some facts concerning FCoV epidemiology need to be considered.

According to different studies, 35 to 79% of cats with FIP shed FCoV in their faeces (Addie et al., 1996; Chang et al., 2010b; Porter et al., 2014; Barker et al., 2017). However, it appears that some cats, once they have developed FIP, stop shedding virus in their faeces. One study (Chang et al., 2010b) on 27 cats with asymptomatic FCoV infection and 28 cats with FIP from the same households revealed that 11 of 17 cats with FIP had no detectable intestinal FCoV and seemingly cleared their primary FCoV infection (Chang et al., 2010b). In those cats suffering from FIP with detectable intestinal FCoV, sequence analysis focusing on the FCoV 3c gene revealed that in all but one cat, the virus was different to that associated with FIP lesions, and thus seemed to have been acquired by FCoV superinfection from other cats in the household (Chang et al., 2010b). The authors concluded that if a cat with FIP restarts shedding, this is likely due to a new FCoV superinfection and not the original FCoV that resulted in FIP. However, in this study (Chang et al., 2010b), the one cat with FIP that was shedding a FCoV strain in its faeces similar to the FCoV strain found in its ascitic fluid was believed to have

arisen due to leakage of systemic virus into the intestines due to, for example, an intestinal granuloma. In another study it was reported that faecal FCoV from cats with FIP can carry the same S gene mutations as FCoV found systemically (Barker et al., 2017), and one study found that the full genomic RNA sequences of field FCoV strains isolated at *post-mortem* examination from the jejunum and the liver of a cat with FIP revealed 100% nucleotide identity between the enteric (jejunum) and non-enteric (liver) derived viral RNA sequences, suggesting that FIP-associated FCoV can be shed under some circumstances (Dye and Siddell 2007). However, even if FIP-associated FCoV is shed in the faeces of cats with FIP, it likely cannot cause FIP following transmission to another cat as one study demonstrated that faeces of cats with FIP did not cause FIP in another cat (Pedersen et al., 2012). The current understanding is that horizontal transmission of FIP, via a FIP-associated FCoV strain, is not believed to occur very frequently, if at all. Although there is no clear evidence that the disease FIP is transmitted from cat to cat under natural circumstances, FIP can be induced experimentally, such as by inoculation of a FIP-associated FCoV intraperitoneally (Kim et al., 2016), a route which bypasses the natural faecal-oral transmission pathway.

Therefore, based on the current knowledge, it is likely to be relatively safe to take a cat with FIP back into a household with cats that have already been in contact with it, as these cats are likely to already be FCoV-infected. It is, however, not recommended that the cat with FIP has contact with any 'naïve' FCoV-uninfected cat, because if the cat with FIP is shedding FCoV, it could infect any naïve cats with FCoV.

In households where a cat with FIP has been euthanised, with no remaining cats in the household, it is recommended that the owner waits for two months before obtaining new cats, as FCoV as it has been suggested that FCoV preserves its infectivity for days to a few weeks, depending on environmental conditions (Scott, 1988), such as in desiccated faeces.

Management of cats with FIP in the veterinary practice

Cats with FIP in a veterinary practice or hospital should be handled and housed like any other cat, as any cat is a potential source of FCoV infection, and routine hygiene measures should be taken. Thus, there is no benefit in isolating the cat with FIP and it is not necessary to keep cats with FIP in infectious disease isolation wards.

Agents used in the treatment of FIP

Supportive, including anti-inflammatory and effusion drainage, treatments for FIP

Table 2 outlines agents that can be used in the supportive treatment of FIP.

FIP is an immune-mediated disease; thus, symptomatic treatment is aimed at controlling the immune response to FCoV and consists of high doses of immune-suppressive or anti-inflammatory drugs that slow down disease progression, such as prednisolone (initially 2-4 mg/kg q24h PO), that might be tapered down slowly if the cat responds to treatment. Although used in many published cases and in the field, the effect of glucocorticoids never has been substantiated in controlled studies. However, two separate double-blind controlled studies that

evaluated feline interferon-omega (Ritz et al., 2007) and propentofylline (Fischer et al., 2011) as treatments for FIP gave all of the cats (both those in the treatment and the control groups) glucocorticoid treatment; the cats given the additional drugs did not survive any longer than those given glucocorticoids only and, indeed, those on glucocorticoids only survived for a median of eight days, confirming a poor outcome with this treatment alone in these cats (Izes et al., 2020c). In one study, cats without effusion treated with both systemic glucocorticoids and the immunomodulator polyprenyl immunostimulant (PPI) had poorer survival than those treated with PPI alone (Legendre et al., 2017); however, a definitive diagnosis of FIP was not established in all cats in this study.

If an effusion is present, some cats benefit from daily removal of the effusion (particularly if pleural effusions are resulting in dyspnoea) and injection of dexamethasone into the abdominal or thoracic cavity (1 mg/kg q24h until effusion is no longer produced, for up to seven days; if effusion is present in both cavities, the dose per cavity should be divided) (Hartmann and Ritz 2008a).

If indicated, cats should also be treated with broad-spectrum antibiotics (e.g., as long as the effusion is being removed) and supportive therapy (e.g., fluids) (Hartmann and Ritz 2008a).

Cyclophosphamide (2-4 mg/kg four times per week PO) alone or in combination with glucocorticoids has been sometimes used but there are no data on its efficacy.

A thromboxane synthetase inhibitor (ozagrel hydrochloride) that inhibits platelet aggregation and cytokine release was used in two cats with some improvement of clinical signs (Watari et al., 1998), but this was not a controlled study.

A placebo-controlled study in a small number of cats (three treated, three placebo) in an experimental model of FIP found a possible beneficial effect of treatment with antibodies acting against feline tumour necrosis factor alpha (TNF-alpha) (Doki et al., 2016). In this study, progression to FIP was prevented in two of the three cats treated with these antibodies, whereas all three cats developed FIP in the placebo group. TNF-alpha is thought to be involved in FCoV replication in macrophages (Takano et al., 2007b) and contributes to development of clinical signs in cats with FIP. An uncontrolled very small study used anti-human-TNF-alpha antibody treatment alongside itraconazole (Doki et al., 2020b); only three of the 10 cats inoculated in this experimental study developed FIP, and two of these three cats improved with treatment. No field studies have been conducted so far.

Pentoxifylline or propentofylline has been applied to cats with FIP because they can down-regulate pro-inflammatory cytokines which in turn are thought to increase vasculitis. However, in a placebo-controlled, double-blind study in cats with confirmed FIP, there was no significant difference in survival time, quality of life, or any clinical or laboratory parameter in cats treated with propentofylline *versus* cats receiving placebo (all cats received glucocorticoids in this study) (Fischer et al., 2011).

Table 2: Drugs that have been suggested for use as supportive treatments for cats with FIP

Drug	Comments	ABCD recommendation
Prednisolone/dexamethasone	Acts as immunosuppressant. No controlled studies available. Some cats benefit from treatment and survive for several months (Ritz et al., 2007). Does not cure FIP.	Currently supportive treatment of choice, especially in cats with effusion (if effusion is present, high dose dexamethasone injection in the body cavity can be considered).
Azathioprine	Aims to immunosuppress (and to lower the prednisolone/dexamethasone dose). Toxic in cats. No published studies available.	Not recommended.
Chlorambucil	Aims to immunosuppress (and to lower the prednisolone/dexamethasone dose). No published studies.	Might be considered in combination with prednisolone/dexamethasone.
Cyclophosphamide	Aims to immunosuppress (and to lower the prednisolone/dexamethasone dose). No published studies.	Might be considered in combination with prednisolone/dexamethasone.
Anti-TNF-alpha antibody	Blocks TNF-alpha that is involved in exacerbating clinical signs of FIP (Takano et al., 2007a; Takano et al., 2007b; Takano et al., 2009). Some efficacy in a placebo-controlled study including few cats (three treated, three placebo) with experimentally induced FIP (Doki et al., 2016). Used in an uncontrolled very small study of cats with experimentally induced FIP alongside itraconazole treatment (Doki et al., 2020b) in which two of three cats with FIP improved.	Placebo-controlled field studies required.
Ozagrel hydrochloride	Inhibits thromboxane synthesis leading to reduction of platelet aggregation and cytokine release. Only used in two cats with some improvement of clinical signs (Watari et al., 1998).	Not recommended until further studies available.
Pentoxyfylline/Propentofylline	Aims at treating vasculitis. One placebo-controlled double-blind study on propentofylline showed no efficacy (all cats were given glucocorticoids) (Fischer et al., 2011).	Not recommended.

Antiviral and immunomodulating treatments for FIP

Table 3 outlines antiviral and immunomodulatory agents that have been used for the treatment of FIP.

Currently, no drugs are licensed in cats to treat FIP, although specific antiviral compounds are now available, albeit non-licensed, but which enable the effective treatment of FIP in cats. For many drugs, evaluation of data is hampered by the lack of well-controlled clinical trials in which new treatments are compared against a standard care or placebo and the fact that the presence of FIP was not always confirmed in these studies before treatment was initiated, which makes an assessment of the outcome impossible (Hartmann and Ritz 2008b). Some very promising drugs have been developed and evaluated for the treatment of FIP but are not yet commercially available to most veterinarians, such as nucleoside analogues and proteinase inhibitors (Sharun et al., 2021). Promising experimental approaches include inhibition of the binding of FCoV spike protein to receptors on the host cell membrane that mediates fusion of the viral envelope with host cell membranes (Kim et al., 2013; Liu et al., 2013), circular triple helix-forming oligonucleotide RNA targeting viral RNA (Choong et al., 2014), cholesterol synthesis and transport inhibitors (including itraconazole) inducing cholesterol accumulation in cells and thereby inhibiting FCoV replication (Takano et al., 2017; Takano et al., 2019; Doki et al., 2020a; Doki et al., 2022) and small interfering RNAs (siRNA) leading to RNA interference and thus, inhibition of virus replication (McDonagh et al., 2011; McDonagh et al., 2015). Some drugs are effective *in vitro*, but are too toxic for cats, such as ribavirin (Weiss and Oostrom-Ram 1989; Weiss et al., 1993a; Weiss et al., 1993b) or the small molecule chloroquine (Takano et al., 2013; McDonagh et al., 2014). Hydroxychloroquine, in *in vitro* studies (Takano et al., 2020), has been suggested as a less toxic alternative to chloroquine. The mechanism of action of the anti-viral properties of the small molecule mefloquine is not known (McDonagh et al., 2014; Yu et al., 2020) but its hepatic metabolism has been studied using an *in vitro* model (Izes et al., 2020a) as well as its pharmacokinetics in healthy cats (Yu et al., 2020) and its plasma protein binding properties in the plasma of healthy cats and cats with FIP (Izes et al., 2020b). Although further studies are needed on its pharmacokinetics and efficacy in cats with FIP, vets in Australia are using oral mefloquine to treat cats with FIP when finances prohibit the use of a full course or increased dose of other antivirals (GS-441524 or remdesivir – see below) as it is cheap and shows some encouraging efficacy as adjunct treatment (Taylor et al., 2021). Other drugs have only been investigated *in vitro*, but *in vivo* efficacy is unknown, such as vidarabine (Barlough and Scott 1990) which inhibits polymerases; nelfinavir, a commercially available protease inhibitor of human immunodeficiency virus; *Galanthus nivalis* agglutinin (GNA), a carbohydrate-binding agent that binds to FCoV-glycosylated envelope glycoproteins, thereby inhibiting viral attachment to the host cell (van der Meer et al., 2007; Hsieh et al., 2010); and ERDRP-0516, a non-nucleoside inhibitor of the RNA-dependent of RNA polymerase (Camero et al., 2022). One study has shown evidence of antiviral synergy between GS-441524 and itraconazole *in vitro*, especially versus a type I FCoV (Doki et al., 2022).

Cyclosporine A

Cyclosporine A can act as an antiviral drug as it binds to cellular cyclophilins thereby inhibiting calcineurin, which is required by many viruses for replication (Tanaka et al., 2012; Tanaka et al., 2013). Cyclosporine A inhibits FCoV replication *in vitro* (Tanaka et al., 2012). Cyclosporine A was also associated with a reduction in pleural fluid volume and a decrease in viral load in one cat with FIP, but the cat succumbed to FIP 264 days after treatment initiation (Tanaka et al., 2015). Thus, cyclosporine A might be an option in combination with other therapeutic agents. So far however, well-controlled clinical studies are missing.

Curcumin

Curcumin, a derivative of turmeric, has anti-inflammatory and antiviral properties. Curcumin-encapsulated chitosan nanoparticles (Cur-CS), created to increase the bioavailability of curcumin, were evaluated *in vitro* and found to decrease the immune-related proteins produced during infection of cell cultures with a FIP-associated virus as well as to inhibit viral replication (Ng et al., 2020). The same study confirmed the enhanced bioavailability of Cur-CS over curcumin in pharmacokinetic analysis in healthy cats. However, another *in vitro* study failed to find any inhibitory effect of curcumin on FCoV proliferation (McDonagh et al., 2014). Thus, further studies on this agent are required.

Interferons

Interferons are frequently used in cats with FIP. Human interferon- α was effective against a FIP-associated FCoV strain *in vitro*, but in a placebo-controlled treatment study including 74 specific pathogen-free cats in which FIP was induced experimentally, neither the prophylactic nor the therapeutic administration of high doses (10^4 or 10^6 IU/kg) interferon- α , feline interferon- β (10^3 IU/kg), the immunomodulator *Propionibacterium acnes* (0.4 mg/cat or 4 mg/cat), or a combination, significantly reduced mortality in treated *versus* untreated cats (Weiss et al., 1990). However, in the cats treated with 10^6 IU/kg interferon- α in combination with *Propionibacterium acnes*, the mean survival time was prolonged, but only by three weeks (Weiss et al., 1990). As an explanation for the limited efficacy of interferon- α , it has been suggested that ORF-7-encoded accessory protein 7a of FIP-associated strains can act as type-I interferon antagonists and counteract the interferon- α -induced antiviral response (Dedeurwaerder et al., 2014).

Feline interferon- ω , which is licensed in many European countries, inhibits FCoV replication *in vitro* (Mochizuki et al., 1994). Preliminary positive results were obtained in one uncontrolled trial, but FIP was not confirmed in the cases that survived (Ishida et al., 2004). In a randomized placebo-controlled double-blind treatment trial in 37 cats with confirmed FIP, feline interferon- ω and immunosuppressive levels of glucocorticoids was not more effective than glucocorticoids alone (Ritz et al., 2007). A published *in vitro* study evaluating the combination of hydroxychloroquine with interferon- ω (Takano et al., 2020) found that the addition of interferon- ω increased antiviral action for Type I FCoV replication, suggesting consideration of combinations of treatment. Oral feline interferon- ω was used as a follow up treatment in a case of FIP that successfully responded to antiviral nucleoside inhibitor treatment (Addie et al., 2020b), and the use of both oral and subcutaneous feline interferon- ω , usually in combination or after other treatments, including antiviral nucleoside inhibitors, has been described in a series of cats with confirmed or suspected FIP (Addie et al., 2022). The additional efficacy of the interferon- ω has not been shown in controlled studies.

Protease inhibitors including GC376

Very promising new drugs include protease inhibitors that prevent viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors needed for the production of infectious viral particles. Inhibitors that target the 3C-like protease with broad-spectrum activity against human and animal coronaviruses have been created (Kim et al., 2013). One 3C-like protease inhibitor, GC376, showed strong activity against FCoV *in vitro* (Kim et al., 2016) and was effective in treating FIP in an experimental setting; of eight cats with experimentally induced FIP, six remained healthy for an eight month follow up period (Kim et al., 2016), although one of these six cats succumbed to neurological FIP subsequently (Pedersen et al., 2018). In a field trial, a cohort of 20 client-owned cats were treated with GC376 at 15 mg/kg SC q12h; this was a higher dose

than that used in the experimental study (Kim et al., 2016) due to treatment failure in the first cat enrolled in the field trial. Nineteen of 20 treated cats regained health within two weeks of treatment. However, disease signs recurred one to seven weeks after primary treatment. Relapses no longer responsive to treatment occurred in 12/19 cats within one to seven weeks of initial or repeat treatments. Most of these relapsed cats developed neurological FIP. At the time of writing, 7/20 cats were in disease remission (Pedersen et al., 2018); most of these were cats that had presented at a young age with effusion. Cats presenting with neurological signs had been excluded from the study as GC376 does not appear to penetrate the CNS. Some side effects occurred and included injection site reactions and retarded development or abnormal eruption of permanent teeth (Pedersen et al., 2018). No untreated controls were used in this study and FIP was not confirmed in all cats with histopathology and/or immunostaining of FCoV antigen, which hampers the interpretation of data. A published retrospective study of cats with suspected FIP (Yin et al., 2021) mentioned some cats that had been treated successfully with GC376, at 6-8 mg/kg/d for at least 4 weeks, together with or without a nucleoside analogue, but full data on route of administration, composition of the formulations used and response to treatment were not provided. Overall, the studies on GC376 suggest that protease inhibitors are a promising approach and might well be more effective if combined with other antiviral drugs, but more field trials are necessary. It is hoped that GC376 will be available as a licensed form for cats within the next few years (Anivive website information on future products 2022).

Nucleoside analogues, including GS-441524 and remdesivir

Another promising treatment approach is the use of nucleoside analogues that act as an alternative substrate for viral RNA synthesis, resulting in RNA chain termination during viral RNA transcription via inhibition of RNA dependent RNA polymerase.

GS-441524

One such nucleoside analogue, the compound GS-441524, which is the active component of remdesivir, was shown to be non-toxic *in vitro* and effectively inhibited replication of FIP-associated FCoV strains and field isolates in two different cell culture systems. In 10 young cats with experimentally induced FIP, GS-441524 (applied SQ q 12 h) caused a rapid reversal of clinical signs and return to a clinically healthy status within two weeks of treatment in all 10 cats. Two of the 10 treated cats had recurrences of clinical signs at four weeks and six weeks post treatment, respectively. These two cats were treated a second time with GS-441524 for two weeks and they responded again, identically to the response seen with the first treatment. All 10 cats remained clinically healthy until the time of publication (> eight months post infection) (Murphy et al., 2018). No signs of toxicity were noted besides a transient "stinging" injection reaction in some cats, such as unusual posturing, licking at the injection site and/or vocalizations, directly after compound administration (Murphy et al., 2018). GS-441524 treatment was then evaluated in a field study of 31 cats with FIP (Pedersen et al., 2019). Cats were diagnosed with FIP based on signalment, history, clinical examination, prior test results, repeat testing and/or effusion analysis, and FCoV RT-PCR performed on effusions in some cats. Tissue IHC for FCoV antigen that confirmed FIP was only performed on five cats that later underwent *post-mortem* examination. Cats with neurological or ocular signs were discouraged from the trial due to concerns from the experimental trial of poor penetration of GS-441524 into the brain and/or eye (Murphy et al., 2018). Of the 31 cats with FIP recruited into this study (Pedersen et al., 2019), five had no evidence of effusion. Cats ranged from 3-73 months of age (mean 14 months). The cats were started with a primary treatment course of GS-441524 at a dosage of 2 mg/kg SQ q 24 h for at least 12 weeks (with more than 12 weeks of treatment given if serum protein levels remained elevated). The dosage was increased to 4 mg/kg SQ q 24 h for later treatments in the trial when cats showed a relapse or when a treatment course of longer than 12 weeks was deemed necessary. The study did not include a

control group treated with a placebo or standard care protocol. Five of the 31 cats died or were euthanized within 26 days of the first treatment. The remaining 26 cats completed 12 or more weeks of GS-441524 treatment and showed rapid clinical improvement within two weeks. Of these 26 cats, 18 remained healthy, while eight others showed FIP relapses (six cats non-neurological FIP and two cats neurological FIP) at a mean of 23 days following treatment. Three of the eight cats with relapses were treated again with GS-441524 at 2 mg/kg SQ q 24 h; one of these three cats relapsed with neurological FIP and was euthanized whilst the two remaining cats responded well but relapsed with FIP again and were treated again with GS-441524 but at a higher dosage of 4 mg/kg SQ q 24 h. Of the original 31 cats, 25 (80.7%) were classified as long-time survivors after successful treatment, but one of these cats was subsequently euthanized due to presumably unrelated heart disease, while 24 remain healthy at the time of publication (Pedersen et al., 2019). Subsequently, a case series describing GS-441524 treatment (at a higher dose of 5-10 mg/kg SQ q 24 h for at least 12 weeks) in four cats with neurological and ocular signs of FIP was published (Dickinson et al., 2020) documenting very promising results. Three of the four cats were alive and off treatment at the time of publication, 354-528 days after treatment had started; two cats had received 5 mg/kg SQ q 24 h and one cat an escalating dose to 10 mg/kg SQ q 24 h. The remaining cat was euthanised 216 days after starting treatment; this cat had not shown complete resolution of signs on treatment (5 mg/kg SQ q 24 h) and rapid clinical regression occurred when treatment was stopped. Additionally, local skin reactions and discomfort around the SQ injections were cited as a reason for euthanasia.

The studies described above have all administered the nucleoside analogues subcutaneously. Further advice regarding how GS-441524 can be used in the treatment of FIP is continually evolving (Pedersen, 2021a), including recommendations on dose, duration, monitoring and possible side effects of treatment. It appears that younger cats without neurological or ocular signs are those that tend to do most favourably with subcutaneous treatment recommended for 12 weeks; an overall success rate of over 80% for GS-441524 has been quoted by those most experienced in its use (Pedersen, 2021a). Minor renal side effects are reported as well as vasculitis type reactions (different to the injection site reactions that can also occur) that can respond to short-term low dose of steroids (Pedersen, 2021a). Neurological and ocular cases need additional considerations with regards to treatment dose and length (Pedersen, 2021b). But more and more published evidence on the success of oral GS-441524 for treatment of FIP is emerging (Krentz et al., 2021).

A retrospective study documenting 393 owner (mostly in the USA) responses to a questionnaire on the use of unlicensed non-standardised GS-441524-like treatment (mostly SQ but a few with oral or initial SQ and then oral treatment) for at least 12 weeks on their own cats for the treatment of FIP (Jones et al., 2021) reported that 88.2% saw an improvement in clinical signs in their cats within a week of starting the SQ 'GS-441524' treatment. Furthermore 54% said that their cats had been cured of FIP, whilst 43.3% said their cats were alive and well but still in a post-treatment monitoring period. Overall, only 12.7% of cats showed a relapse of FIP-associated clinical signs, whilst 3.3% had died despite treatment. Reported complications of treatment were similar to those reported previously (Dickinson et al., 2020, Murphy et al., 2018) and included vocalisation and pain on injection. Diagnosis of FIP in this study was based on the owners' individual veterinarian's opinion and was not confirmed in the study, but the signalment and clinical signs reported were very suggestive of FIP, with most cats (57%) having effusions but around 43.2% had neurological and/or ocular signs too. Varied unlicensed GS-441524-like compounds were used in the study (the most common one was 'Mutian', although this product is also known as Xraphconn) and in this study only 8.7% of owners received help from their veterinarian for treatment of their cat and most learnt about treatment from websites or Facebook pages. Treatment was expensive with the average cost being USD 4920 per cat in this study. Although the authors were not advocating the unauthorised use of the GS441524-like compounds, the study is valuable in describing the experiences of owners and apparent efficacy

of these compounds. The dosages used varied greatly and were significantly higher at the end of treatment courses compared to the beginning. It is difficult to be sure of the dosages used as the lack of quality control data for the formulations used, as well as a lack of confirmation of the active compound in the formulation, makes it difficult to be sure the concentration of the active compound used was as described by the manufacturers.

A published retrospective study of cats with suspected FIP (Yin et al., 2021) reported that 23 of 24 (95.8%) cats treated with GS-441524 at 2-4 mg/kg/d for at least 4 weeks (route of administration not documented) were cured; again the true composition of the compounds administered were not confirmed.

One case report of the successful treatment of FIP with an oral preparation of a nucleoside analogue was published in 2020; this was in a cat with ocular signs in the absence of effusions (Addie et al., 2020b). The oral nucleoside analogue preparation used in that case was 'Mutian'; the manufacturers of Mutian had not released the identity of the active agent in their formulation although it was suggested to be GS-441524 (Pedersen 2020a) and this has now been confirmed by analysis of Mutian tablets in another study (Krentz et al., 2021). In the case report (Addie et al., 2020b) the Mutian GS-441524 preparation was administered PO for 50 days, initially at a dosage of 8 mg/kg/day based on the company's information on the Mutian constituents. Within two weeks of starting treatment, the cat had shown a marked improvement in weight, ocular signs and various haematological (e.g. normalization of haematocrit) and biochemical (e.g. marked reductions in AGP and globulin measurements and an increase in the A:G ratio) measurements. The cat was also given anti-inflammatory prednisolone for the first 6 days, and then feline interferon-omega PO subsequent to finishing Mutian, which was ongoing at the time of publication. The cat appeared to have been cured of FIP. No side effects of treatment were noted although the cat did have increased symmetric dimethylarginine (SDMA) concentrations during treatment (but baseline pre-treatment levels were not measured) which decreased following discontinuation of the Mutian. This cat was also given SAME supplementation alongside Mutian to protect the liver.

The promising results in that case report have been followed by a larger study describing the successful treatment of 18 or 18 cats with confirmed, or highly suspected FIP, with oral Mutian (Krentz et al., 2021). This prospective study used dosages of either 5 mg/kg/day, or 10 mg/kg/day, depending on the absence, or presence, of neurological/ocular signs respectively in a treatment course lasting 12 weeks (84 days) for each cat; these dosages were those recommended by the manufacturers, and it was assumed that the concentration of the active component in the capsules was as described on the package inserts. Although this study confirmed that GS-441524 was the main component of the Mutian capsules, the amount of GS-441524 in the capsules was not determined, and so the true dose administered is not known, as is the case with most unlicensed and non-standardised formulations offered for sale (Pedersen, 2021). Additionally, although Mutian contains other ingredients to GS-441524, such as herbal compounds, these are not believed to be responsible for efficacy in FIP, although future studies should evaluate treatment with GS-441524 alone. In the study (Krentz et al., 2021), the oral GS-441524 was given on an empty stomach with food following 30 minutes later. Treatment was associated with a rapid improvement in clinical and laboratory parameters and marked reductions in FCoV loads in both blood and effusion samples. Additionally, no serious side effects were seen with only a mild increase in liver enzymes noted in some cats, and in some cats, lymphocytosis and eosinophilia. No renal side effects were reported. The curative response seen in all 18 cats treated with oral GS-441524 was remarkable with the shortest follow up being 99 days after completion of the 84-day treatment course. All cats received veterinary care during hospitalisation during the first 8 days of treatment, and this intensive supportive care (e.g. intravenous fluids, appetite stimulants, anti-emetics, analgesia) might have contributed to the success rate, highlighting the importance of veterinary involvement in the care of sick cats with FIP.

An even larger study documented the successful treatment, with Mutian, of 116 of 141 (82.2%) pet cats with suspected effusive FIP (Katayama et al., 2021); the remaining 25 cats died despite treatment. A similar protocol of 84 days of treatment was given with most cats receiving oral rather than SQ therapy. Of the 116 survivors, 3 cats relapsed in the 4 weeks after stopping oral Mutian treatment but were said to be responding to a higher dosage of Mutian at the time of publication. Although the method of confirmation of diagnosis was not stated (but most cats [139/141] were FCoV RT-PCR on effusion samples), the study gave valuable information on what parameters may be useful to look at to predict response to Mutian treatment. The cats that survived treatment, at assessment before the start of treatment, had better appetites and activity levels, and interestingly higher temperatures, than those that did not survive. Additionally, survivors had significantly lower bilirubin concentrations, with the likelihood of surviving following treatment following the bilirubin concentration, as shown below:

Total serum bilirubin ($\mu\text{mol/l}$)	Number of surviving cats over total number in category	Survival %
≤ 8.6	28/29	96.6
$> 8.6 - 17.1$	24/27	89.0
$> 17.1 - 34.2$	15/20	75.0
$> 34.2 - 68.4$	9/18	50.0
≥ 68.4	1/7	14.3

Another small study of 42 cats with confirmed or suspected FIP documented the successful use of serum AGP measurements in differentiating cats that fully recovered from FIP (26 cats) from those that would relapse from remission (16 cats) (Addie et al., 2022). In this study an AGP concentration of $< 0.5 \text{ mg/ml}$ was associated with recovery from FIP and was more reliable to track than resolution of lymphopenia or hyperglobulinaemia, suggesting that serum AGP concentration could be used as an indicator to stop antiviral treatment with nucleoside inhibitors. Further prospective studies are required to confirm this.

Mutian has been used to eliminate FCoV shedding in cats (Addie et al., 2020), and further details on this can be found below in the section 'Elimination of FCoV shedding' below.

Remdesivir

Remdesivir, GS-5734, is a prodrug of GS-441524; remdesivir is altered by infected cells to yield the active ingredient GS-441524 and it has to be injected as it is not active orally (unlike GS-441524 which is active both orally and as an injectable (Xie and Wang, 2021)). Remdesivir has been suggested as a treatment for respiratory coronavirus diseases in humans, most notably with Covid-19 due to SARS-CoV-2, although clear evidence for a beneficial effect in humans is lacking with available data (Ansems et al., 2021). Remdesivir has been considered for coronavirus treatment in cats (Izes et al., 2020c) but the safety and efficacy of remdesivir for FIP in cats has not been established in peer-reviewed publications (Pedersen, 2020b). However, anecdotal evidence from field cases in Australia and the UK using a 'specials' formulation of injectable remdesivir legally available for cats in those countries (Bova - a veterinary specials manufacturer, Australia 2022; Bova - a veterinary specials manufacturer, UK 2022) suggests that injectable remdesivir is very effective for the treatment of FIP (Spanner, 2020); comparative studies with GS-441524 are not yet available.

In other countries, human-licensed preparations of remdesivir could be used legally in cats. However, the cost of a full 12-week treatment course of remdesivir is extremely high, precluding its use in many cats. Protocols, based on the experience of Australian vets, have been established (Taylor et al., 2021) for the use of injectable remdesivir for a week or two, followed by oral GS-441524 for the remainder of the 12-week course, as the switch

to GS-441524 allows for more affordable treatment, as well better compliance due to the avoidance of needing injections, which can be problematic due to stinging etc. The protocol (Taylor et al., 2021) contains additional advice on how to improve compliance if this is problematic.

Further miscellaneous considerations for use of protease inhibitors & nucleoside analogues

As described above, efficacy in cats with naturally occurring FIP appears greater with GS-441524 (Pedersen et al., 2019; Dickinson et al., 2020) than with GC376 (Pedersen et al., 2018), as only six of 20 cats treated with GC376 remain in remission (Pedersen, unpublished data, 2018; quoted in (Pedersen et al., 2019) compared to 25 of 31 cats treated with GS-441524 (Pedersen et al., 2019), and three of the four cats with neurological and ocular signs treated with the higher dose of GS-441524 also went into remission (Dickinson et al., 2020). Disease relapses not responding to retreatment were far more common with GC376 compared to GS-441524, and most of the relapses seen in the GC376 trial were neurological in nature, in contrast to the GS-441524 trial. Both treatments caused similar injection site reactions and appeared to be relatively safe, although GC376 interfered with the development of permanent teeth when given to younger kittens. Although the results of the field studies appear to favour GS-441524 treatment, some of the difference might have been influenced by how the two drugs were administered as the efficacy of GC376 might have been better if all 20 cats had been treated without interruption for 12 weeks, rather than with progressively longer periods starting at just two weeks at the start of the trial in the first five cats. This was done because the 10 experimentally infected cats treated with GS-441524 (Murphy et al., 2018) were also initially given only a two-week treatment course, although two cats required a second treatment. Thus, another GC376 field treatment trial would be warranted using a longer treatment with a higher dosage and a larger number of cats before a final comparison can be made. It might also be indicated to evaluate both types of drugs in combination, although an *in vitro* FCoV study found no additional benefit of combining antiviral agents over the use of monotherapy (Addie 2012) (Cook et al., 2021). Neither GC376 nor GS-441524 are currently commercially available nor licensed for use in animals, although owners are sourcing agents via the internet and administering these agents themselves. Veterinarians whose clients are using these drugs might need to contact their professional regulatory bodies for guidance on their legal position in dealing with such cases. The UK (Bova - a veterinary specials manufacturer, UK 2022) and Australia (Bova - a veterinary specials manufacturer, Australia 2022) have unlicensed, but regulated, preparations of oral GS-441524 and injectable remdesivir available to purchase by veterinarians as 'specials' for the treatment of cats in their care; these formulations are quality assured and contained confirmed concentrations of the antiviral agents and give an opening to legal treatment of FIP by vets in these countries, with protocols having been developed for their use in the treatment of FIP (Taylor et al., 2021). Provision of supportive care to the cats undergoing treatment and their owners in these circumstances is generally recommended and was believed to be an important component of curative therapy with oral GS-441524 in one study (Krentz et al., 2021).

Drug resistance is relatively common for anti-viral agents, especially with prolonged drug exposure, high viral mutation rates and sometimes genetics can play a part. In the GC376 treatment study (Pedersen et al., 2018), the 3C-like protease gene sequences of a number of cats were compared from the time of presentation to after GC376 treatment in samples obtained at post-mortem examination after euthanasia for persistent or relapse of FIP; only one cat showed a change in its 3C-like protease gene sequence. This cat had shown a relapse with FIP-caused effusion 30 weeks after starting treatment (which comprised two treatment courses totaling 16 weeks). The three gene changes/mutations found in that cat were then studied for resistance to GC376 (Perera et al., 2019); only one of the mutations conferred a change, a small reduction, in susceptibility to GC376. In the

study evaluating GS-441524 treatment (Pedersen et al., 2019), only one cat in that study was thought to have shown evidence of drug resistance, although sequencing studies were not performed. Thus, drug resistance does not appear to be common. It was suggested at a FIP conference that only a small percentage of cats (~3%) were resistant to GS-441524 (Pedersen N, personal communication). However, resistance is a real threat and resistance to remdesivir, that is now being used in humans, has been associated with amino acid mutations in RNA polymerase and proofreading exonuclease in tissue culture propagated coronaviruses (Agostini et al., 2018). Thus, it may be important to evaluate different types of anti-viral drugs (e.g. GC376, GS-441524) in combination, as done for HIV infection and hepatitis C in humans (Pedersen et al., 2019) although synergistic effects of FCoV antivirals *in vitro* has not been shown (Cook et al., 2021). Concerns regarding antiviral resistance are behind the reluctance to use antivirals in healthy FCoV infected cats, as described below in the section 'Elimination of FCoV shedding'.

Immunomodulatory drugs

Immunomodulators are also commonly used in cats with FIP. The idea behind immunomodulator treatment is that these products might stimulate the immune response toward a cell-mediated response or to reduce an overactive Th2 response. An imbalance in T cell *versus* B cell response has been suggested to attribute to the development of FIP; however, this hypothesis has been questioned (Pedersen et al., 2014). Non-specific stimulation of the immune system might in fact even be contraindicated, since the clinical signs develop and progress because of an immune-mediated response. Therefore, treatment with these drugs is not recommended as long as there is a lack of documented efficacy in well-controlled studies (Hartmann and Ritz 2008b; Hartmann 2018). Some old case reports suggest some effect through immunomodulator treatment, such as tylosine, promodulin, acemannan, or "para-immunity inducers", but FIP was not confirmed in these studies (Colgrove and Parker 1971; Robison et al., 1971; Ford 1986; Bolcskei and Bilkei 1995a; Bolcskei and Bilkei 1995b; Hartmann and Ritz 2008b).

Polyprenyl immunostimulant is a drug that has shown promise for immunomodulation. PPI is a commercially available oral agent that is given three times a week and that is considered to act by upregulating Th-1 cytokines. In a case series of three cats with FIP without effusion (confirmed by histopathology in one of the three cats only), PPI was associated with prolonged survival (Legendre and Bartges 2009). In a field study, treatment with PPI was evaluated in 60 cats that were suspected to have FIP without effusion by primary care and specialist veterinarians, but again confirmation of FIP was not established in all cats and no untreated controls were included (Legendre et al., 2017). Of the 60 treated cats, 16 survived over 100 days; of these, eight survived over 200 days, including four who survived over 300 days. Veterinarians of treated cats that survived over 30 days reported improvements in clinical signs and behaviour. The survival times were significantly longer in cats that were not treated with systemic glucocorticoids concurrently, although topical ophthalmic glucocorticoids did not affect survival (Legendre et al., 2017). Thus, use of PPI might hold some promise for treatment of cats with FIP without effusion, although the number of cats responding to treatment in that study was low overall and FIP was not always confirmed. Controlled studies in cats with confirmed FIP would be necessary to evaluate efficacy.

Table 3: Antiviral & immunomodulating drugs that have been suggested for use in cats with FIP

Drug	Comments	ABCD recommendation
GC376	Inhibits 3C-like protease. Promising results <i>in vitro</i> and in one <i>in vivo</i> experimental study, especially in cats with effusion (Kim et al., 2016), and one field study, although FIP was not confirmed in all cases (Pedersen et al., 2018).	Not yet commercially available. Further controlled field studies required.
GS-441524	Acts as nucleoside analogue that terminates the RNA chain of viral RNA-dependent RNA polymerase. Very promising results <i>in vitro</i> , in one <i>in vivo</i> experimental study (Murphy et al., 2018), and in three field studies, although FIP was not confirmed in all cases (Pedersen et al., 2019; Dickinson et al., 2020; Krentz et al., 2021) as well as in two retrospective studies of cats with suspected FIP (Yin et al., 2021). Prospective study showed 100% efficacy in 18 cats with FIP treated with oral GS-441524 (Krentz et al., 2021).	Not commercially available in most countries but available as a 'special' (non-licensed) product for veterinary use in Australia and UK. Shows excellent promise.
Remdesivir – GC-5734	Nucleoside analogue and prodrug of GS-441524. Not active orally so must be given subcutaneously or intravenously. No published studies on efficacy but information available on its use favourable (Taylor et al., 2021) from observational studies.	Commercially available as a human drug but very expensive, available as an affordable 'special' (non-licensed) for veterinary use in Australia and UK. Field studies required.
Ribavirin	Inhibits FCoV replication <i>in vitro</i> , but very toxic in cats (Weiss et al., 1990; Weiss et al., 1993a; Weiss et al., 1993b).	Not recommended.
Vidarabine	Inhibits FCoV replication <i>in vitro</i> , but <i>in vivo</i> efficacy unknown (Barlough and Scott, 1990). Toxic to cats if given systemically.	Not recommended.
Nelfinavir	Acts as protease inhibitor that showed synergistic effects against FCoV with Galanthus nivalis agglutinin <i>in vitro</i> (Hsieh et al., 2010). No <i>in vivo</i> data available.	Not recommended until <i>in vivo</i> studies available.
Galanthus nivalis agglutinin (GNA)	Binds to FCoV-glycosylated envelope glycoproteins, thereby inhibiting viral attachment to the host cell. Showed synergistic effects against FCoV with nelfinavir <i>in vitro</i> (Hsieh et al., 2010). No <i>in vivo</i> data available.	Not recommended until <i>in vivo</i> studies available.
Cyclosporine A and non-immunosuppressive derivatives (e.g., alosporivir)	Inhibits cyclophilins and thereby blocks replication of FCoV <i>in vitro</i> (Tanaka et al., 2012; Tanaka et al., 2013). Reduced viral load in one cat (Takano et al.,	Further field studies needed.

Drug	Comments	ABCD recommendation
	2015). Can lead to immunosuppression, depending on the cyclosporine A derivative.	
Indomethacin	Acts as cyclopentenone cyclooxygenase (COX) metabolite with activity against several RNA viruses, including canine coronavirus (Amici et al., 2006). No data on efficacy against FCoV <i>in vitro</i> or in cats with FIP available.	Not recommended until further studies available. Safety in cats unknown (e. g., general risk of non-steroidal anti-inflammatories in cats).
Chloroquine	Inhibits FCoV replication <i>in vitro</i> and has anti-inflammatory effects <i>in vivo</i> (Takano et al., 2013). Can increase liver enzyme activities. Effective as a small molecule inhibitor of FCoV replication <i>in vitro</i> (McDonagh et al., 2014)	Not recommended until further field studies available.
Hydroxychloroquine	Inhibits type I and II FCoV replication <i>in vitro</i> , with less evidence of cytotoxicity than chloroquine (Takano et al., 2020); addition of interferon-omega increased antiviral action against type I FCoV replication.	Not recommended until further studies available.
Mefloquine	Effectively inhibits replication <i>in vitro</i> as a small molecule inhibitor of FCoV (McDonagh et al., 2014). Hepatic metabolism studied <i>in vitro</i> (Izes et al., 2020a), and its pharmacokinetics in healthy cats (Yu et al., 2020). Its plasma protein binding properties have been studied with blood from cats with and without FIP, and a simple high performance liquid chromatography assay developed to measure mefloquine (Izes et al., 2020b). Causes vomiting if not given with food but generally appeared safe in healthy cats. Used in Australia as adjunct treatment for FIP (Taylor et al., 2021) but no published studies yet.	Field studies required.
Itrazonazole	Inhibits cholesterol transport in type I FCoVs <i>in vitro</i> (Takano et al., 2019) and thus, inhibits FCoV replication. <i>In vitro</i> synergism with GS-441524 shown with type 1 FCoVs (Doki et al., 2022). Used in an uncontrolled very small study of cats with experimentally induced FIP alongside anti-human-TNF-alpha antibody treatment (Doki et al., 2020b) in which two of three cats with FIP improved.	Further controlled field studies needed.
Feline interferon-omega	Inhibits FCoV replication <i>in vitro</i> and reduced FCoV shedding in 9/11 cats (without FIP) in a shelter (Gil et al., 2013), but was not effective in one placebo-controlled study as treatment in cats with FIP with effusion that were concurrently treated with glucocorticoids (Ritz et al., 2007).	Lack of efficacy in one placebo-controlled study, but further studies in cats without effusions and with lower concurrent glucocorticoid doses would be useful.

Drug	Comments	ABCD recommendation
Curcumin	Decreased the immune-related proteins produced during infection of cell cultures with FIP-associated FCoV and inhibited viral replication <i>in vitro</i> as curcumin-encapsulated chitosan nanoparticles (Ng et al., 2020). Enhanced bioavailability as curcumin-encapsulated chitosan nanoparticles over curcumin in pharmacokinetic analysis in healthy cats. Not effective as a small molecule inhibitor of FCoV replication <i>in vitro</i> (McDonagh et al., 2014).	Further studies needed.

Vaccination

Efficacy of FIP vaccines

At present there is one intranasal vaccine commercially available in the USA and in some European countries. It contains a temperature-sensitive mutant of the type II FCoV strain DF2; type I coronaviruses are, however, more prevalent in the field in most countries (Addie and Jarrett 2001; Addie et al., 2003; Kummrow et al., 2005). The vaccine aims to induce local mucosal immune responses through the induction of IgA and cell-mediated immunity. However, it also induces development of systemic antibodies against FCoV, although usually with low titres. The efficacy of this vaccine is in question. Results from experimental studies have been inconsistent, with preventable fractions between 0 and 75% (Gerber et al., 1990; Gerber 1995; Hoskins 1995a; Hoskins 1995b; McArdle et al., 1995; Scott et al., 1995a; Scott et al., 1995b). Results from field studies have been equally inconsistent (Fehr D et al., 1995; Postorino Reeves 1995; Fehr et al., 1997). No difference in the development of FIP between the vaccinated and placebo groups was found during the first 150 days after vaccination when the vaccine was used in Persian breeding colonies (Fehr D et al., 1995). However, after 150 days, significantly fewer cases of FIP occurred in the vaccinated cats compared to the placebo group (Fehr D et al., 1995) and retrospectively virus was found in the blood samples of the cats who developed FIP. In another trial, a preventable fraction of 75% was found when the vaccine was tested in a large cat shelter in the USA (Postorino Reeves 1995), although the published study description was very short making it difficult to interpret the study fully. In this study, all kittens were antibody-negative prior to vaccination. The conclusion is that the vaccine is likely not effective in antibody-positive cats that have already been exposed to FCoV. The ADE of infection that was a feature of some experimental vaccine trials (McArdle et al., 1995; Scott et al., 1995a; Scott et al., 1995b), where more vaccinated than control cats developed FIP, has not been observed in field studies, suggesting that the vaccine can be considered safe (Fehr D et al., 1995; Postorino Reeves 1995; Fehr et al., 1997).

Use of the FIP vaccine

The ABCD considers the presently available FIP vaccine to be non-core. There is no benefit in the use of this vaccine in FCoV antibody-positive cats, which severely limits its use as many cats are FCoV antibody-positive. FCoV antibody-negative kittens could potentially benefit from vaccination, particularly if they subsequently enter

an FCoV-endemic environment and thus would be at risk of developing FIP. The fact that in multi-cat environments most kittens are already infected at the age of 16 weeks further limits the usefulness of the vaccine (Addie and Jarrett 1992; Lutz et al., 2002; Pedersen et al., 2008).

Primary course

If vaccination is to be given, the 1st dose should not be given before 16 weeks of age with a 2nd dose being given three weeks after the 1st dose.

Booster vaccinations

If primary vaccination has been performed, annual boosters might be considered. Although studies on the duration of immunity are lacking, it is thought to be short-lived (Addie et al., 2003).

Control of FCoV and FIP in specific situations

FIP is especially a problem of cats kept in larger groups, particularly in breeding catteries and rescue situations. Breeding catteries are high-risk environments for FIP. In Europe, it is likely that the vast majority of breeding catteries have endemic FCoV infection (Felten et al., 2020). Very rarely an unusually high number of cats (>10%; (Barker et al., 2013)) develop FIP within a multi-cat environment. Such “mini-outbreaks” (referred to as epizootics) are very occasionally reported (Graham et al., 2012; Barker et al., 2013; Wang et al., 2013) and more have been observed by the authors of these guidelines (unpublished data). Several factors might contribute to these “mini-outbreaks”. These factors include FCoV that have a high chance of becoming FIP-associated FCoV (thus, need a low number of mutations to become FIP-associated), high viral replication and thus viral loads in the environment and spread of these FCoV within a highly susceptible cat population.

General approach to FCoV & FIP control

Reducing FCoV transmission

Since FCoV is transmitted predominantly via the faecal–oral route, hygiene is the mainstay of FIP control in any multi-cat environment. FCoV infection is maintained in a household by continual cycles of infection and re-infection (Foley et al., 1997; Addie et al., 2003), the source of infection being faeces in the litter tray. Rarely is FIP a problem among cats leading an indoor–outdoor lifestyle or in stray cats that bury their faeces outside unless these cats originate from multi-cat environments (Riemer et al., 2016).

The goal in every cat household must be to reduce the FCoV infection pressure and risk of transmission. This can be achieved by keeping not more than three well-adapted cats per room (and keeping such cat groups stable), observing strict hygiene, and providing outdoor access if possible. If outside access is not possible, enough litter boxes should be provided (one more than the number of cats). Litter trays should be positioned in different rooms away from food and water bowls. They should have faeces removed at least twice a day, and litter tray utensils should be cleaned daily. Litter trays should be completely emptied at least weekly and cleaned using detergent. A study (Addie et al., 2020a) suggested that a Fuller’s earth-based litter that tracked minimally was associated with a reduced viral load in a multi-cat household compared to another Fuller’s earth litter; this effect was believed to be due to a binding effect of the clay in the Fuller’s earth as well as the non-tracking

property of the litter to help reduce spread. Further larger studies are required. Although FCoV is only rarely shed in saliva, food and water bowls should be cleaned daily using detergent or in a dishwasher at a cycle of at least 60°C, because of the risk of fomite contamination.

Managing FCoV shedders

In some breeding catteries, attempts to at least control FCoV spread by segregation of cats has been made. Faecal shedding in cats occurs over several months or sometimes lifelong, especially in multi-cat households (Addie and Jarrett 2001; Addie et al., 2003; Pedersen et al., 2008). Identification of cats that are persistently shedding a high FCoV load, and their separation from low shedders and faecal RT-PCR-negative cats, has been suggested for reducing transmission rates (Addie and Jarrett 1995; Kass and Dent 1995). Persistent high FCoV load shedders can be detected using quantitative RT-PCR on faecal samples or rectal swabs, which can be collected by the cat owner. Repeated individual RT-PCR of four faecal samples or swabs collected one week apart has been recommended to correctly identify non-shedders. The recommended frequency and interval of faecal sample collection for the identification of shedding cats in multicat households by RT-PCR has been suggested as at least three faecal samples collected at between one week and one-month intervals (Klein-Richers et al., 2020). The use of FCoV antibody testing on blood samples at a single time point, instead of repeated faecal sampling for RT-qPCR, to identify FCoV shedders was not found to be useful in another study (Felten et al., 2020), despite the finding of a positive correlation between antibody titres and the likelihood and frequency of faecal FCoV shedding and viral load (Felten et al., 2020). This was because, despite this correlation, the study identified antibody-positive cats that did not have any FCoV RNA in any of their four sequentially collected faecal samples (15 of 82 cats) and antibody-negative cats that had FCoV RNA in all of their four sequentially collected faecal samples (2 of the 82 cats).

The laboratory performing the RT-PCR from faecal swabs should provide the FCoV loads and/or an interpretation of the results as to whether or not a cat is considered a persistent high FCoV load shedder. When persistent high load shedders are identified within a multi-cat environment, access to their litter trays by non-shedders should be strictly avoided. Ideally three categories of cats are formed to separate from each other: the persistent high shedders, low shedders and non-shedders. However, if not feasible and for practical reasons, at least separation of the high shedders from the low/non-shedders can be recommended. In addition, the recommendations on how to reduce the FCoV infection pressure listed above should be strictly enforced, and FCoV-infected cats should not be exposed to stressful situations. The screening for persistent high FCoV load shedders gives a temporary picture and results can change over time. Cats can be retested six to nine months later to evaluate whether the situation has changed.

Elimination of FCoV shedding

One published study (Addie et al., 2020c) has described the use of an oral novel nucleoside analogue Mutian (containing GS-441524 (Krentz et al., 2021) to eliminate FCoV shedding (identified by faecal RT-qPCR) in 29 cats within four households with endemic infection. A second study also describes the use of oral Mutian to eliminate FCoV infection in four cats in a household that lived with a cat that was treated successfully for FIP with oral Mutian (Addie et al., 2020b). Both of these studies describe households in which the owners were wanting to eliminate FCoV shedding due to previous FIP cases in their households, including to prevent re-infection of cats recovered from FIP by other cats. In both studies a 4-day course of 4 mg/kg orally q 24 h was effective in eliminating FCoV shedding; follow up periods to confirm duration of elimination were variable in the studies. In the larger study (Addie et al., 2020c), two cats that completed the study in a household of four cats were still

negative 155 and 157 days after stopping Mutian, whilst the other cats from other households that had follow up periods were negative between three to 51 days after stopping Mutian. The study was not placebo-controlled, so spontaneous elimination of FCoV by the treated cats cannot be ruled out, but the abrupt fall in FCoV shedding seen when Mutian treatment was started was consistent with the effect being due to the treatment. The rationale behind using Mutian to eliminate FCoV shedding is to establish households that are free of FCoV to remove the risk of FIP arising. One potential problem with this approach, however, is that because FCoV is so common in cat populations, and can be carried on fomites, it is likely to be hard to maintain a FCoV-negative status long term and it is highly unlikely that treatment leads to more permanent prevention of re-infection than natural infection (Pedersen 2020a; Pedersen 2021b). Additionally, it is of note that many advise that the use of antivirals should be preserved only for the treatment of cats with FIP, in view of the potential for resistance to develop (Pedersen 2021b; Katayama et al., 2021), and even though Mutian is not licensed for treatment of FIP, it is known to be one of the agents that is used to treat the disease in the field (Pedersen 2020a). More studies are required on induction of viral resistance, and currently, for these reasons, ABCD does not recommend that this agent is used for control of FCoV infection in cats without FIP.

Breeding catteries

Breeding catteries are those households in which the reduction of FCoV infection pressure is of particular importance. A study of 37 breeding catteries in Germany, that performed RT-PCR on faecal samples collected from cats in the catteries, did not find any to be free of FCoV (Klein-Richers et al., 2020), showing how highly prevalent FCoV is in such environments. In this study, in which all households had ≥ 5 cats, only having cats of < 1 year of age was associated with an increased risk of FCoV shedding; management and husbandry measures (e.g. thoroughness of cleaning, number of litter trays, cleaning and disinfection frequency) was not associated with prevalence of faecal shedding (Klein-Richers et al., 2020). Special measures in kittens can be considered. FIP usually occurs after the kittens have left the breeder and are in a new household (Cave et al., 2002). It has been suggested that most kittens are considered protected from FCoV infection by maternally-derived antibodies (MDA) until they are five to six weeks of age. In some studies, FCoV transmission has been prevented by isolating pregnant queens two weeks before birth and then moving their kittens away to a clean environment away from other cats when they are five to six weeks old and maintaining them there until they go to a new home (Addie and Jarrett 1992; Addie and Jarrett 1995). For this method to succeed, the breeder is required to follow strict quarantine hygiene methods. However, the procedure failed in another study in which kittens were found to shed FCoV already as early as at the age of two weeks (Lutz et al., 2002), questioning the protection through MDA. In addition, specialist veterinary behaviourists often advise against early weaning due to a risk of socialisation problems in these kittens (Philip and Seitz 1959; Guyot et al., 1980; Bateson 1981).

There are PCR tests commercially available which purport to detect cats that are resistant to FIP (discussed briefly in the section above on Pathogenesis and Immunity). These tests are currently not recommended as a bases for breeding decisions. Positive selective breeding for “resistance to FIP” in a colony of laboratory cats was shown to decrease the survival of the offspring after intraperitoneal infection with FIP-associated FCoV (Pedersen et al., 2016). The diminished resistance to FIP in these cats was associated with decreased genomic heterozygosity.

Rescue facilities, shelters and boarding catteries

Preventing FCoV infection in rescue facilities, shelters, and boarding catteries is extremely difficult. In catteries and shelters with more than six cats, FCoV infection is virtually always present (Pedersen, 2009). Incoming cats

should be kept in quarantine for a minimum of three weeks. After entry into a shelter, shedding of FCoV increases dramatically within one week amongst cats that were already infected at entry, and more than one half of initially negative cats were shedding FCoV a week later (Pedersen et al., 2004). Strict hygiene precautions, like movement control, hygiene protocols for care workers, cleaning and disinfection, must be enforced to reduce virus contamination and viral spread. Special care should be given to sterilising litter trays between use in different cats, having litter trays and scoops dedicated to each cat pen, and avoiding fomite transmission on cleaning utensils such as brushes. Ideally, cats should be kept in small groups of three or less cats per room (Addie et al., 2009) (see above) and with limited exchange of animals. Stress reduction is of particular importance, as stress may lead to increased virus production and a risk of development of FIP. New catteries should be designed with infectious disease control and stress reduction as priorities (Möstl et al., 2013; Wagner et al., 2018a; Wagner et al., 2018b). More information on control of infectious diseases in shelters can be found in the ABCD guidelines on this topic.

Management of apparently healthy FCoV-infected cats

FCoV-infected cats can be detected by faecal RT-PCR. Stress experienced by FCoV-infected cats (e.g., surgery, boarding, adoption) (Rohrer et al., 1993; Riemer et al., 2016) or immunosuppression caused by co-infection with immunosuppressive viruses, e.g. feline immunodeficiency virus, predisposes the cats to develop FIP (Poland et al., 1996). Minimisation of stress and avoidance of secondary infections are therefore important features of prevention of the development of FIP in FCoV-infected cats.

A FIP vaccine is not useful in FCoV-infected cats. The question has been raised whether FCoV-infected cats should receive other vaccinations, since vaccination was identified as a stressor preceding onset of FIP in one study (Riemer et al., 2016). However, studies are lacking to support that FCoV-infected cats should be vaccinated less often than non-infected cats. Therefore, until the contrary has been demonstrated, healthy FCoV-positive cats should receive vaccination similarly to non-infected cats.

Any treatment inducing immunosuppression might increase the risk of FIP development in FCoV-infected cats (Addie et al., 2015). However, cats might have diseases that require immunosuppressive treatment in the presence of FCoV infection.

Maintaining a FCoV-negative status

Once a household or a geographic area has achieved a FCoV-negative status, every effort should be made to keep it FCoV-free. Rectal swabs for RT-PCR, taken four times, a week apart, and a serum or plasma antibody test can help to prevent introduction of infected cats into a FCoV-free home or geographical area (Addie et al., 2012). The veterinarians of the Falkland (Malvinas) Islands instituted a policy that only FCoV antibody-negative cats could be imported to the Islands, protecting the islands' cats from FIP by preventing the introduction of FCoV (Addie 2012). However, as outlined above in the section on 'Measures for reduction of FCoV infection pressure and risk of FCoV transmission in multi-cat environments', there might be limitations to antibody testing as method of control as some antibody-negative cats may shed FCoV in their faeces (Felten et al., 2020) and testing faeces RT-PCR is the preferred way to identify shedders.

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