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Feline Infectious Peritonitis: Certain death?
A FIP summary and literary review of new treatment research

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Budapest, Hungary
2021

1. Abstract

Feline infectious peritonitis (FIP) is a coronaviral disease of domesticated cats (*Felis catus*) that almost invariably leads to death. For 60 years, scientists, researchers, and veterinarians have strived to find a treatment and cure for this disease and to find accurate methods for diagnosing this enigmatic virus that seemingly refuses to give up its secrets. This paper will summarise the most important information that has been meticulously gathered piece by piece by devoted researchers throughout the years. It will also present a review of the latest treatment options of FIP, which are bringing a great amount of optimism to researchers, veterinarians, and owners alike. However, this new antiviral therapy is still not available on the markets, and thus cannot be prescribed by practitioners, which has led to some unique ethical circumstances, which will also be discussed.

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Key abbreviations: 3CLpro: 3C-like proteases CMI: Cell-mediated immunity, FCoV: Feline coronavirus, FECV: Feline enteric coronavirus, FeLV: Feline leukaemia virus, FIP: Feline infectious peritonitis, FIPV: Feline infectious peritonitis virus, FIV: Feline immunodeficiency virus, ORF: Open reading frame, S: Spike.

2. Introduction

Feline infectious peritonitis (FIP) is a highly fatal immune-mediated disease caused by a viral biotype of the feline coronavirus (FCoV) (Izes et al., 2020; Pedersen, 2009). The virus is considered to be common on a worldwide basis and affects domesticated cats as well as several wild felids (Addie et al., 2009; Drechsler et al., 2011; Pedersen, 2014a). It is the most common cause of death in cats less than two years of age, and it is estimated that it kills 1 in 100-300 cats worldwide (Pedersen, 2009; Rohrbach et al., 2001). FCoV in domesticated cats usually presents in the form of Feline Enteric Coronavirus (FECV); a common, mild enteric disease that is self-resolving (Kennedy, 2020). Cats usually appear completely healthy, or with mild symptoms such as diarrhoea (Pedersen, 2014b). However, in some cats, the FCoV will undergo spontaneous genetic alterations that cause the mild enteric disease to transform into the highly pathogenic and fatal variant: Feline Infectious Peritonitis Virus (FIPV) (Felten and Hartmann, 2019; Vennema et al., 1998). In contrast to the often symptom-free (sub-clinical) FECV, the closely related biotype FIPV results in a highly inflammatory, systemic disease that is nearly 100% fatal once the clinical signs develop (Haake et al., 2020). As many as 10% of FCoV infections result in FIP (Tasker, 2018)

FIPV has proven to be complex and enigmatic. Although research into FCoV and FIP has been ongoing since the 1960s, the virus has evaded almost every attempt at finding not only an infallible diagnostic test, but also in finding a functional treatment or cure. Research into prevention of the disease, as well as vaccine development, has so far not yielded any favourable results (Pedersen, 2009).

FIP continues to be devastating news to owners, as its diagnosis delivers the message that their cat will almost certainly die. The disease is also frustrating for veterinary practitioners because it leaves them powerless to offer anything other than supporting treatment or euthanasia. However, in recent years there has been considerable progress in the research on antiviral medications for human and animal diseases. With the Covid-19 pandemic taking place at the time this paper was written, the interest in the development of medication that could treat or prevent coronaviral diseases has certainly escalated. This paper will summarise the current knowledge and understanding about FIP, as well as the recent research into new antiviral medications that have made researchers hopeful for a cure.

3. Summary of current FIP knowledge

3.1 Causative agent

The disease was first described in a paper by Holzworth in 1963, and the causative agent was classified to be in the *Coronaviridae* family in 1970 by Ward. FCoV belongs to group 1, or alphacoronaviruses, along with canine coronavirus and porcine transmissible gastroenteritis virus (Drechsler et al., 2011). The virus is divided into two serotypes, type I and type II, based on serological and genomic properties, specifically the amino acid sequence on the spike (S) protein (Addie et al., 2009; Motokawa et al., 1995). Although both types can cause FIP, type I FCoV is much more prevalent in natural infections worldwide (Benetka et al., 2004; Hohdatsu et al., 1992; Wang et al., 2014), while Type II FCoV have been used more in research as it is easier to propagate in tissue cultures in vitro (Addie et al., 2009; Pedersen, 2009)

Coronaviruses are spherical, enveloped, single-stranded, positive-sense RNA viruses. The FCoV genome consists of >29,000 nucleotides (King et al., 2011, Pedersen, 2014b), and is considered to be one of the largest RNA genomes (de Vries et al., 1997). Viral polymerases of RNA viruses are prone to making mistakes and have no proofreading abilities when copying and replicating the viral genome (Kennedy, 2020). With such a large genome, errors in the viral replication happen naturally and with some regularity, and mutation of the virus as a result is not uncommon. Therefore, the longer a virus replicates in a host, the higher the chances are for pathogenic mutations to occur, especially in chronically infected cats. Reinfection also increases the risk of virus mutation (Drechsler et al., 2011; Kennedy, 2020; Pedersen, 2014a).

To better understand the mutations that occur, as well as understanding the resulting disease and problematic diagnostics, it is advantageous to have some knowledge about the viral genomics of the FCoV. There are 11 open reading frames (ORF's) that encode structural, non-structural, and accessory genes. The first one ORF (1a and 1b) consists of a little over 20,000 nucleotides, in other words about two-thirds of the whole genome, and encodes more than 10 individual proteins used in the RNA synthesis required for creating messenger RNA (mRNA) and the genome in the viral replication process (Drechsler et al., 2011; Kennedy, 2020; Pedersen, 2014a) (**Fig. 1**).

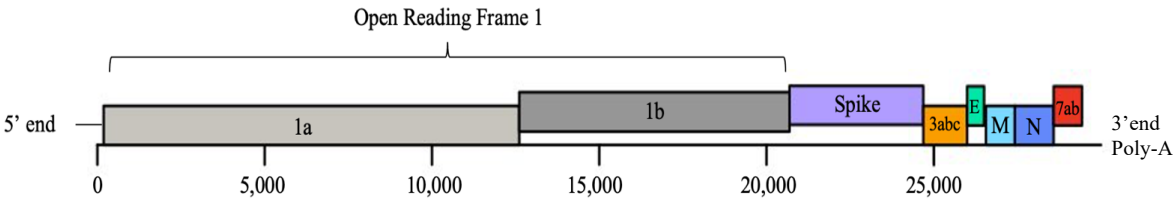

Figure 1. *Schematic of the FCoV genome with base numbers. The 5' end contains a cap structure, while the 3' end contains a poly-adenylate tail, which are typical structures on an RNA genome for creating proteins within a cell. The bars indicate the ORF's for FCoV proteins; ORF 1a and 1b, Spike protein, ORF 3a, 3b, and 3c, Envelope (E) protein, Membrane (M) protein, Nucleocapsid (N) protein, and ORF 7a and 7b (Modeled after Drechsler et al., 2011; Kennedy, 2020).*

The next ORF (ORF S) encodes the major envelope protein, the S glycoprotein, which is used by the virion as a means to attach to the target cell. ORF S is followed by ORF 3a-c that encode non-structural proteins whose functions are still not quite clear. The E gene encodes for a minor, hydrophobic envelope protein, the M gene encodes for an integral structural membrane protein that interacts with both the core and the envelope of the virus, and the N gene encodes the nucleocapsid protein (**Fig. 2**). Finally, the last ORF is the 7a-b which, like the 3a-c ORF, also encodes for non-structural proteins of unknown function. Most of the research that has investigated the possible sites for the mutation of FECV into FIPV has mainly focused on the Spike gene, the M gene, and ORFs 7a-b and 3a-c (Bank-Wolf et al., 2014; Borschensky and Reinacher, 2014; Kennedy, 2020; Pedersen, 2009).

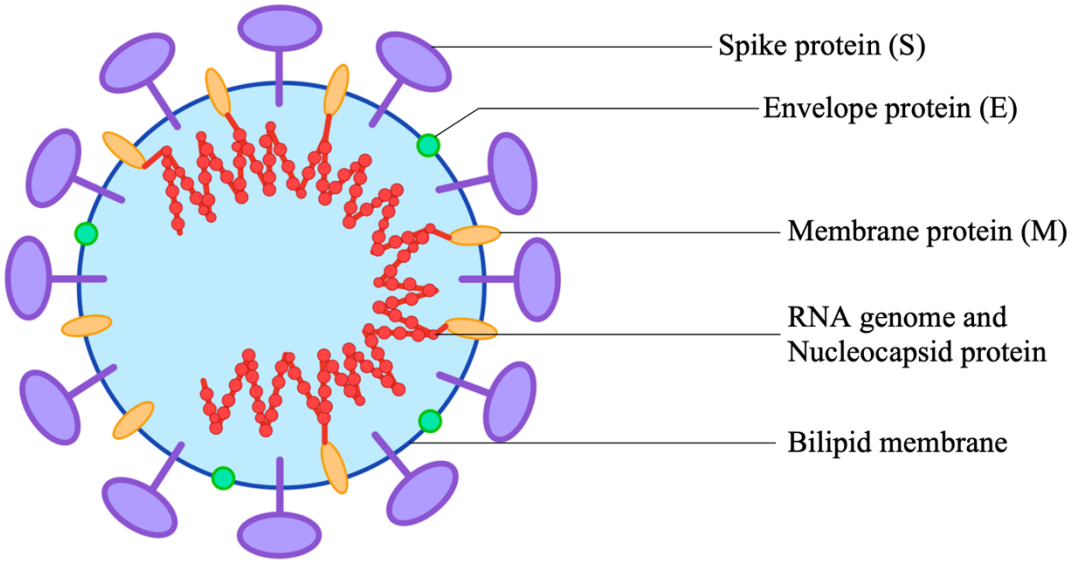

Figure 2. *Schematic of coronavirus virion (viral particle). with structural proteins. The S, M, and E proteins are embedded in a bilipid membrane of host cell origin. (Modelled after Drechsler et al., 2011; Haake et al., 2020)*

Crucial point mutations that have been suggested to be responsible for the conversion of FECV into FIPV include amino acid differences in the gene encoding the fusion peptide on the S protein (Chang et al., 2012), a change or substitution in a furin cleavage site between the receptor-binding (S1) and fusion (S2) domain on the S protein (Licitra et al., 2013), and mutations in the ORF 3abc resulting in a reduced 3c protein (Bank-Wolf et al., 2014). Mutations of the S protein and 3c protein are often found together, but a single mutation in either of them appears to be enough to drastically change the tropism of FECV, and allow for increased pathology (Bank-Wolf et al., 2014; Haake et al., 2020). As the S protein is the means of the attachment, fusion, and subsequent viral release of the virion into the host cell, the S protein is crucial in determining the host species, tissue, and cell tropism for each coronavirus. Mutations to this gene, in particular, could explain how the virus is able to change which species are susceptible, and which cells are targeted for replication within a host (Haake et al., 2020).

It is still not completely understood why these viral genetic mutations happen only in some cats. Some researchers have suggested that there are inheritable genetic factors that cause some breeds or familial lineages of cats to be more predisposed for FECV to mutate into the fatal form (Pedersen et al., 2016, 2014; Pesteanu-Somogyi et al., 2006). Specific breeds that have been found to be more susceptible to the disease include Abyssinian, Bengal, Birmans, Ragdoll, and Rex cats. (Pesteanu-Somogyi et al., 2006; Worthing et al., 2012). FIP-like disease has also been documented in several wild feline species, such as European wild cats, African lions, mountain lions, leopards, cheetahs, jaguars, lynx, servals, caracal, sand cats, and Pallas cats (Haake et al., 2020).

Other research has suggested that the disease occurs more frequently in cats that have been exposed to living conditions where the number and density of cats are high, such as in shelters or catteries (Addie et al., 1995; Pedersen, 2009). Simple and frequent mutations of the FCoV genome are common, and mutations that do not have a negative impact on the survival of the virus can accumulate in a population or geographical area, and can contribute to sporadic local outbreaks (Pedersen, 2014a; Vennema et al., 1998).

3.2 Pathogenesis

As FECV replicates in the epithelium of the intestines, cats with this enteric form of FCoV will usually shed the virus in their faeces, and can thereby be a source of infection of other cats through the faecal-oral route (Drechsler et al., 2011; Pedersen, 2009). The shedding of FECV in the faeces can be transient, recurrent, or chronic, and has been demonstrated to be consistently present in the faeces of infected cats, starting from 2 days to 2 weeks post-infection. The duration of the shedding can last from a few weeks or months, which is more typical, to life-long shedding in rare instances (Addie et al., 2009; Haake et al., 2020; Pedersen et al., 2008).

While the FECV replicates in enterocytes, FIPV utilises monocytes and macrophages for their replication (Addie et al., 2009; Drechsler et al., 2011). It is hypothesised that the mutations in the FCoV genome cause the virus to lose tropism for enterocytes, while it gains tropism for monocytes and macrophages, leading to a systemic spread of the virus (Kennedy, 2020; Pedersen, 2014a). As the tropism of the virus shifts from enterocytes to macrophages, the virus will no longer be shed, or only shed in negligible amounts, in the faeces. Therefore, FIPV is not transmissible between cats like FCoV is, and endemic outbreaks like those seen in for example panleukopenia virus or feline calicivirus are relatively rare (Kennedy, 2020).

The main target of FIPV is a specific population of precursor monocytes and macrophages that have a particular affinity for the endothelium of venules in the serosa, omentum, pleura, meninges, and the uveal tract. The FIPV will spread rapidly with expanding cycles of macrophage infection, viral replication, virus release from dying infected macrophages, and infecting yet more macrophages. The hosts own immune system reacts to the disease progression and concurrent inflammation by mounting a stronger immune response, which in most cases seems to be counterproductive, seeing as the virus uses these very immune cells to replicate (Pedersen, 2014a, 2009). Components of the host immune system that are known to affect the occurrence and internal spread of FIPV include major histocompatibility complex characteristics, quality of cytokine responses, and features of cell-mediated immune (CMI) response. (Addie et al., 2004). Natural killer cells release type I interferons (IFN-α and IFN-ß) in response to viral infection, which promotes an antiviral state. However, coronaviruses have shown great capacity to suppress this antiviral mechanism (Drechsler et al., 2011).

While a strong T-cell mediated (or CMI) response seems to be the only proficient defence against the disease progression, B-cell mediated (or humoral) immunity, seems to have little to no effect. The viral load also plays an important role in whether or not the immune system will be able to combat the virus or not (Addie et al., 2009). It is important to note that due to the absence of viral antigens on the surface of infected cells, monocytes and macrophages can remain infected even in the presence of high antibody levels. During FIP inflammation, monocytes and macrophages have been found to produce tumour necrosis factor (TNF-α), an inflammatory cytokine that can lead to depression, apoptosis and severe depletion of T-cells. (Addie et al., 2009; Drechsler et al., 2011; Poland et al., 1996; Rottier et al., 2005). Cats infected with FIP have consistently been found to have high levels of TNF-α, and low levels of IFN-γ, which is an interferon that plays a significant role in further activation of the CMI. (Drechsler et al., 2011). Researchers have suggested that these mechanisms play a key role in how the FIPV is able to suppress the cats' cell-mediated immunity, leaving them in an immunocompromised state. If the cat additionally becomes exposed to an environment of high stress, like a shelter, they are predisposed to stress, which increases glucocorticoid release. Glucocorticoids further decrease the IFN-γ production and impair T-cell production. Seeing as shelters usually also have a higher FECV concentration, the cat is likely to be exposed to a higher FCoV load. All these elements combined leaves the cat with poor odds of successfully fighting off the virus and returning to normal health (Drechsler et al., 2011).

Macrophages with high viral loads, as well as viral particles and proteins, accumulate in small venules, where they react with immune cells to form immune complexes. The complexes can lodge in smaller venules and trigger macrophage factors that cause tissue damage, resulting in the lesion that is distinct for FIP; the pyogranuloma, also defined as pyogranulomatous vasculitis, a type-III hypersensitivity (immune-mediated / Arthus-type) vasculitis. (Jacobse-Geels et al., 1982; Pedersen, 2009; Pedersen and Boyle, 1980). The pyogranuloma is characterised by oedema and an outflow of proteinaceous fluid from the vessels, that contains plasma proteins, breakdown products from haemoglobin, inflammatory proteins, and activated clotting factors (Pedersen, 2014a).

3.3 Clinical signs

The clinical signs can be highly variable and complex, but mortality is extremely high once the clinical signs appear (Pedersen, 2014b). The emergence of clinical signs usually marks the beginning of the end, because it signals that the immune system has lost the battle with the virus, and return to health is extremely uncommon. On rare occasions, a cat can recover and return to health, but it has been shown that it will relapse months or years later and succumb to the disease. The progression from FECV to the clinical signs of FIP can take as little as 2-3 weeks, to several months, and in rare cases years (Legendre and Bartges, 2009; Pedersen, 2014b). The median time from official diagnosis to euthanasia is approximately 8-9 days (Ritz et al., 2007).

3.3.1 Predisposed patients

FIP is usually seen in young cats less than 3 years of age, especially in cats <1 year, but it can affect cats of any age. Geriatric cats and cats with immunosuppression are also at greater risk of contracting the disease. It seems that FIP is more likely to occur in primary infections in kittens because there is a higher level of FECV replication and a decreased resistance to mutations that might occur. (Drechsler et al., 2011; Haake et al., 2020; Kennedy, 2020; Pedersen et al., 2008). Intact males seem to have a higher risk of contracting the disease, while spayed females have a lower risk (Pedersen, 2009).

The history of the cat can be quite important in the diagnosis of FIP. The onset of clinical signs is usually preceded by a long history of non-specific poor health, poor growth, and failure to thrive (Pedersen, 2009). Recent history of a stressful event, such as surgery (e.g. neutering and spaying), moving to a different home or different owners, or changes to social hierarchy has also commonly been noted in cats who are diagnosed with FIP (Hartmann, 2005). Cats that come from multi-cat environments such as catteries, breeders, or shelters have a higher risk of being repeatedly exposed to FCoV, and are therefore at higher risk of developing FIP (Drechsler et al., 2011; Pedersen, 2009). Cats suffering from other concurrent diseases, especially immunosuppressive diseases, are also at greater risk of developing FIP. Due to the fact that FIPV is immunosuppressive in itself, it can also lead the cat to be more susceptible to other diseases, for example Feline leukaemiavirus (FeLV), which is found in about one-third to one-half of all cats with FIP. (Drechsler et al., 2011; Kennedy, 2020; Pedersen, 2009).

The clinical signs of FIP can be particularly fickle, as the distribution of vasculitis and pyogranulomatous lesions can vary between cats. FIP has two forms, characterised as being effusive "wet", or non-effusive "dry", reflecting the nature and presentation of symptoms, although they are not mutually exclusive. Both forms have common, non-pathognomonic features like anorexia and weight loss, fever, apathy, and depression. The wet form is hypothesised to arise when there is a weak CMI response, and the dry form arises when there is a strong CMI response (Addie et al., 2009; Drechsler et al., 2011; Pedersen, 2009).

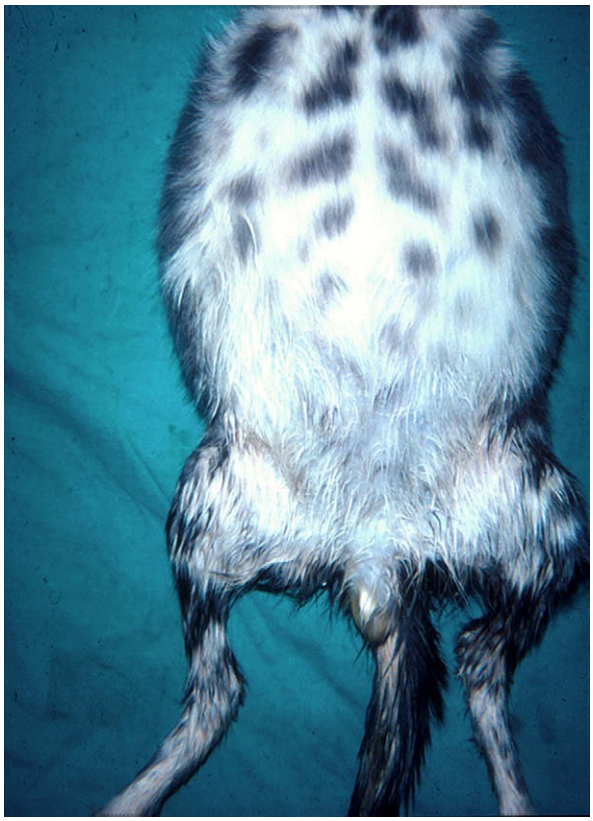

Figure 3. *Significantly distended abdomen of a kitten with effusive feline infectious peritonitis. Scrotal enlargement due to inflammation of the tunica vaginalis can also be observed (Courtesy of Pedersen, 2009).*

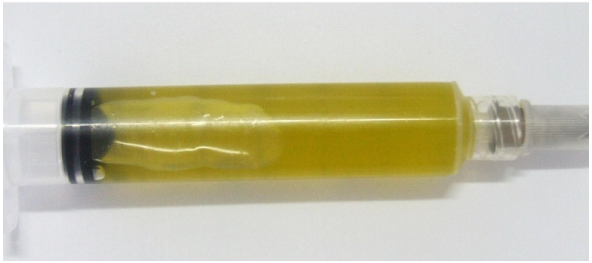

Figure 4. *Peritoneal effusion from a cat with wet (effusive) FIP collected by abdominocentesis. (Courtesy of Daniel Gerardi, Universidade Federal do Rio Grande do Sul, Brazil.)*

3.3.2 Wet FIP

The wet form is often characterised by a distended abdomen, with large amounts of fluid in the abdominal cavity (ascites), and is seen in 60-70% of cats with FIP (**Fig. 3**). This is the most distinct clinical symptom, and FIP should always be considered in these patients before other conditions such as heart failure. Less commonly the cat will have thoracic effusions of the pleura and sometimes pericardial effusions, which will present as dyspnoea and cardiac tamponade. There can also be a combination of both abdominal and thoracic effusions. (Addie et al., 2009; Pedersen, 2014a, 2009). The effusions, when extracted, are clear to moderately cloudy, yellow-tinged due to the presence of bilirubin, and exudative due to the large amounts of protein in the fluid (**Fig 4**). A less common clinical presentation is serositis of the tunica vaginalis of the testes of intact males, leading to scrotal enlargement. Generalised synovitis can also sometimes be seen in the form of fever and lameness (Addie et al., 2009; Drechsler et al., 2011; Pedersen, 2014b, 2009).

3.3.3 Dry FIP

The designation of 'dry' FIP indicates that there is an absence of effusion in the abdominal and/or thoracic cavity, or the amount is too sparse to be detected outside of necropsy. Dry FIP is usually more complicated to diagnose because it depends on which organs are affected by the vasculitis and multifocal pyogranulomatous lesions. Signs relating to abdominal organs occurs in 40% of cats, where lesions extend from the serosal or pleural surfaces into the underlying parenchyma, hence yielding the alternative designation 'parenchymatous FIP' (Pedersen, 2009). The involvement of the kidneys can lead to renomegaly, which can sometimes be detected upon abdominal palpation (Addie et al., 2009). Lesions and enlargement of the mesenteric lymph nodes can also be palpated abdominally, but can often be mistaken for neoplasia (Kipar et al., 1999).

The involvement of the eyes and/or central nervous system (CNS) can be seen in 60% of cats with dry FIP, and half of all feline patients presenting with inflammatory CNS disease have FIP, as well as one-sixth of CNS signs from any cause (Pedersen, 2009). Lesions involving the brain and spinal cord can be expressed as posterior paresis, ataxia, incoordination, hyperaesthesia, seizures, and palsy of the brachial, trigeminal, facial, and sciatic nerves. Nystagmus and behavioural changes can also be observed in some cats (Holliday, 1971; Marioni-Henry et al., 2004; Quesnel et al., 1997). Ocular diseases and/or abnormalities are also much more likely to occur in cats with dry FIP, but can also be observed in cats with wet FIP on occasion. Uveitis and chorioretinitis are the main ocular symptoms. (Pedersen, 2009) (**Fig. 5**).

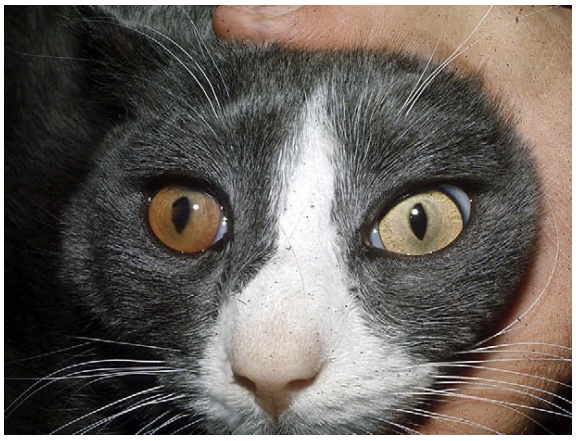

Figure 5. *Uveitis can be seen in the right eye of a cat diagnosed with the dry form of FIP. Colour changes in the iris can be seen, as well as changes to the shape of the pupil. The anterior chamber is somewhat hazy, and keratic precipitates has formed a pigmented lesion in the centre of the cornea (Courtesy of Pedersen, 2009).*

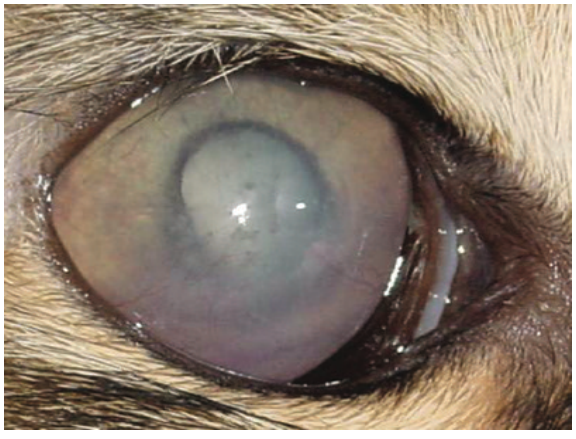

Figure 6. *Keratic precipitates and ocular lesions in a Maine Coon with dry FIP (Courtesy of Addie et al., 2009).*

Uveitis can lead to changes in the colour of the iris, dyscoria, or anisocoria secondary to iritis, sudden blindness, and hyphaemia. Keratic precipitates in the caudal part of the cornea are also a trademark of FIP, and are due to an accumulation of fibrin, macrophages, and other inflammatory cells (Addie et al., 2009; Norris et al., 2005; Pedersen, 2009) (**Fig. 6**).

Cutaneous signs that include multiple nodular lesions caused by pyogranulomatous-necrotising dermal phlebitis, skin fragility, and toxic epidermal necrolysis have also been observed in cats with dry FIP (Cannon et al., 2005; Pedersen, 2009; Trotman et al., 2007). Icterus can also be seen in some patients if the liver is amongst the affected organs (Addie et al., 2009). Lesions in the colon or ileocaecocolic junction may also occur in some patients, and can be seen as chronic diarrhoea and/or vomiting (Addie et al., 2009).

It is important to note that cats usually do not "exclusively" have wet or dry FIP. Some cats have transitional stages, where the disease can progress from being dry to wet, or wet to dry (Pedersen, 2009). The disease course from infection, to clinical signs, to death is variable, and cats can live with FIP for weeks, months, and sometimes even years. However, survival time is generally shorter in young cats and effusive FIP (days to weeks after onset of clinical signs), than in older cats and dry FIP (weeks to months after the onset of clinical signs) (Fischer et al., 2011; Hugo and Heading, 2015; Pedersen, 2014b; Tsai et al., 2011).

There are many important diseases that should be considered in cats that are suspected of having FIP. The most important differential diagnoses include congestive heart failure, toxoplasmosis, Feline leucaemia virus (FeLV)/Feline immunodeficiency virus (FIV), inflammatory liver disease, neoplasia (e.g. lymphoma, abdominal carcinoma), sepsis, and bacterial/septic peritonitis/pleuritis. Other diseases that can also have symptoms in common with FIP include Mycobacterial infection (including tuberculosis), lymphocytic cholangitis, pancreatitis, and rabies (Addie et al., 2009; Tasker, 2018).

3.4 Diagnostic tools

Finding an accurate and definite test that can quickly and correctly determine the diagnosis of FIP has proven to be incredibly challenging. To this day the history, signalment, clinical signs, and different diagnostic tests are required in combination to confirm FIP in a patient (Kennedy, 2020). Even though FECV and FIPV vary greatly in their virulence and pathology, they are both a biotype of FCoV, and are indistinguishable by most diagnostic methods (Felten and Hartmann, 2019).

3.4.1 Haematology

Haematology is a good place to start when a cat is suspected of having FIP, because haematology parameters are often altered in this disease (Addie et al., 2009). Complete blood count analysis often includes non-specific leucocytosis, a varying degree of lymphopenia, and a non-regenerative anaemia (<30% haematocrit) (Addie et al., 2009; Kennedy, 2020). These findings are not unique to FIP and can be found in a variety of different diseases. However, if a normal lymphocyte distribution on flow cytometry is found, FIP can be ruled out with almost 100% certainty. (Addie et al., 2009; Kennedy, 2020). Degree and time of the onset of lymphopenia have been found to be predictors of development and severity of the disease; the greater and earlier the onset of lymphopenia, the faster and more severe the disease progression will be (Pedersen et al., 2015). The decrease in lymphocytes, specifically T-lymphocytes, correlates with previous findings that macrophages and monocytes infected with FIPV secrete an increased amount of cytokines, specifically TNF-α, which results in the apoptosis of the lymphocytes (Takano et al., 2007).

Another change that is common to find is an increase in the total serum protein, caused mainly by an increase in gamma globulins and a decrease in the albumin-to-globulin ratio (A:G) (Paltrinieri et al., 2002; Riemer et al., 2016). Hyperglobulinaemia was, in one study, found in about 50% of cats with effusive FIP, and about 70% in cats with non-effusive FIP (Sparkes et al., 1994). A study by Jeffery *et al.* (2012) found that an A:G ratio greater than 0.6 to 0.8 is a good negative predictive value. Even though low A:G has a low specificity value, a higher A:G is a helpful parameter when it comes to ruling out FIP. Serum protein electrophoresis may uncover both polyclonal and monoclonal hypergammaglobulinaemia, in addition to an increase in acute phase proteins (Addie et al., 2009; Kennedy, 2020)

The biochemistry profile can also reflect which organs are involved in the disease. Liver enzymes, urea, and creatinine can be elevated, depending on the organ or organs involved, and the degree of their damage. However, these parameters are generally not particularly useful in the diagnosis of FIP, because they are seen in several different diseases (Addie et al., 2009; Kennedy, 2020). Many cats with FIP have bilirubinaemia, bilirubinuria, and icterus. This is usually due to the destruction of erythrocytes and accumulation of haemoglobin, which is often seen in FIP, rather than hepatic involvement (Pedersen, 2014b). Serum elevation (>1500 μg/ml) of the acute phase protein α1-acid glycoprotein (AGP) can be a good marker for FIP, but only in combination with other results. It is important to remember, however, that AGP is also elevated in several inflammatory diseases. In addition to this, healthy cats can have high levels of AGP while they have the milder version of the disease, FEVC (Addie et al., 2009; Duthie et al., 1997; Paltrinieri et al., 2007). In conclusion, none of the parameters that are possible to examine in a blood test are pathognomonic or able to conclude with the diagnosis of FIP on their own.

3.4.2 Investigation of effusion / fluids

Effusion, especially of the abdominal cavity, is one of the most recognizable symptoms of FIP, but it is important to remember that about only 50% of cats with effusion is affected by the disease. The non-effusive, dry FIP can be much more challenging to diagnose because it can manifest with vague signs together with non-specific blood parameters. If there is effusion, it is important to obtain a sample, because tests on effusion are much more helpful than blood tests. Ultrasound can be particularly useful in order to identify small amounts of fluids in the abdomen, pleural cavity, or even pericardium for sampling and diagnostic tests. Effusion fluid in the case of FIP is a cellular-, protein-, and fibrin-rich transudate, typically clear-to-cloudy, yellow in colour, and sticky in consistency. But the presence of this effusion alone is not enough for a conclusive diagnosis (Addie et al., 2009; Kennedy, 2020; Tasker, 2018). Electrophoresis of effusion fluid has a high positive predictive level if the A:G <0.4, and a high negative predictive value if the A:G value is >0.8 (Addie et al., 2009; Shelly et al., 1988). A recent study by Tasker *et al.* (2018) found AGP in the effusion to be the best acute phase protein to distinguish between cats with and without FIP. A cut-off value of 1550 μg/ml had a sensitivity and specificity of 93% each for diagnosing FIP.

The Rivalta test is a simple, inexpensive test to differentiate transudate from exudate, and is an easy and practical test that any veterinarian can confidently perform. A positive test shows the effusion to be exudate and is indicative of FIP, but it can also be positive in other cases, such as bacterial/septic peritonitis and lymphoma. If the test is positive, there is a positive predictive value of 58.4%. A negative result, showing the effusion to be transudate, is a strong indication that the cat does not have FIP, with a negative predictive value of 93.4% (Addie et al., 2009; Fischer et al., 2011; Tasker, 2018).

If there are neurological signs, which can often be observed in the case of dry FIP, and there is a lack of effusion, cerebrospinal fluid (CSF) should be sampled to reach a diagnosis. An increased protein content (50-350 mg/dl; normal value <25mg/dl) and pleocytosis (100-10 000 nucleated cells/ml), with mainly neutrophils, lymphocytes, and macrophages can often be seen in cats with FIP. CSF cytology often shows a mixed or suppurative inflammation, where mononuclear infiltration can be seen. This result is not diagnostic on its own, however, as these changes can be present in cats with other neurological diseases, and some FIP cats with neurological signs will have no changes to the CSF at all. (Addie et al., 2009; Boettcher et al., 2007; Felten and Hartmann, 2019; Singh et al., 2005; Tasker, 2018).

3.4.3 Antibody testing

There has been a lot of work and research done to ascertain whether or not serum antibody testing can contribute to diagnostic information, but there are still some problematic features that have yet to be overcome. The main problem with antibody tests is that both FECV and FIPV antibody responses are virtually identical, due to the fact that they are both biotypes of FCoV. Titres can be high in healthy cats that are only infected with FECV, and low in cats with FIP, indicating that they are seronegative. (Addie et al., 2009; Pedersen, 2014b). Nevertheless, if the antibody titres are accurately performed, they may have some value. Healthy cats with FECV usually have a titre, by indirect immunofluorescence assay, of 1:100 to 1:400 (Pedersen et al., 2008). Cats FIP may have similar results, but the higher the titre, the more likely it is that the cat has FIP. Not many healthy cats have titres of 1:1600, and titres of ≥1:3200 are highly suggestive of FIP (Hartmann et al., 2003). The fact that cats with FIP can have low antibody titres has been suggested to be caused by the binding of the antibodies in the abundance of the virus, forming immune complexes, or simply immune exhaustion. (Addie et al., 2009; Kennedy, 2020).

3.4.4 PCR / RT-PCR

The use of PCR and reverse transcriptase (RT)-PCR may be the most common mode of virus detection today (Kennedy, 2020). However, there are still challenges to differentiate FECV from FIPV. Positive FCoV RT-PCR results from blood have been found in healthy cats that did not develop FIP over a period of some 70 months (Gunn-Moore et al., 1998; Herrewegh et al., 1997). In addition to this, it has been discovered that cats with negative FCoV RT-PCR results do sometimes indeed have FIP, and this especially applies to cats with dry FIP (Addie et al., 2009; Hartmann et al., 2003).

Researchers have repeatedly attempted to identify the specific mutation(s) that happen in the viral genome of FCoV in order to develop new, more accurate diagnostic tests that are able to separate FECV from FIPV, but so far there is still no common conclusion (Kennedy, 2020). It was long speculated that ORF 7a-b mutations would be the key to antibody-testing, but this was based on a faulty assumption that FECV was lacking the 7b gene, which was later proven to be incorrect. The assumptions had been made using a specific laboratory version of FECV (WSU-79-1683) that did indeed not have the 7b gene, but wild-types of FECV often do contain this gene, and cannot, in this way, be distinguished from FIPV (Pedersen, 2014b, 2014a, 2009). Brown *et al*. (2009), as well as Hora *et al*. (2013), suggested that point mutations to the M gene correlated with the development of FIP. Pedersen *et al*. (2012) found that deletional mutations to the 3c gene were crucial in the systemic spread of the virus, and the loss of tropism towards replication in intestinal cells. They also found that all FCoV isolates shed in the faeces had an intact 3c gene, which characterised their intestinal replication. Bank-Wolf *et al*. (2014), found that mutations of the 3c gene, along with mutations of the S gene was a good predictor for FIP, while they refuted that changes to the M protein were required. Chang *et al*. (2012) found that 95% of 183 FIPV isolates had mutations to the S protein.

All these inconsistent and seemingly contradicting results highlight the challenges researchers face when trying to reveal the evasive nature of FCoV and understand its pathogenesis, as well as the mutability of the FCoV genome (Kennedy, 2020; Pedersen, 2014a). The presence of point mutations in the S protein gene has led to the development of PCR assays that can identify these mutations. Although not all cats with FIP will have these specific point mutations, especially cats that appear healthy or have dry FIP, this PCR test may certainly be a good diagnostic tool, along with other tests (Kennedy, 2020).

3.4.5 Histopathology and Immunohistochemistry

Researchers widely agree that the "golden standard", and the best way to confirm the diagnosis of FIP, is histopathology and using immunohistochemical staining (IHC) on affected tissues to find FCoV. Unfortunately, this is usually only possible to do on post-mortem examination, as the patients often are too poorly to have any invasive treatment like laparotomy/laparoscopy done (Addie et al., 2009; Izes et al., 2020; Kennedy, 2020). The gross and histologic lesions are usually very characteristic, and can be an accurate way to confirm a diagnosis (**Fig. 7**) (Pedersen, 2014b).

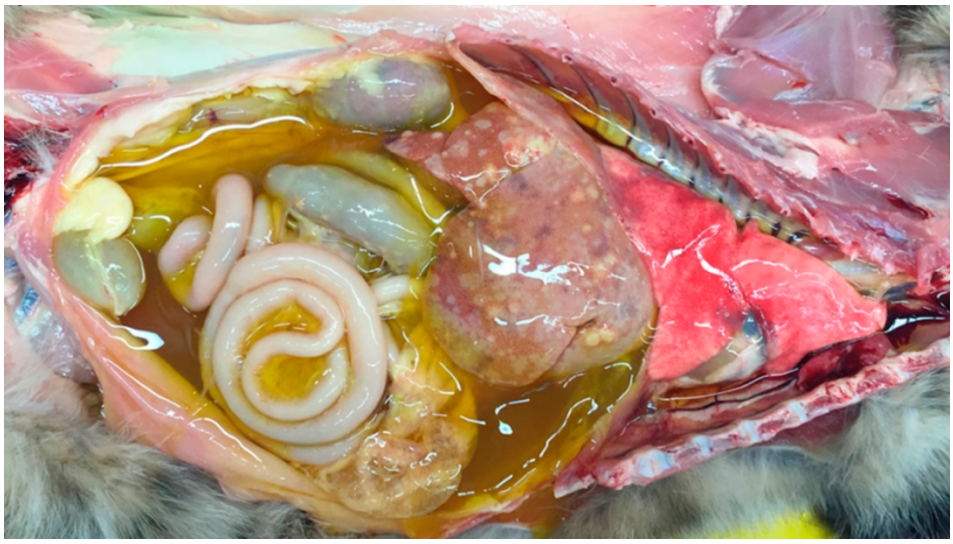

Figure 7. *Gross pathology of wet, or effusive, FIP in the abdominal and thoracic cavity in a cat. Abundant, semi-translucent "straw coloured" effusion can be seen in the peritoneum and thorax. Fibrinous and granulomatous serositis is present, along with multifocal granulomatous lesions on the liver (Courtesy of Haake et al., 2020, Chrissy Eckstrand)*

Haematoxylin and eosin stained tissue sections usually have localised inflammation with macrophages, neutrophils, lymphocytes, and plasma cells (**Fig. 8**). Vascular lesions surrounded by large amounts of inflammatory cells are characteristic for wet FIP, while focal accumulations of inflammatory cells and necrotic-proliferative lesions are characteristic of the pyogranulomatous lesions of dry FIP. Immunohistochemical staining of tissue can allow for the detection of FCoV antigens in the tissue, but it cannot differentiate between FECV and FIPV. However, as FIPV replicates in larger numbers, higher concentrations of the viral antigen can be a good indicator for a FIP infection (Sharif et al., 2010).

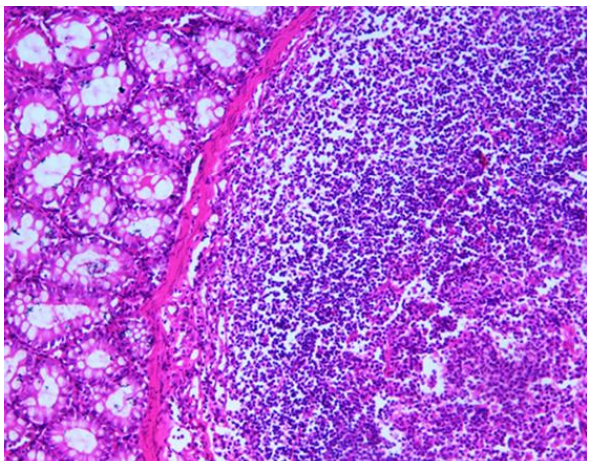

Figure 8. *Granulomatous colitis found in a cat with "wet" effusive FIP. Infiltration of inflammatory cells can be seen (Courtesy of Sharif et al., 2010).*

Ultrasounds-guided fine needle aspiration (FNA) of various organs and lymph nodes to diagnose FIP is a much-discussed subject. Research into whether tissue from affected organs like spleen, liver, and mesenteric lymph nodes could be used to directly diagnose FIP, but so far this is not possible. FNA used for IHC could not confidently confirm or rule out FIP. However, if S gene mutations or high viral loads of FCoV are detected with RT-PCR, this strongly supports a diagnosis of FIP (Felten and Hartmann, 2019). As demonstrated, the diagnosis of FIP with definite proof can be quite challenging. The gold standard of diagnosis is still post-mortem histopathology with IHC (Kennedy, 2020). A combination of tests is required to increase the certainty of the diagnosis, and the diagnostic tests performed must be adapted to the clinical signs of the patient. For example, analysis of effusion is the best place to start in a cat with ascites, and testing of CSF might be a better place to start in a cat with uveitis and neurological signs, in addition to haematological values in both types of patients (Addie et al., 2009; Felten and Hartmann, 2019).

3.5 Therapeutics

Treatment of cats with FIP can be quite challenging for several reasons. As previously discussed FIP is hard to diagnose with 100% certainty and proof, which means that some cats inevitably end up being treated for different diseases, while others are treated for FIP and do not actually have the disease. As the diagnosis is usually synonymous with death, euthanasia is the most common outcome once the clinical signs progress to a state of severe suffering for the cat (Pedersen, 2009). It is also worth stating that some cats have been found to have small quiescent lesions that are characteristic of FIP in surgery on otherwise healthy cats, or in post-mortem examination where the cats have been examined for other reasons, indicating that while uncommon, it is possible that cats with FIP can make a spontaneous recovery. This unexpected, natural recovery can often coincide with medical treatment, leading to claims of efficient treatments or cures or FIP (Colgrove and Parker, 1971; Madewell et al., 1978; Pedersen, 2009, 1976).

3.5.1 Treatment

So far there have been three main approaches to therapeutics, used either alone or combined, to treat FIP (Pedersen, 2014b). The first approach focuses on the use of non-specific immunostimulants to up-regulate the CMI, assisting the cats own immune system to fight off the virus (Izes et al., 2020). Examples of this include staphylococcal A protein, lymphocyte T-cell immunomodulators (Pedersen, 2014b), *Propionibacterium acnes* (an immunostimulatory compound produced by gram-positive bacteria) (Weiss et al., 1990), and plant extracts like poly-prenyl immunostimulant (Legendre et al., 2017; Legendre and Bartges, 2009). Usage of these compounds have unfortunately been unsuccessful, or have had only limited success. The usage of poly-prenyl immunostimulants have in some studies been found to prolong the survival time, but they have not been efficient as a cure (Izes et al., 2020; Legendre et al., 2017).

The second approach focuses on the use of immunosuppressant drugs in order to limit the inflammatory response that resides at the core of FIP. These drugs would in theory limit the amount of immune cells available to the virus, and thus hindering its replication and systemic spread (Addie et al., 2009; Hartmann and Ritz, 2008; Pedersen, 2014b). Examples of drugs that dampen the immune system are glucocorticoids (e.g. prednisolone, dexamethasone), alkylating agents (e.g. cyclophosphamide, chlorambucil) (Addie et al., 2009), and cytokine inhibitors (e.g. pentoxifylline and propentofylline) (Fischer et al., 2011). While glucocorticoids have been reported to reduce the clinical signs in cats with FIP, no significant improvement on survival time has been proven. In fact, none of the studies using immunosuppressant medication enhanced survival time, and the studies that claimed to have found significant improvement on survival time had failed to confirm a diagnosis of FIP (Izes et al., 2020).

A review by Pedersen (2014b) found that there is a paradoxical practice in veterinary clinics, where a combination of both immunosuppressants and immunostimulants are used concurrently, even though these drugs work in contrast with each other. It recognised that this form of treatment is expensive, which may cause the veterinary practitioner to medicate the cat in smaller and more infrequent doses to save the cost for the owner.

There is also a great deal of uncertainty around accurate dosage use and treatment efficiency due to a lack of solid pharmacokinetic and bioactivity studies, placebo control, randomisation, double-blinding, and sufficient case numbers with accurate determination of disease status.

The third and final main approach to FIP treatment has been the use of antiviral agents. The targets of these drugs can vary and can, for example, be aimed at the hosts own cellular replication processes that the virus utilises for its own replication purposes, or the specific viral mechanisms related to infection and/or replication. (Kennedy, 2020; Pedersen, 2014b). There is some agreement amongst researchers that drugs that are the least effective are the ones that target the hosts own cellular mechanisms of replication, because their end result tend to have a negative effect on the host, while only somewhat slowing the virus down temporarily (Pedersen, 2014b). Some drugs, like chloroquine and Cyclosporine A, were found to inhibit or block coronaviral replication, and even improved survival time, but they were also found to have unforeseen toxic effects (Pedersen, 2014b; Pfefferle et al., 2011; Takano et al., 2013). Research in more recent times has focused on identifying specific viral genes or viral processes that could successfully be targeted by drugs,

3.5.2 Prevention

Any cat can be a potential source of FCoV, in view of the fact that it is an extremely common virus in environments where cats reside. Some cattery owners and/or breeders have attempted to test for FCoV in the faeces with PCR in an attempt to identify FECV carriers within groups of cats to try to eliminate shedders from the environment, in order to prevent FIP losses. FECV is shed in large amounts in the faeces, but because the shedding is intermittent, the number of shedders can be 40-80% higher in multi-cat environments than detected. This testing is not only challenging to perform at a satisfactory level, but the tests can be quite expensive as well, and are not really a good alternative. (Addie et al., 2009; Pedersen, 2009). Research has shown that it is virtually impossible to maintain a FCoV-free clowder of cats. Dust and microscopic particles from litterboxes contain high levels of the virus, and can easily spread through the air, covering cages, feeding bowls, and clothing.

Therefore, strict quarantine involves separate quarters, caregivers, litter, food and water pans, air space, clothing, isolation of new cats, etc. These measures may work for very small catteries and breeders, but they are measures that are challenging, if not impossible, to maintain for shelters and larger catteries. Losses caused by FIP increase notably when shelters or catteries experience overcrowding, and decreases when the overcrowding is controlled (Pedersen, 2009).

In households where a cat had been diagnosed with FIP, it is recommended to wait for at least two months before obtaining a new cat, to allow for environmental FCoV virions to die off (Addie et al., 2009). FCoV can survive for 7 weeks in a dry environment and may be transmitted indirectly (e.g. via litter trays, shoes, hands, and clothes), including at cat shows. However, FCoV is readily inactivated by most household detergents and disinfectants (Scott, 1988).

There have been multiple attempts to develop a vaccine for this disease, but results have been controversial and tenuous. The most notable attempt is a temperature-sensitive mutant of FIPV-79-1146, an exceptionally aggressive strain. The vaccine is administered intranasally twice, 3-4 weeks apart, starting at 16 weeks or older. Immunity is attributed to a local IgA response. The results from various studies have been inconsistent, with reported preventable fractions from 0-75%. Even so, the vaccine is available in the USA and some European countries, and a number of shelters have reported a decrease in FIP cases, which have been attributed in part to a vaccine programme, paired with quarantine measures (Addie et al., 2009; Pedersen, 2009).

The vaccine is not recommended by the World Small Animal Veterinary Association (WSAVA). The reason for this is that the vaccine is recommended only to cats that are known to be FCoV antibody-negative, because these are the only cats who would be likely to develop some form of protection. The recommended age of vaccination is 16 weeks, and it is exceedingly unlikely that kittens of 16 or older have never encountered FCoV (Day et al., 2016). 16 weeks is when the immune system of kittens is considered to be at a near-adult level of maturation (Pedersen, 2009). Cats have been experimentally vaccinated before 16 weeks of age, but this did not result in any form of protection (Addie et al., 2009).

4. New treatments

The focus on finding antivirals that could target specific regions of the viral genome have been in the interest field of researchers for a long time. Most aspects of modern medicine and research knowledge have been applied in the search for a cure for FIP, including a wide range of antibiotics, antivirals, immune system regulators, plant extracts; the list goes on and on (Catella et al., 2021; Dunowska and Ghosh, 2021; Pedersen, 2014b). Numerous studies that have been conducted have significant flaws, like testing treatment on cats that do not have a conclusive FIP diagnosis. Many have also not been tested in further studies, for example only testing potential drugs on feline kidney cells in vitro, and not moving on to in vivo studies. (Pedersen, 2014b).

As previously mentioned (*3.4.4 PCR / RT-PCR*) scientists have long discussed the value of identifying FIPV-specific genomic regions that could best be used for the identification of FIPV, and make it possible to separate FIPV from FECV. This investigation has also carried into the research for developing an antiviral drug that will specifically, efficiently, and safely target and eliminate FCoV from the patients' system. The most successful antiviral drugs so far involve drugs that target specific genomic regions in order to inhibit or disrupt infection and replication processes. These types of antivirals have shown very promising results in the treatment of chronic viral infection of humans, such as HIV/AIDS, hepatitis B and C, and herpesvirus (Pedersen, 2014b; Pedersen et al., 2018). These viruses are ideal targets for virus inhibitors such as RNA-dependent RNA polymerase and protease. (Carter et al., 2017; Kim et al., 2013; Pedersen et al., 2018)

Kim *et al*. (2012) made a significant leap towards "cracking the code" of FIP antiviral medication development when they started researching, and later synthesising, peptidyl compounds that target 3C-like proteases (3CLpro) and evaluated them for their efficacy against FCoV, as well as feline calicivirus. Their research led them to recognise a number of compounds that showed strong inhibitory activity against various coronaviruses, including FCoV, and had a wide margin of safety. The in vivo results of their 3CLpro in mice infected with murine hepatitis virus A59, a murine coronavirus, showed that there was a significant reduction in virus titres and pathological lesions (Kim et al., 2015, 2013).

4.1 GC376

In 2016, Kim *et al*. performed a pharmacokinetic study of GC376, a 3CLpro inhibitor, on two healthy, specific pathogen-free (SPF) cats with 10mg/kg/dose injected subcutaneously (SC) to determine plasma drug concentration. Then, they performed a safety study on four SFP cats, with 10mg/kg/dose, twice per day for four weeks. The results showed that the drug had a very large safety margin, and a good bioavailability in cats. Finally, they tested GC376 in vivo on cats experimentally infected with serotype I FIPV, which is responsible for 80-90% of naturally-occurring FIP. The first round of treatment was tested on four cats with only mild to non-apparent signs, and the second round of treatment was tested on four cats with profound clinical signs (8 cats in total). Cats in both groups were 8-10 months old. Typical clinical signs included lymphopenia, inappetence, weight loss, jaundice, and mild-to-profound ascites. Under normal circumstances, at this stage in the disease progression, it is certain that the cat will soon succumb to the disease.

The antiviral treatment with GC376 was given at either 5 or 10 mg/kg/day SC, twice per day, for 14-20 days. It caused a rapid reversal of clinical signs, lymphopenia, and a reduction of the viral titres in the macrophages from the ascites, and a cessation of active infection after 14-20 days. Two cats in the second (profound clinical signs) group, however, had to be euthanised after 4 and 7 days respectively. The remaining six cats showed no sign of relapse after 8 months. Upon pathological examination of the two euthanised cats it was discovered that the granulomatous lesions that are typically found in various organs in cats with FIP were either greatly reduced, or not found at all. Their results demonstrated that continuous viral replication is crucial in the progression of the immune-mediated pathogenesis of FIP, and that by inhibiting this replication with an antiviral compound that targets the coronaviral 3CLpro, reversal of the disease is indeed possible. This paper provided the first evidence of direct-acting antiviral agents being effective in reversing coronaviral immune-mediated disease, even when the disease was clinically advanced.

It is important to note, however, that the sample size was rather small, and the cats used in this research were all experimentally infected. There was no placebo group, but perhaps this was because it is well known what happens to cats with FIP who have received no treatment.

Pedersen *et al*. (2018) continued the research of Kim *et al*. (2016) on 3CLpro inhibitor GC376 on 20 naturally infected cats that were brought in by owners. Diagnosis of FIP was based on signalment, clinical history, physical examination, examination of prior laboratory testing, and blood and effusion analysis. Further confirmation of FIPV presence was examined by real-time quantitative RT-PCR from either abdominal or thoracic effusion taken at time of admission or necropsy. Cats that were not adopted into permanent homes, and cats with overt neurological disease, were excluded from the study. The cats were 3.3-82 months of age (mean of 10.4 months) with either wet FIP or dry-to-wet FIP. They were given a dosage of 15 mg/kg SC every 12 hours for two weeks. Because this study was based on the one from Kim *et al*. (2016), the originally planned dose given to the cats was 10 mg/kg, but after the first cat of the study failed to respond to the dose, it was raised to 15 mg/kg. They decided not to use a placebo group because the history of FIP is very well known, and "the lasting ethical position on placebo-controlled clinical trials is that whenever effective treatment exists for a given condition, it is unethical to test a new treatment for that condition against placebo" (Chiodo et al., 2000; Miller and Brody, 2002).

Nineteen of 20 cats regained their outward health within their two weeks of treatment. However, disease signs recurred (relapsed) within 1-7 weeks. Relapse cases, and every cat after the first five cats (who were the initial patients to only be treated for two weeks), were treated for a minimum of 12 weeks. 13 out of the 19 cats experienced relapses 1-7 weeks after initial or repeated treatment(s). Severe neurological disease occurred in 8 of the 13 cats, and 5 cats had recurrent abdominal lesions. All in all, 5 cats remained in complete disease remission until the publishing of the paper (mean 11.2 months), while two were still alive, but had relapsed.

New side effects of this drug were discovered during this drug trial. GC376 is synthesized in a highly concentrated and pure form, and it is required to be mixed as a concentration of 53 mg/ml in 10% ethanol and 90% polyethylene glycol, which is injected SC. The drug administration caused "stinging" on the injection site, subcutaneous swellings if injections happened repeatedly to the same site, and one cat developed a deep localised ulceration. On the seven long-term survivors, permanent focal subcutaneous thickening was found, three had 1-3 small focal areas with permanent hair loss at the injection site(s), and one cat obtained 4 pea-sized fibrous nodules between the shoulder blades.

The most notable side effect, however, was that the normal formation, growth, and eruption of permanent teeth were delayed in the youngest patients (3.3-4.4 months). Canines, incisors, fourth premolars, and molars were the least affected, while the 2nd and 3rd premolars were the most affected. The adult teeth, when /if they eventually grew out were also deemed to be smaller than average.

It is noteworthy that many of the cats that went into one or more relapses into the disease, and some became unresponsive to a new round of treatment. Kim *et al*. (2016) did an in vitro serial passage of viral resistance under drug pressure on FIPV-1146 in Crandell Rees feline kidney (CRFK) cells. After 20 passages they did not find any development of drug resistance to GC376. And, because FIPV is very rarely transmitted from cat to cat, it is unlikely that drug resistance would be transferred between cats. Pedersen *et al*. (2018) did not suspect that the cats suffering from recurrent disease vas due to the virus becoming drug resistant. However, one cat that relapsed twice and died 8 months after the start of the initial treatment examined on necropsy was found to have minor amino acid mutations on the viral 3CLpro in cells from the spleen and lung, compared to virions in the abdominal effusion fluid in pre-treatment samples. This could suggest that the resistance to GC376 could occur faster in vivo than in vitro.

This study clearly demonstrated that there was a distinct difference between experimentally- and naturally-infected cats. While 75% of experimentally infected cats survived, only 35% of the naturally infected cats survived, with only 25% in complete remission with no relapses. All the cats with neurological symptoms had relapses, and the antiviral medication only seemed to slow the progression of the neurological disease, and not stop or reverse it. GC376 seemed to work best in cats less than 18 months of age, and in cats without neurological disease. Even if the number of cats that died during this treatment was high, there were still seven survivors, and the cats that went into temporary remission most likely lived longer than they normally would have. One solution could be to test the effect of a higher dosage regimen, as the safety margin or this drug is very high.

4.2 GS-441524

The small molecule nucleoside analogue GS-441524 is a molecular precursor to a pharmacologically active nucleoside triphosphate molecule. Because it is an analogue, it acts as an alternative substrate and RNA-chain terminator or viral RNA dependent RNA polymerase. In other words, it functions as a competitor for the natural nucleoside triphosphates required for viral RNA synthesis. Murphy *et al*. (2018) performed an expansive study on GS-441524. They found that the drug had a wide safety margin and good bioavailability. In vitro studies on CRFK cells found the effective dose for inhibiting replication of FIPV in macrophages was 10 uM, and partial inhibition at 1 uM. No cytotoxicity could be found at >100 uM. They also found that the drug had sustained and effective plasma levels for 24 hours after administration in vivo when injected either subcutaneously or intravenously.

Murphy *et al*. (2018) also tested GS-441524 on 12 experimentally FIPV infected adolescent cats for two weeks. Ten cats who showed clinical signs and were diagnostically confirmed to have disease progression were divided into groups of two, where group 1 received a dosage of 2 mg/kg, and group 2 received 5 mg/kg SC every 24 hours. Two cats did not develop the disease after challenge-exposure to FIPV and served as controls for normal lymphocyte count and rectal temperature. Rapid reversal of disease signs started within 24-48 hours, and 10 cats made a full recovery. Two cats relapsed at four and six weeks post-treatment, one from each group, and received a second treatment for an additional two weeks. All 10 cats were still completely healthy 8 months later.

Again, a promising result is shown for a FIPV antiviral drug. The sample size is small, but the pharmacokinetic, cytotoxic, and experimental infection studies show an immense amount of positive results. For GC376 the laboratory experiments were also positive, but the field testing of the drug yielded a somewhat disappointing result, where only 7/20 cats survived. There is also uncertainty around how well it would work in cats with dry FIP. GC376 only had a 3% plasma drug concentration in the brain, and was deemed unlikely to be capable of treating cats with severe neurological disease in FIP (Pedersen et al., 2018). GS-441524, however, showed a blood plasma level of 11-12.9 uM, aqueous humour levels of 2.4-4.3 uM (22-23% of plasma), and CSF levels of 0.8-2.7 uM (7-21% of plasma), which could be promising in a drug treatment trial in cats with dry FIP.

Pedersen *et al*. (2019) performed a field study with GS-441524 on 31 cats with naturally-occurring FIP, 26 with wet or dry-to-wet FIP, and five with non-effusive FIP. All the cats were recruited from owners or veterinarians. Cats with severe ocular or neurological signs were discouraged to enter the trial due to concerns about the drug being able to cross the blood-brain barrier efficiently enough.

All cats were set on a schedule of a 2.0 mg/kg SC dosage every 24 hours for at least 12 weeks. Dosage was increased to 4.0 mg/kg in cases where treatment had to be extended or if a relapse occurred. The timeline was based on the findings of Murphy *et al*. (2018), and previous experiences with the field testing of GC376 (Pedersen et al., 2018). Treatment was extended by one or more weeks in cases where the cats still had abnormal serum protein values. The cats were also taken off all other treatments, such as antibiotics, corticosteroids, anti-inflammatory medications, or pain relief when they entered the treatment to ensure that there were no drug interactions, and to see the results of the use of GS-441524 alone. Blood and ascites samples were taken every 1-3 days for monitoring. Four cats were euthanised or died within the first 2-5 days due to severe disease complications. A fifth cat was euthanised on day 26 due to a lack of response to treatment. The remaining 26 cats showed drastic improvement, with fever resolving within 12-36 hours, rapid improvement of appetite, and weight gain. Ascites disappeared over a 1-2 week period, and clinical signs of thoracic effusion were no longer apparent after 7 days. A total of 8 cats went into disease relapse within 3-84 days. In total, 25/26 cats in this antiviral drug trial achieved sustained remission after initial or repeated treatment, though one later died of an unrelated heart condition.

Side effects in this study included mainly immediate pain and vocalisation upon injection, injection-site reactions. One cat had a rise in blood urea nitrogen and SDMA 8 weeks into the third round of treatment, but the cat completely recovered once taken off the medication, and the abnormalities could not be found with testing one month later. The drug dilutant (5% ethanol, 30% propylene glycol, 45% PEG 400, 20% water) had a pH of 1.5, which is well below the 4.5 threshold of the U.S. Food and Drug Administration. The synthesised GS-441524 comes in a highly purified powder form and is difficult to solubilise and stabilise at a more physiologic pH. Nevertheless, considering the clearly expressed pain in the patients, and the fact that 16 of 26 cats had injection site reactions, 7/16 developed open sores, and 3 developed scars, more effort should be made to improve the method of administration.

GC376 and GS-441524 are both possible antiviral treatments of feline infectious peritonitis, even though they function in different manners. While GC376 blocks viral polyprotein cleavage, GS-441524 terminates the viral RNA transcription. Both methods have been demonstrated to work well in humans (De Clercq and Li, 2016). When the abovementioned studies are compared, it can be noted that even though both drugs had a virtually identical result in laboratory in vitro and in vivo test results, GS-441524 performed much better in field studies with 25/31 cats surviving and going into long-term remission, versus 5/20 cats treated with GC376. 70% of the cats that were treated with GC376 went into disease relapse, while the number for GS-441524 was only 30%. It is also particularly interesting that out of 19 relapse cases in the GC376 treatment, 13 were no longer responsive to treatment (resistance development), and 8/13 of these cases resulted in severe neurologic disease and were euthanised. GS-441524, on the other hand, only had 8 relapse cases out of 26, and only one resulted in neurological disease and euthanasia, which shows a significant improvement of survivors. It could be argued that if GC376 had been given at higher doses, and had been a continuous treatment for 12 weeks (instead of an initial two weeks, then repeat treatment of 12 weeks after) the results could have been better. GS-441524 also showed better results for the relapse cases, where all cats that were treated with a higher dose recovered. Both of these treatments have room for improvement, as patients in both GC376 and GS-441524 had pain at injection, and injection site reactions. They both appear to be quite safe to use in FIP patients, but GC376 seemed to interfere with the development of permanent teeth in particularly young kittens (Kim et al., 2016; Murphy et al., 2018; Pedersen et al., 2019, 2018).

Dickinson *et al*. (2020) tested GS-441524 on four cats with neurological FIP, to investigate if the antiviral drug would affect these cats or not. The cats were given doses of 5-10 mg/kg SC once daily for 12-14 weeks. Three of the cats went into long term remission (528, 516, and 354 days after treatment initiation), while one was euthanised 216 days after treatment initiation following a relapse. Although the sample size was small, the result of three cats having recovered from a severe progression of FIP could definitely be counted as progress. Part of the decision to euthanise one cat was due to increased (behavioural) resistance to drug administration SC. A cure where the patient must resign to great amounts of pain could pose an ethical dilemma, especially considering the length of the treatment, as well as the emotional distress to the patient, owner, and veterinarian. Future research on pain relief administered before the antiviral medication could have great potential.

5. Discussion

5.1 Black market

Even though multiple studies have proved the safety and efficacy of new antiviral drugs, especially with the promising results of GS-441524 in 2019, there are still no widely distributed commercially available medications for veterinarians to prescribe. This is due, in part, to the fact that Gilead Sciences, Inc. (Foster City, Da, USA) halted further drug development of GS-441524 (to which they own the patent) therapy in cats (Jones et al., 2021). This is hypothesised to be because of the potential use in human medicine development, which often takes priority over veterinary medicine. Remdesivir, a drug very closely related to GS-441524, is also patented by Gilead, and was the first drug to be approved (on an emergency basis) for treatment of COVID-19, a coronaviral disease in humans. The drug has also been considered as a treatment for Ebola virus disease (Wogan, L., 2021).

The halt in licencing for the medication, along with published articles about its positive effect, created a demand for GS-441524 amongst pet owners whose cats were diagnosed with FIP. This has culminated in multiple unlicenced, "black market" drug manufacturers (often from China) who sell the medication (referred to as GS-441524-like) online. Large-scale, crowd-sourced social media FIP groups and other internet treatment and support forums have subsequently been created by despairing cat owners all over the world in the pursuit of a cure for their death-sentenced cats. Some of these groups actively help new members with acquiring the medication, planning treatment protocols, and drug administration advice (Jones et al., 2021; Kennedy, 2020).

Jones *et al*. (2021) distributed surveys on the most prominent FIP social media groups, and targeted owners who had bought these GS-441524-like drugs online, and subsequently treated their own cats, to evaluate how successful these treatments were. Although it would be close to impossible to determine if the drugs these owners received from online purchases really was a GS-441524-like drug, and whether these cats really had FIP, the results from the study still revealed a fair amount of interesting information.

With 393 cases meeting the inclusion criteria, it became clear that most owners had found this cure online (30.3% from their own research, 23.2% from Facebook), while a smaller faction had heard about it from their veterinarian (14.5% directly from a veterinarian, 12% indirectly). While a majority of owners (291 responses, 74.3%) answered that they had at least some degree of veterinary help, usually with disease diagnosis or diagnostic monitoring, only 8.4% (34 responses) reported that they received veterinary help with actual injection or oral administration of the medication. On a concerning note, 101 (25.7%) owners reported that they had not received any veterinary help other than initial diagnosis, which is cause for worry when it comes to animal welfare, especially if owners are untrained and inexperienced when it comes to administrating injections and caring for potential wounds. The price of this treatment also came at a considerable financial burden for owners, with a mean cost of USD 4920, the lowest reporting at USD 500, and the highest at USD 21 000.

While 57% of owners reported that their cat had signs of effusive FIP, 43% reported signs of neurological and/or ocular disease. In total, 96.7% (380) of the owners reported their cat to be alive (88.5% were treated for 12 weeks, 11.4% were treated for an additional 4 weeks), 12.7% suffered a relapse, and only 3.3% of the cats had died. This might reflect a survey bias, where owners whose cats had died simply would not want to take the study, or they might have left the social media group after the cat was no longer alive. The average starting dosage was 6.7 mg/kg, and the average ending dosage was 8.5 mg/kg, both dosages higher than that reported in the study by Pedersen *et al*. (2019). Owners with cats that had neurological and/or ocular symptoms were also encouraged by social media page administrators to start treatment at higher dosages (8.0 mg/kg). Pain accompanying injection was common (89%), and injection site ulceration and/or bleeding was reported for 43% of the cats.

While there were many limitations to this study, like potential reporting bias, inability to analyse administered drugs, no definite antemortem diagnostic tests, the results are still worth attention. The protocol for many of the FIP social media pages that offer assistance to owners require that the cat has an official diagnosis of FIP from a veterinarian, and often ask for official clinical and laboratory data. If it is assumed that all the cats from this survey did indeed have FIP, a survival rate of 96.7% is nothing short of astonishing.

There have also been recent reports of oral GS-441524-like medication being offered from unlicenced drug manufacturers. Addie *et al*. (2020b) tested an unlicenced oral medication called Mutian X (GS-441524-like) on 29 cats with FCoV infections, to see if faecal shedding could be decreased or stopped entirely. The drug was not analysed by the researchers, but a dose of 4mg/kg stopped all shedding within 7 days in 95% of the cats. This research could indicate that GS-441524 could potentially be used in a preventive measure, in addition to good hygiene measures, for example in shelters or with breeders, to limit the viral load of FCoV, and potentially limit the development of FIP. Additional studies by Addie *et al*. (2020a) and Krentz *et al*. (2021) showed that other oral unlicenced GS-441524-like drugs were capable of resolving both effusive and neurological FIP.

5.2 Future

The fact that an oral GS-441524 drug application can potentially work just as well as an injectable medication is great news. The ethical predicament of causing a patient to suffer in order to save their life can be highly distressing for all parties involved. It can be a burdensome decision to choose between suffering or survival, when the outcome is not guaranteed. Using chemical modifications of antiviral agents to make them more tolerable has previously been seen in human drug development, such as HIV/AIDS and HCV (Pedersen, N., 2019). Using these modifications to lessen the pain for patients in the veterinary field, especially those who are already severely affected by the disease, is of great importance.

Another ethical dilemma veterinarians face with these new drugs is that they are not yet licenced and available to be prescribed to patients. Being under both legal and ethical constraints, refusing to utilise unlicensed drugs are well within their rights. However, some may choose to help with administering the drug at the clinic, or perform regular diagnostic monitoring, as they may feel obligated to ensure the wellbeing of their patients. After all, the veterinary oath of most countries includes the promise to promote animal welfare and health, avoid pain and suffering in animal patients, and using professional knowledge and competence to the best of their ability.

The human pandemic of COVID-19 may have contributed to a favourable development for antiviral treatment in animal patients. As of August 2121, Remdesivir became a legal and licenced product for treating FIP in the UK. The treatment is currently only available as an injection, but there is hope that a tablet form will be available by the end of the year (Barker, 2021). In Australia, a pharmaceutical company has obtained the formula to allow the manufacturing of Remdesivir to make it available for feline patients. This drug is listed as "off-label" for usage in animals, but veterinarians are free to prescribe them (AVA, 2021). Hopefully, more countries will soon follow the licencing of this medication for use in the treatment of FIP.

6. Summary

Feline infectious peritonitis continues to be a major cause of mortality in young cats around the world. This coronaviral disease kills around 0.3-1.4% of all cats worldwide (Pesteanu-Somogyi et al., 2006; Riemer et al., 2016; Rohrbach et al., 2001). The research and treatment of FIP has come a long way since its discovery in the 1960's. Some aspects of this coronaviral disease still remain somewhat of a mystery to scientists, and accurate diagnostic tests continue to be elusive. However, in the last couple of years, a functional and effective treatment has finally been developed. Although the antiviral medication itself is still not available on the market in most countries to be prescribed by veterinary practitioners, there is great hope that this will soon change, with enough solid research papers, as well as production by pharmaceutical companies and licencing by local administrations. Hopefully, in the near future, cats dying from Feline infectious peritonitis will be a concept of the past.

7. Acknowledgement

I would sincerely like to thank Janikke L. Wisnes, Helene Solli, and Silje M. Terland for their invaluable input, advice, and unwavering support. This paper would not have been written without you.

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