Review

An update on feline infectious peritonitis: Diagnostics and therapeutics

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ABSTRACT

This review is concerned with what has been learned about feline infectious peritonitis (FIP) diagnostics and therapeutics since the publication of an extensive overview of literature covering the period 1963–2009. Although progress has been made in both areas, obtaining a definitive diagnosis of FIP remains a problem for those veterinarians and/or cat owners who require absolute certainty. This review will cover both indirect and direct diagnostic tests for the disease and will emphasize their limitations, as well as their specificity and sensitivity. There is still no effective treatment for FIP, although there are both claims that such therapies exist and glimmers of hope coming from new therapies that are under research. FIP has also been identified in wild felids and FIP-like disease is now a growing problem among pet ferrets.

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Introduction

Feline infectious peritonitis (FIP) is a coronaviral disease that can affect cats of any age, but is most prevalent among cats <3 years of age and especially from 4 to 16 months of age (Pedersen, 2009). FIP occurs commonly in catteries (pedigreed cats), shelters, kitten foster/rescue facilities and dense free-roaming colonies. Typical of an enzootic infection, the incidence of FIP can vary widely over time. The mortality is extremely high once clinical signs appear, although some cats can live with the disease for weeks, months or, rarely, years. A detailed clinical description of FIP can be found in earlier comprehensive reviews and will not be covered herein (Addie et al., 2009; Pedersen, 2009; Drechsler et al., 2011).

FIP virus (FIPV) arises through specific mutations in a common feline enteric coronavirus (FECV) that is ubiquitous in cats throughout the world and not in itself an important pathogen (Pedersen, 2009). FECV is shed in the feces of most apparently healthy cats in large multi-cat environments (Pedersen et al., 2004) and transmission results from direct ingestion of feces or contaminated litter and other fomites. Kittens usually become infected at around 9 weeks of age (Pedersen et al., 2004, 2008). Mutants of FECV capable of causing FIP are probably generated in large numbers during this initial infection, when levels of FECV replication are extremely high (Pedersen et al., 2008; Vogel et al., 2010). However, only a small proportion of cats exposed to these mutant viruses will develop FIP. Resistance to FIP is complicated and involves genetic susceptibility, age at the time of exposure and a number of stressors that occur at the same time as infection and have a negative impact on the ability of the infected cat to eliminate the virus. The time period between initial FECV exposure and clinical signs of disease can be as short as 2–3 weeks, as long as several months or, rarely, years. This period could reflect the time it takes for mutant FIPVs to evolve, or for the disease to progress from a subclinical to clinical state. Subclinical infections are usually limited to the mesenteric lymph nodes and can resolve or progress (Pedersen and Black, 1983; Legendre and Bartges, 2009; Pedersen, 2009). The onset of overt disease is a signal that the cat's battle with the virus has been lost and a return to normal health is extremely uncommon. There are rare occasions when a cat will make an apparent recovery, only to have clinical signs recur months and even years later (Legendre and Bartges, 2009).

The disease course between onset of clinical signs and death is also variable, but is generally shorter in younger cats and cats with effusive disease than in older cats and cats with non-effusive disease. Some cats, even with effusive FIP, can live for many months and the author has worked with a Birman cat that died of dry FIP at 6 years of age; based on its extensive clinical history, the cat appeared to have subclinical disease for its entire life. In one study concerning mainly cats with relatively mild presenting signs of non-effusive disease, the 1 year survival rate was only 5%.[1]

Owners that have acquired a kitten or young cat often become deeply attached to the animal before the first signs of FIP occur. The diagnosis of FIP, especially with its extreme mortality rate and lack of any effective treatment, has a great psychological effect on many owners. It also is the trigger for an owner communication most feared

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[1] See: http://www.vetmed.ucdavis.edu/ccah/research/FIP%20and%20PI%20info%20page.cfm (accessed 11 May 2014).

by both breeders and shelter managers. Because some cats with FIP are still reasonably well at the time of diagnosis and can often live weeks or months longer with only symptomatic treatment, owners might be reluctant to accept the diagnosis or the fact that there is no effective treatment. This can lead to a series of additional tests that often purport to be highly sensitive and specific, but seldom provide the desired answer and might even further cloud the diagnosis. The lack of an effective therapy often complicates matters, and some owners will go to great lengths to research the disease on the Internet and other sources. This can lead them to individuals who claim to have found treatments for the disease that are either curative or will prolong life. These claims are frequently supported by anecdotal statements from owners who have found such treatments beneficial. Unfortunately, due to problems with interpreting available diagnostic tests, not all cats diagnosed with FIP actually have the disease. If these cats have a self-limiting condition other than FIP, they will appear to respond well to almost any non-harmful treatment that is administered. Such cases give credibility to a particular treatment when none is deserved.

The purpose of this review is to update the knowledge of FIP diagnostics and therapeutics since the subjects were last reviewed (Pedersen, 2009). A number of studies concerning these subjects have appeared over the last 5 years and our knowledge of FIP has greatly increased. However, there still is no easy way to prevent the disease, no simple way to diagnose it definitively and no way to treat it effectively. Hopefully, this will change with our increasing knowledge of the factors causing FIP and drugs that target essential steps in FIP viral replication.

Diagnostic tests for feline infectious peritonitis

The diagnosis of FIP is based first and foremost on consideration of the cat's age, origin, clinical signs and physical examination. Cats 4–36 months of age from high-density environments that manifest a persistent but undulating antibiotic unresponsive fever are immediate suspects for FIP. Very few infectious diseases other than FIP have this signalment. More specific signs of FIP observed by the owner or on physical examination will narrow the diagnostic choices even more. Abdominal distension with ascites, dyspnea with pleural effusion, jaundice, hyperbilirubinuria, discernible masses on the kidneys and/or mesenteric lymph nodes, uveitis and a range of neurological signs associated with brain and/or spinal cord involvement are all common in cats with either the effusive ('wet') or non-effusive ('dry') form of FIP. At this point, the diagnosis of FIP can be made with reasonable certainty. However, given the high mortality, many veterinarians and owners feel uncomfortable with a diagnosis based on 'reasonable certainty'.

The difficulty then becomes choosing tests that will either further increase the odds that the clinical signs are caused by FIP (indirect tests), or that might provide a definitive diagnosis (direct tests). It is important to remember that the sensitivity and specificity of any indirect test will vary greatly depending on the likelihood that the cat has FIP based on other parameters. That is to say, the positive predictive value of a test such as a complete blood count (CBC) or albumin:globulin (A:G) ratio to predict FIP will be far greater in cats that have a signalment compatible with FIP than for those with a signalment not typical of FIP. It is also important to mention that the results of additional indirect tests are just as capable of confusing the diagnostic process as strengthening it.

Indirect tests

Complete blood count, albumin, globulin and bilirubin

The diagnosis of FIP is usually achieved by weighing signalment, clinical findings, abnormalities present in common diagnostic procedures and, when possible, postmortem examination and

histopathology (Sharif et al., 2010). Classic indirect tests for FIP include CBC, total serum protein, albumin and globulin levels, A:G ratio and basic blood chemistries (Addie et al., 2009; Pedersen, 2009; Drechsler et al., 2011). Common abnormalities usually include a chronic non-regenerative anemia (anemia of chronic disease), leukocytosis with an absolute increase in neutrophils and an absolute decrease in lymphocytes, elevated serum protein associated with high globulin and low albumin, and a low A:G ratio.

Hyperbilirubinemia and hyperbilirubinuria are common in cats with FIP, especially those with the effusive form. Elevations in serum and urine bilirubin (or biliverdin) are usually not associated with elevations in liver enzymes (Addie et al., 2009) and the liver is often spared in cats with FIP; evidence of cholestasis is not observed. Therefore, elevations in blood and urine bilirubin are not due to liver disease, as has been previously suggested, but rather are due to the increased destruction of RBCs in both lesions and in the circulation and difficulties in clearing hemoglobin breakdown products. Cats are notoriously poor at glucuronidation (Court and Greenblatt, 2000), thus limiting the rate that bilirubin and biliverdin are metabolized and recycled. If these common abnormalities are coupled with the usual signalment and clinical signs, a diagnosis of FIP can be made with high certainty. Even though many cats with FIP have characteristic CBCs, albumin and globulin levels and A:G, it is not reasonable to expect that every targeted parameter is always abnormal in the right direction. It is more important to look at the total picture and always in context of the signalment, clinical and physical features.

Analysis of effusions

The presence of a characteristic type of fluid in the peritoneal cavity or, less frequently, the pleural cavity is one of the most diagnostic features of the effusive (wet) form of FIP. Wet FIP predominates in most purebred and random bred cats, except for Birman and Burmese, which are more commonly diagnosed with the dry form. The fluid is usually yellow tinged due to the presence of bilirubin and, rarely, green-tinged due to the presence of biliverdin. As with hyperbilirubinemia and hyperbilirubinuria, the yellowish discoloration is a product of microhemorrhage and the breakdown of erythrocytes by macrophages.

FIP effusions are clear to moderately cloudy, viscous (egg-white consistency, often with threading) and high in protein (near serum level or higher). They often form partial clots when placed in a serum tube. FIP fluids are frequently labeled 'modified transudates' based on their perceived lack of cellularity. However, they are inflammatory exudates in the purest sense and do not meet the established physical or physiologic criteria for a modified transudate (Zoia et al., 2009). Most FIP effusions contain a fair number of cells (500–5000/μL), including macrophages, neutrophils and a low proportion of lymphocytes. FIP effusions are usually not outwardly hemorrhagic in appearance, with the exception of some pleural effusions. However, they often contain microscopic numbers of RBCs and visible fibrin tags. The exudate of FIP is unlike that seen in rare cases of bacterial peritonitis; the fluid accompanying bacterial peritonitis is clearly purulent in appearance, with very high neutrophil counts, and is not viscous or yellow-tinged. Transudates and modified transudates associated with liver and heart disease, lymphatic duct rupture and neoplasia do not have the same physical and cellular characteristics as FIP effusions.

Ultrasonography

The analysis of ascitic or pleural exudates in cats with FIP depends on the ability to realize the presence of such effusion and to obtain a fluid sample by simple centesis. Large pleural or peritoneal effusions often cause noticeable dyspnea or abdominal distension. However, there are many effusions that go unsuspected on initial physical examination or that are of minimal volume. In those cases,

ultrasonography or radiology can be employed to identify or confirm these effusions and assist in sample collection. Radiology is of more limited value with abdominal effusions, but might be more sensitive in detecting small amounts of pleural fluid.

Although ultrasonography and radiology can be very helpful in identifying the presence of fluid, these are not FIP-specific tests. Ultrasonography was reviewed retrospectively in 16 cats that had a reasonably confirmed diagnosis of effusive or non-effusive FIP (Lewis and O'Brien, 2010). The liver was judged to be normal in echogenicity in 11 (69%) cats, diffusely hypoechoic in three cats, focally hyperechoic in one cat and focally hypoechoic in another. Five cats had a hypoechoic subcapsular rim in one or both kidneys. Free fluid was present in the peritoneal cavity in seven cats and in the retroperitoneal space in one cat. Abdominal lymphadenopathy was noted in nine cats. The spleen had normal echogenicity in 14 cats and was hypoechoic in two. One cat had bilateral orchitis, with loss of normal testicular architecture. The authors concluded that, while none of these ultrasonographic findings were specific for FIP, such findings when considered along with appropriate clinical signs should raise the level of suspicion for FIP.

Albumin:globulin ratio and serum protein electrophoresis

The A:G ratio has been advocated as a useful predictor of FIP infection . However, the predictive value of the A:G ratio is very much dependent on the presence of other historic, clinical/physical or laboratory abnormalities associated with the disease. In a study of FIP cases seen in a referral practice, it was concluded that, when the prevalence of FIP is low, a high A:G ratio is useful to rule out FIP, but a low A:G ratio is not helpful in making a positive diagnosis of FIP (Jeffery et al., 2012).

Elevated total protein and/or globulin are often seen in chemistry panels from cats with FIP. In the absence of other common laboratory abnormalities, it is sometimes helpful to analyze these proteins by gel electrophoresis to determine the exact cause of the elevation. Taylor et al. (2010) performed a retrospective study of all serum protein electrophoresis (SPE) results from cats presented to the University of Bristol Feline Centre, UK, from 2002 to 2009; 155 results met the inclusion criteria. Clinical cases with a final diagnosis were classified as having normal or abnormal serum protein electrophoretic patterns compared to 77 clinically normal cats. Of the 155 cases, 136 (87.7%) had abnormal serum electrophoresis profiles, most commonly from a polyclonal increase in gamma globulins. The most common disease classification associated with serum protein abnormalities was infectious/inflammatory disease (80/136; 58.8%), including 39 cats diagnosed with FIP. Monoclonal gammopathy was observed in four cats, including one with FIP, one with lymphoma and two cases of splenic plasmacytoma.

Intraocular and neurologic signs

The majority of young cats with intraocular or neurologic disease have the non-effusive form of FIP (Pedersen, 2009). Ocular FIP is usually manifested by a uveitis, with idiopathic uveitis being one of the main differential diagnoses. Wiggans et al. (2013) compared the two disorders, particularly the diagnostic utility of aqueous humor cytology. They noted that the duration of disease tended to be shorter in cats with FIP than with idiopathic uveitis. In cats with idiopathic uveitis, microbial nucleic acids, antigens, or antibodies against a causative agent were seldom found in blood/serum, while serum feline coronavirus titers ≥1:6400 were detected only in cats with FIP. On cytological examination of the aqueous humor, plasma cell numbers were correlated with keratic precipitates and disease duration. As expected, clinically detectable hyphema correlated with erythrocyte numbers, while there was no correlation between cell numbers in the anterior chamber and aqueous flare. Although aqueous humor contained predominantly neutrophils in cats with

FIP and large reactive lymphocytes and plasma cells appeared more frequently in cats with idiopathic uveitis, neither clinical nor cytologic assessment of anterior chamber contents differed significantly between cats with idiopathic or FIP-associated uveitis.

The involvement of the brain or brain and spinal cord in non-effusive FIP is well known (Pedersen, 2009); however, sole involvement of the spinal cord is less commonly diagnosed. In another review of records, Marioni-Henry (2010) found that inflammatory/infectious diseases and FIP were the most commonly listed causes of feline myelitis, representing approximately 50% of all cases. Neoplasms were documented in approximately 25% of cases, with lymphosarcoma being the most common cancer of the spinal cord of cats. FIP and lymphosarcoma tended to occur in younger cats, while spinal disease in cats >10 years of age was more likely to be caused by other spinal cord tumors, intervertebral disc disease or fibrocartilaginous embolism.

Feline coronavirus antibody titers

Controversy still exists over the use and interpretation of feline coronavirus antibody titers in serum or plasma (Pedersen, 2009). The main problem with antibody tests is that both FECVs and FIPVs, being virtually identical to each other, evoke the same antibody responses. Titers also tend to be high among healthy cats in the same environments that foster FIP. Feline coronavirus antibody titers, if accurately performed, are nonetheless of some value. Although many healthy FECV exposed cats have titers by indirect immunofluorescence assay from 1:100 to 1:400 (Pedersen et al., 2008), as do many cats with FIP, the likelihood of a titer being associated with FIP increases with its magnitude. Fewer healthy cats have titers of 1:1600, while titers ≥1:3200 are highly suggestive of FIP (Hartmann et al., 2003). Healthy cats with titers <1:100 infrequently shed FECV in their feces, while cats with titers of 1:400 are usually positive for feline coronavirus in the feces (Pedersen et al., 2008).

The presence of low coronavirus titers in cats with confirmed FIP has always been problematic. Meli et al. (2013) attempted to explain this discordance by investigating whether virus in the blood or effusions of such cats was binding antibody and thereby lowering antibody titers in serum/plasma or effusions. Thirteen effusions, one plasma sample and three undefined samples from cats with FIP that had unexpectedly low serum coronavirus antibody titers were examined by real-time reverse transcriptase (RT)-PCR. Increasing amounts of virus correlated with lower signals in indirect immunofluorescent, ELISA and rapid immunomigration antibody tests in 70% of samples. However, five sera or effusions with low coronavirus antibody titers were negative by real time RT-PCR, so the presence of virus alone was not the sole explanation for false negative serum coronavirus antibody test results.

There have been attempts to make antibody tests more specific for FIPV, just as there have been attempts to make PCR-based tests more FIPV specific. A classical test, which is still marketed, is the '7b' antibody test. This test was developed on a faulty premise that FECVs lack the ORF 7b gene and hence do not produce the ORF 7b protein product, while FIPVs have the ORF 7b gene, produce the protein and therefore also evoke an antibody response to the ORF 7b protein. It is now well known that virtually all field isolates of FIPVs and FECVs possess an intact ORF 7b gene and therefore both evoke anti-ORF 7b antibodies. This fact, as it relates to the the 7b antibody test, was reconfirmed by Kennedy et al. (2008); 95 serum samples submitted for various diagnostic assays and 20 samples from specific-pathogen-free cats (free of coronavirus infection and antibodies) were tested for antibodies against the purified ORF 7b protein. As expected, expression of the ORF 7b protein, as indicated by detection of antibodies against the protein, was found in most feline coronavirus (i.e. FECV or FIPV) infected cats and it was concluded that seropositivity for this protein was not specific for FIPV and could not be used to diagnose FIP. In essence, the 7b

antibody test is no different than any other feline coronavirus antibody test.

ELISAs have been popular for antibody detection in many different diseases, but have some disadvantages compared to indirect immunofluorescent antibody testing. Takano et al. (2013a) developed three new immunochromatographic assays using recombinant feline coronavirus nucleocapsid protein. Two of the three tests were plagued by non-specific binding, while a third assay incorporating a protein A blotting step proved equal in specificity and sensitivity to standard ELISAs in detecting antibodies in plasma, serum, whole blood and ascitic fluid, while presumably being simpler to run.

α_1-Acid glycoprotein (AGP)

AGP has been used extensively, particularly in Europe, as an indicator test for FIP. AGP was found almost a decade ago to be hyposialylated in cats with FIP, but not in normal cats or in cats with other pathologies (Ceciliani et al., 2004). This study confirmed that serum AGP is a powerful discriminating marker for FIP, but only when coupled with other high risk factors (Paltrinieri et al., 2007). A Bayesian approach demonstrated that, when the pretest probability of FIP was high based on history and clinical signs, moderate serum AGP levels (1.5–2 µg/mL) could discriminate cats with FIP from others. However, only high serum AGP levels (>3 µg/mL) were highly suggestive of FIP in cats with a low pretest probability of disease (Paltrinieri et al., 2007).

Giori et al. (2011) studied the specificity and sensitivity of several tests in 12 cats, four of which were confirmed not to have FIP by histopathology and immunohistochemistry, and eight cats with FIP confirmed by histopathology and immunohistochemistry. Results from serum protein electrophoresis, analysis of effusions, anti-feline coronavirus serology, serum AGP concentrations and histopathology were then compared with the confirmed diagnosis. No concordance was found for serology and analysis of effusions, poor concordance was noted for histopathology, fair concordance for serum electrophoresis and perfect concordance for AGP. The conclusion was that immunohistochemistry must always be performed to confirm FIP and, if this is not possible and histopathology is not definitive, elevated AGP concentrations might support the diagnosis of FIP. However, the small numbers of cats in this study make it difficult to validate such conclusions and the earlier study of Paltrinieri et al. (2007) is probably a more accurate assessment of AGP testing for FIP. Like most indirect tests for FIP, the positive predictive value increases with the number of other risk factors that are present.

Paltrinieri et al. (2012) also investigated the levels of leukocyte-bound AGP in normal cats and cats with diseases including FIP by flow cytometry using an anti-feline AGP antibody. A total of 32 healthy cats (19 feline coronavirus seropositive), 13 cats with FIP (presumably all coronavirus seropositive) and 12 cats with other diseases (six coronavirus seropositive) were studied. The proportion of cats with AGP-positive leucocytes in each group or in cats with different intensities of inflammatory response (as measured by CBC, serum electrophoresis and serum AGP levels) was compared. AGP positive leucocytes were found in 23% of cats; most were diseased, but a small number were healthy. AGP positive leukocyte staining was associated with inflammation and not with leukocytosis per se. Staining among healthy cats was unrelated to coronavirus antibody status. Cats with FIP were more likely to have positive staining leukocytes than healthy cats, but not as likely as cats with other diseases. It was concluded that AGP positive leucocytes are present in feline blood, especially during inflammation. Staining leukocytes for AGP binding does not appear to have any value over serum AGP testing, especially when considering the potential cost and effort involved in this method.

Postmortem and histopathologic findings

Although studies such as that of Giori et al. (2011) downplayed the reliability of histopathology, the gross and histologic lesions of FIP are stereotypic. A thorough necropsy with adequate histopathologic examination of diseased tissues can be an accurate way to confirm a diagnosis. However, as with other aspects of this disease, veterinary pathologists are often reluctant to assign a definitive diagnosis to a cat with FIP based on what they see grossly and microscopically, even though they often do so for neoplasia. The tendency is then to describe what is found and hopefully list FIP as the most likely cause. This will then lead to immunohistochemistry so that a definitive diagnosis can be made, but this might not be definitive in all cases.

Rivalta test

The Rivalta test is widely touted, especially in Europe, and has been long used for diagnosing FIP-associated exudates (Hartmann et al., 2003). The test involves placing a few drops of ascites or thoracic fluid into a tube containing a weak acetic acid solution. The appearance of a white flocculent material is seen in a positive test. A positive Rivalta test was once believed to be highly specific for FIP fluid. In a study of 497 cats with effusions, 35% of which had confirmed FIP, the Rivalta test had a sensitivity of 91% and a specificity of 66%, with a positive predictive value of 58% and a negative predictive value of 93% (Fischer et al., 2012). As would be expected, these values increased when cats with lymphosarcoma or bacterial infections were excluded, or when only cats of ≥2 years of age were considered. The Rivalta test appears to be reproducible in samples stored for 21 days at room, refrigerator or freezer temperatures, and with some modifications of acid concentration (Fischer et al., 2013). However, reading of the test is subjective and results are therefore somewhat dependent on the evaluator.

Real time reverse transcriptase-PCR for feline enteric coronavirus shedding in feces

Some cat breeders will test fecal samples for FECV for various reasons, including diagnosing FIP or implicating a certain cat as the source of their FIP problems. Although cats with FIP will often shed FECV, it is inconsistent and the virus is shed at lower levels than in healthy cats; feline coronavirus in these cases is usually of the enteric and not the FIP biotype (Pedersen et al., 2009, 2012; Chang et al., 2010, 2012). This application of the test should be discouraged, because the odds that a significant proportion of cats in the cattery will be shedding FECV at any given time are high, while the odds of an adult FECV shedder developing FIP are very low. There is evidence, however, that the higher the proportion of cats in a cattery that shed coronavirus at a given time, and the higher the level of shedding, the more likely FIP will occur in the population (Foley et al., 1997).

Fecal testing for feline coronaviruses by real time RT-PCR is not only a good way to detect shedding, but also a good way to quantitate the level and frequency of shedding. Real time RT-PCR is commercially available and highly accurate if appropriately performed. However, the presence of inhibitors needs to be taken into consideration and can affect quantitative measurements of viral levels (Dye et al., 2008). Large amounts of virus are shed in the feces for many weeks, and even months, after initial infection, but with time and lack of re-exposure most cats will stop shedding (Pedersen et al., 2008). However, some cats can shed at high levels for prolonged periods of time and some cats that have stopped shedding become susceptible to reinfection (Pedersen et al., 2008).

FECV is the most common of a large and diverse group of enteropathogens detected in feces from diarrheic and healthy shelter cats and is the only enteropathogen that had even a weak statistical association with diarrhea (Sabshin et al., 2012). Even so, the enteric signs are uncommon and usually mild and short lived (Pedersen et al.,

2008). Given the fact that the majority of shelter and cattery cats shed the virus at any given time, coupled with its low intrinsic disease potential, there is little need to test for its presence in shelters or catteries other than as a means toward eliminating FECV from the premises and thus lowering the chances of cats dying of FIP. However, without keeping cat and especially kitten numbers low, and evoking an extremely strict quarantine, FECV is both difficult to rid from premises and easily re-acquired. Moreover, establishing and maintaining a FECV free cattery or shelter run counter to the purpose of such facilities. Even if a coronavirus free status is achieved, kittens from such an environment will ultimately become exposed when they are sent elsewhere or new cats introduced. Although fecal testing for FECV is not a very productive procedure, there is some predictive value in knowing the proportion and frequency of fecal shedders. The greater the proportion and frequency of shedding, the more likely there will be FIP losses (Foley et al., 1997).

FECV shedding can also be indirectly measured by serologic tests. Serum feline coronavirus antibody levels, as measured by indirect immunofluorescence, appear within 2 weeks of infection to titers of around 1:100–1:400 and then drop to levels of 1:25 or lower in cats that develop immunity and cease shedding (Pedersen et al., 2008). Feline coronavirus serology can also be used as an indirect measure of FECV shedding. Cats having antibody titers of 1:100–1:400 or greater are usually FECV shedders and cats with titers of 1:25 or lower are usually negative. However, some cats with low or negative antibody titers in serum or effusions might still contain considerable virus in their feces or effusions.

Direct tests

A great deal of credence has been placed on definitively diagnosing FIP. Although this author believes that a diagnosis of FIP can be made with sufficient certainty based on the signalment, disease signs, physical examination and results of basic indirect tests, the grim implications of the diagnosis often drive veterinarians and owners to seek a definitive diagnosis. A definitive diagnosis for FIP requires identification of viral RNA or proteins within macrophages in characteristic lesions or fluids from diseased tissues. There are currently two types of tests available for definitive diagnosis: (1) identification of viral proteins by immunohistochemistry and (2) identification of viral RNA by some sort of test based on PCR.

PCR-based tests

Tests based on PCR have been used to help diagnose FIP for almost two decades (Li and Scott, 1994). PCR-based tests for feline coronaviruses start with the purification of RNA from feces, blood or tissues; the RNA is then reverse transcribed to complementary DNA (cDNA). A small region of this cDNA that encodes a feline coronavirus-specific sequence is then amplified thousands of times and the product identified as a single distinct band by gel electrophoresis. Further refinements have been made to increase the sensitivity and specificity of PCR. Nested PCR is a method that greatly increases the ability of the test to detect very small amounts of feline coronavirus RNA as cDNA (Gamble et al., 1997). Nested PCR involves amplifying a larger fragment of the viral cDNA in the first step, purifying this PCR product and then amplifying a smaller piece from within the larger amplified DNA in a second reaction. A nested PCR was reportedly >90% sensitive and specific in detecting FIPV in ascites from cats with effusive FIP (Gamble et al., 1997). Although very sensitive, nested PCRs are plagued by DNA contamination with PCR products, which causes false positive reactions.

The problem of laboratory contamination with PCR products can be avoided by using real time RT-PCR and, for this reason, virtually all PCR-based diagnostic tests for FECV/FIPV RNA are based on this format. It is generally conceded that real time RT-PCR is quite sensitive and specific in detecting and semi-quantitating fecal coronavirus

(FECV) shedding in both experimental and naturally infected cats (Pedersen et al., 2008, 2009, 2012; Kipar et al., 2010; Vogel et al., 2010; An et al., 2011; Addie et al., 2012; Amer et al., 2012; Wang et al., 2013). However, a high sensitivity and specificity is conditional on a number of variables. Samples must be collected to maximize virus content, the viral RNA within these samples must be properly extracted and preserved, the RNA has to be properly purified and reverse transcribed, any PCR inhibitors (usually in feces) must be negated (Dye et al., 2008), all reagents must be properly designed and maintained in active form, and samples properly run by knowledgeable technicians on reliable instruments. A remaining problem is the high degree of viral genome diversity, which affects the binding of primers used in RT-PCR tests. An attempt was made to address this by designing a RT-PCR that takes into account a degree of genomic variability in various feline coronaviruses (Hornyák et al., 2012). Most of these technical aspects, although daunting, can be overcome with attention to detail. Therefore, it is generally assumed that real time RT-PCR is a sensitive way to detect coronavirus RNA in feces and diseased tissues/effusions. Nevertheless, in one study testing its accuracy, PCR-based testing was found to be only 80–90% accurate in confirming the presence of FIPV in diseased tissues (Sharif et al., 2010). In another study, only 377/854 (44%) of peritoneal effusion specimens from cats suspected but not confirmed of having FIP tested positive by RT-PCR (Soma et al., 2013). Strangely, positivity was 78-92% in purebreds vs. 35% in mixed breed cats.

Even though real time RT-PCR can be highly accurate in detecting FECVs in feces and FIPVs in diseased tissues and fluids, the ultimate test is one that can detect FIPV in blood. The problem of differentiating FECV RNA from FIPV RNA was identified quite early (Herrewegh et al., 1995) and, based on the theory that FECVs were found only in the intestine and not in a replicating form in tissues, a test was developed that would only detect the replicating forms of feline coronavirus RNA (i.e. subgenomic RNAs); this test was claimed to correctly diagnose 93% of cats with confirmed FIP and none with other disorders (Simons et al., 2005). However, in a second study using the same procedure, 54% of healthy cats, especially in the 6–12 month age range, also tested positive (Can-Sahna et al., 2007). This was later explained by experimental infection studies of FECV by Kipar et al. (2010) and Vogel et al. (2010), both of whom demonstrated that viremia accompanied intestinal infection. FECV was identified in blood monocyte/macrophages in 40% of experimentally FECV infected cats by day 14 and 14% remained viremic at day 48 post-infection; FECV was detected in several internal organs after fecal shedding ceased (Kipar et al., 2010).

The problem of FECV co-infections can be overcome by designing tests that identify FIPV-unique mutations. Mutations in the ORF 3c and at the S1/S2 gene cleavage site are unique to each FIPV and therefore are not good candidates (Licitra et al., 2013; Pedersen et al., 2009) . The most FIPV-specific mutations are two single nucleotide changes within the fusion protein region of the spike or surface (S) protein (Chang et al., 2010). Either one of these mutations occurs in >98% of FIPVs detected in diseased tissues, but no results have been reported regarding whether these mutations are present in blood at detectable levels. It is also possible that these mutations could be found in healthy cats with abortive or subclinical infections (Porter et al., 2014). Even if highly sensitive and specific FIPV RNA detection tests could be developed, it appears that many cats with naturally occurring FIP do not have detectable levels of viral RNA in their blood, either within plasma or concentrated in the white cell fraction.

Immunostaining methods

Immunostaining of diseased tissues (immunohistochemistry) or fluids by immunofluorescence or immunoperoxidase methods can be as reliable as RT-PCR, but the accuracy of these tests is limited by the quality of the reagents used, the tissues sampled, and the ex-

pertise of the microscopist in discerning true positive staining within macrophages in tissue lesions or from effusions. Immunofluorescence is more sensitive than immunoperoxidase staining, but requires frozen sections (with or without glycerol/resin protection medium), while immunoperoxidase staining can be performed on formalin fixed tissues. Formalin fixation and wax embedding should be completed as rapidly as possible following tissue collection. Although immunohistochemistry is considered to be accurate for the definitive diagnosis of FIP (Giori et al., 2011), there are cases of FIP that test negative depending on the quality of the tissues, the presence of adequate lesions within the material examined, and the quality of reagents and test performance.

Immunohistochemistry of effusions or fluids from cats suspected of having FIP should be used much more often than at present. Effusions often contain numerous virus positive macrophages that can be concentrated onto slides. This technique has also been used successfully to detect FIPV infected macrophages within the cerebrospinal fluid of a cat with neurologic disease (Ives et al., 2013).

Non-specific positive staining of macrophages might also be a problem. Litster et al. (2013) compared results from direct immunofluorescence on ante-mortem feline effusions with postmortem results in 17 cats with abdominal or thoracic effusions. Histopathologic examination of tissues collected at necropsy confirmed FIP in 10/17 cases and ruled out FIP in 7/17 cases. Ante-mortem direct immunofluorescence testing was positive in all 10 cases confirmed as FIP at postmortem examination. In the seven cats where FIP was ruled out at postmortem examination, direct immunofluorescence was negative in five cases and positive in the remaining two cases. The calculated sensitivity of immunohistochemistry testing was 100% and the specificity was 71.4%. Duplicate effusion specimens from eight cats that were initially positive by immunofluorescence were stored at 4 °C or room temperature (22–25 °C) and subjected to serial testing to determine the duration of positive results. Direct immunofluorescence positive specimens stored at both temperatures retained their positive status for at least 2 days.

Immunoperoxidase staining has been used to diagnose FIPV in macrophages in the skin of two cats with atypical skin lesions (multiple popular lesions; Declercq et al., 2008; Bauer et al., 2013) and in various tissues of a diseased Mountain lion (Stephenson et al., 2013).

Feline infectious peritonitis therapeutics

Several approaches have been used to treat cats with FIP. The proven approach for other virus infections (e.g. human immunodeficiency virus type 1, HIV-1; hepatitis B and C viruses) is to use drugs that specifically inhibit viral replication. A second approach is to inhibit key aspects of the inflammatory response with substances such as interferon. The second approach rarely works independently, but has been successful when combined with specific anti-viral drugs (e.g. interferon-α used with antiviral drugs such as ribavirin, tenofovir and entecavir for hepatitis B and/or C virus infections). A third approach is to stimulate the immune system non-specifically in the hope that it will be able to overcome the infection. Some approaches combine one or more of these types of therapies. Regardless of the approach used, a properly controlled clinical trial for both safety and efficacy should be a prerequisite for any scientific publication claiming a treatment for FIP (Hartmann and Ritz, 2008).

Antiviral drugs

Antiviral drugs are of two basic types: one type targets the cellular machinery that viruses usurp to aid their replication, while the other targets some activity specific to viral infection and replication. Drugs that affect the cellular machinery are least likely to be effective because they tend to have a negative effect on both the host and virus. The most successful antiviral therapies have involved drugs that target specific regions of the viral genome that regulate key processes in infection or replication. Feline coronaviruses have several genes that are similar in activity to those of HIV-1, including RNA-dependent polymerase and viral proteases. The retroviral protease is another important target for HIV-1 and a combination of reverse transcriptase, protease and integrase inhibitors have effectively reduced HIV-1 to a chronic subclinical infection in most patients. Based on experience with other viral protease inhibitors, similar drugs are now being developed against the main protease (3CL) encoded by both coronaviruses and noroviruses (Kim et al., 2013). Many of the structural proteins of coronavirus are first transcribed from mRNA as polyproteins which are cleaved by proteases into their constituent parts. Although cellular proteases have been assumed to play a major role in cleaving FIPV polyproteins, preliminary in vitro tests show 3CL protease inhibitors are effective in inhibiting FIPV replication at levels that are not toxic to cells (Kim et al., 2013).

The drug chloroquine, which is used to treat malaria, has been shown to inhibit FIPV replication in vitro and has anti-inflammatory properties (Takano et al., 2013b). It was then tested on experimental FIP infection; although the clinical scores of cats in groups treated with chloroquine were better than those of cats in groups not treated with chloroquine, alanine aminotransferase levels increased in the chloroquine-treated groups, indicating an untoward toxic effect.

Cyclosporine A has also been shown to possess anti-coronavirus activity. Pfefferle et al. (2011) demonstrated that a number of immunophilins strongly interact with coronavirus nonstructural protein 1 (Nsp1) and that cyclophilin inhibitors, such as cyclosporine A, block the replication of coronaviruses of all genera, including human, feline and avian species. This confirms a role for cellular immunophilins (cyclophilins) in coronavirus replication. The authors postulated that non-immunosuppressive derivatives of cyclosporine A might not only serve as broad-spectrum inhibitors of emerging human coronaviruses, but also of the more ubiquitous coronavirus pathogens of humans and livestock. The ability of cyclosporine A to inhibit feline coronavirus replication in cell culture has been confirmed, but it has not been tested in vivo (Tanaka et al., 2013). The problem with antiviral agents such as chloroquine and cyclosporine is that they work through pathways common to cellular and viral activities. Coronaviruses usurp normal cellular pathways to facilitate their own replication and the anti-viral effects of compounds such as chloroquine and cyclosporine cannot be separated from their other effects on cells and, therefore, the host. For example, the antiviral activity of chloroquine in vivo was inferior to in vitro activity and there were toxic effects in the host (Takano et al., 2013b).

Hseih et al. (2010) screened 16 compounds for their antiviral activity against a local feline coronavirus strain in *Felis catus* whole fetus-4 cells (Hseih et al., 2010). *Galanthus nivalis* agglutinin (GNA) and nelfinavir (a HIV-1 protease inhibitor) effectively inhibited feline coronavirus replication. When the level of virus infection was increased to mimic that of target cells from FIP-infected cats, GNA and nelfinavir were no longer inhibitory. However, when the two agents were added together to FIPV-infected cell cultures, a synergistic effect was observed and virus replication was inhibited. No further reports of these drugs in cats with FIP have been published. It is doubtful whether a natural agglutinin would have any anti-viral effect in vivo, whereas protease inhibitors such as nelfinavir tend to be virus specific.

Raaben et al. (2010) investigated the role of the ubiquitin–proteasome system in different steps of the coronavirus infection cycle using ubiquitin inhibitors such as MG132, epoxomicin, and velcade. These compounds impaired entry and subsequent RNA synthesis and protein expression of mouse hepatitis virus (MHV), FIPV and severe acute respiratory syndrome (SARS) coronavirus. Virion assembly and release, as judged by MHV, were not affected. Viral

protein expression was reduced in cells in which ubiquitin was depleted by using small interfering RNAs. Under these conditions, the susceptibility of the cells to virus infection was not affected, excluding an important role of ubiquitination in virus entry. This study indicated that the ubiquitin–proteasome system was involved in multiple steps of the coronavirus infection cycle and identified it as a potential drug target against coronavirus infection. However, it must be noted that the ubiquitin–proteasome system was not found to be involved in the replication cycle of the SARS coronavirus in a subsequent study, leaving uncertainty about its involvement with MHV and FIPV (Schneider et al., 2012).

Virus inhibitory peptides

Liu et al. (2013) designed five overlapping peptides using a plaque reduction assay that spanned the heptad repeat (HR) region of the S protein of the type II FIPV strain NTU-156. The peptides inhibited virus replication by blocking the intercalation of the HR1 and HR2 regions, which is necessary for the activation of S protein-mediated fusion. They were able to inhibit FIPV replication by 97% using one of the five peptides at a concentration <20 μM. A synergistic effect was found with human interferon-α . The authors concluded that this peptide could be a valuable addition to current FIP prevention methods, but it should be remembered that using such peptides in vivo is quite different to using them in vitro.

Anti-inflammatory and immunosuppressive drugs

A number of drugs have both anti-inflammatory and immunosuppressive activity. Prednisolone and alkylating drugs such as cyclophosphamide have been used to reduce clinical signs in cats with FIP, but there is no evidence that they altered the disease outcome. Rather than using this less specific therapeutic approach, attempts have been made to inhibit specific cytokines deemed to be important in the pathogenesis of FIP. Tumor necrosis factor (TNF) inhibitors have been used for some time to alleviate some of the signs of FIP. One of the most popular of these drugs is pentoxyfilline (Fischer et al., 2011). Pentoxyfylline was widely used in FIP because of its use in controlling vasculitis in humans, vasculitis being an important component of the pathophysiology of FIP. A study of 23 cats with proven FIP failed to detect an effect of pentoxyfylline on the survival time, the quality of life or any FIP-associated clinical or laboratory parameters (Fischer et al., 2011).

Non-specific immunostimulant drugs

The use of non-specific immunostimulants has been popular in veterinary medicine for decades, often based on purported efficacy for specific signs of feline leukemia virus (FeLV) and/or feline immunodeficiency virus (FIV) co-infections with FIP, and there are anecdotal reports of cats presumed to be infected with FIP being cured or having their lives prolonged by such treatments. These include immunostimulants such as staphylococcal A protein, ImmunoRegulin (*Propriobacterium acnes*), Acemannan (mucopolysaccharide extract of *Aloe vera* leaves) and Imulan (lymphocyte T cell immunomodulator). Polyprenyl immunostimulant (PI) is the latest plant extract claimed to prolong the lives of some cats with mild forms of dry FIP.[1] The current rush to use this biologic agent was based on a study of three cats, one of which was reportedly cured of FIP after long-term treatment (Legendre and Bartges, 2009). However, all three cats had non-effusive disease localized to a single mesenteric lymph node; two had subclinical disease and were healthy at the onset of the trial and the third appeared only mildly affected. The authors reported that the treatment had no effect on cats with more severe disease, such as effusive FIP, although no results were provided on this point. The authors also failed to con-

sider that cats with such localized disease could potentially undergo spontaneous remission.[1] Subsequent trials with PI that excluded cats with effusive FIP and cats with severe disease (presumably from dry FIP) showed only a 5% survival at 1 year.[1] Therefore, there is no evidence that PI is curative of FIP in any form and no evidence that it can significantly prolong life in even cats with the mildest disease.

It must be emphasized that the use of all such biologic immune stimulants for the treatment of FIP is off-label, as most were conditionally licensed or licensed for use in cats with FeLV or FIV infections, or in the case of PI, feline herpesvirus. Furthermore, these compounds can be expensive, with no evidence of significant benefit. The dosage regimen for such drugs is often uncertain and frequently based more on limiting costs to the owners by administering small doses or treating less frequently. This lack of information is in itself evidence of the quality of pre-testing. None of these immunostimulants have been subjected to vigorous pharmacokinetic and bioactivity studies, although all appear to be safe. In the absence of adequate pretesting, it is important to do valid clinical testing prior to recommending these agents for general use. Proper clinical testing requires the use of a placebo control, randomization, double blinding, sufficient case numbers for statistical validity and accurate determination of disease status. It is also paradoxical that many veterinarians use these types of drugs in combination with more traditional prednisone/prednisolone treatment, since one drug reportedly stimulates the immune system while the other drug suppresses it.

Feline infectious peritonitis-like disease in other animal species

Wild felids

FIP has been well documented in virtually every species of Felidae. FIP of domestic cats and cheetahs has been historically intertwined (Pearks Wilkerson et al., 2004). Initial studies of genetic diversity among cheetahs concluded that they were extremely inbred as a species, to the point of accepting skin grafts from each other, and that this inbreeding was a result of an extreme bottleneck that occurred some 12,000 years earlier. If correct, this degree of inbreeding would have made the species highly vulnerable to the introduction of a novel and highly fatal pathogen. This paradigm was thought to have been realized when a fatal epizootic of what appeared to be FIP caused by feline coronavirus occurred among captive cheetahs at Winston Safari Park in Oregon, USA, in the early 1980s. The outbreak started with two confirmed FIP deaths among cheetahs recently acquired from Sacramento and, within months, other cheetahs in the park fell ill. Retrospective serum samples tested for antibodies demonstrated that, prior to 1982, all cheetah sera were negative, but within 6 months of the arrival of the index cases, 100% of the cheetahs had seroconverted. Ninety percent of the 60 cheetahs in the park became ill over the next 2 years, manifesting a range of clinical signs such as weight loss, diarrhea, jaundice, hepatic and renal disease, and gingivitis, all ascribed to coronavirus, often without firm evidence. The conclusions of these early studies were that captive cheetahs were highly susceptible to feline coronavirus due to extreme inbreeding and that the introduction of coronavirus into wild cheetahs could be catastrophic. This was later found to be incorrect. Castro-Prieto et al. (2011) tested a much larger sampling of wild cheetahs and reported that they had much more MHC class I and II diversity and immune competence than previously believed, contradicting previous speculation that the cheetah is a paradigm species for inbreeding vulnerability. This was confirmed in another manner by studies of feline coronavirus infection in wild and captive cheetahs (Gaffney et al., 2012). Cheetahs in the wild were found to have an extremely low rate of exposure to many common cat infections, including feline coronavirus, and were rarely affect-

ed by infectious diseases. However, almost half of healthy captive cheetahs shed feline coronavirus in their feces persistently, transiently, or intermittently, and FIP losses after the initial outbreak remained very low. Therefore, it appears that domestic cats and cheetahs handle feline coronavirus in a similar manner. Although already briefly described in the mountain lion or puma (*Puma concolor*), a detailed report of FIP in a California mountain lion has been recently published (Stephenson et al., 2013).

Ferrets

An epizootic of catarrhal enteritis caused by a novel coronavirus was first described in pet ferrets in the USA in 1993 (Murray et al., 2010). Subsequently, a disease identical in appearance to dry FIP of cats was also described in ferrets in the USA (Murray et al., 2010). The causative virus of the epizootic catarrhal enteritis (ECE) was named ECE virus (ECEV), while the causative virus of FIP-like disease was named ferret systemic coronavirus (FRSCV). These infections have subsequently been reported in pet ferrets in Japan and Europe (Michimae et al., 2010; Wise et al., 2010; Provacia et al., 2011). A genetic analysis of ECEVs and FRSCVs indicated that the two coronaviruses were closely related but genetically unique (Wise et al., 2010). Interestingly, functional mutations in the ORF 3c gene resembling those of FIPV were seen in 2/3 FRSCV isolates from ferrets, but were not present in the ECEVs. Terada et al. (2014) detected coronavirus in the feces of 44/79 (56%) pet ferrets in Japan and classified the viruses into two genotypes based on S gene sequences. Genotype 1 was related to the FRSCV reported by Wise et al. (2010), whereas all published ECE strains fell within genotype 2 and resembled ECEV. These two ferret coronaviruses appear at first glance to resemble FECVs and FIPV in cats. Whether viruses causing FIP-like disease and ECE in ferrets are indeed genotypically distinct, or a spectrum of closely related strains, remains to be determined. What is certain is that ferret infectious peritonitis is a relatively new and increasing problem in pet ferrets and is hauntingly similar to the appearance of FIP for the first time in cats in the late 1950s (Pedersen, 2009). The fact that ferret coronavirus exists in several genetic forms indicates that coronaviruses have been in the species for some time. Has a new ferret coronavirus evolved that is more likely to mutate to cause infectious peritonitis? As ferrets have become more popular as pets, do they face the same risk factors that faced cats as they became more popular as pets? Are similar mutations responsible for systemic disease in both feline and ferret coronaviruses? These questions remain unanswered.

Conclusions

The diagnosis of FIP should not be difficult. It is a disease mainly of kittens and young cats from multi-cat environments, such as shelters, cat/kitten rescue organizations or catteries. The presenting historical and physical signs, whether in the effusive or non-effusive form, are relatively stereotypic and the results of routine CBCs, serum protein, albumin and globulin determinations often confirm the suspicions obtained from the history and physical examinations. Yellow-tinged, mucinous, inflammatory peritoneal (or pleural) effusions are characteristics for the effusive form of disease, while neurologic signs, uveitis and masses in the ileo-cecocolic region, kidneys and mesenteric lymph nodes are characteristics for the non-effusive form of FIP. Coronavirus antibody titers can be misleading in the range of 1:25–1:1600, but are increasingly relevant at higher titers. Gross and microscopic findings at postmortem examination are often undervalued and should be given greater importance. Immunohistochemistry and real time RT-PCR are very useful for identifying FIPV antigens in tissues with lesions or effusions and, if done properly, can provide a definitive diagnosis. The holy grail for FIP diagnostics is a simple blood test that would detect FIPVs associated with active disease and not FECVs or mutants of FECV that are not yet capable of causing FIP. Currently, there is no effective treatment for FIP and mortality is extremely high. However, the fact that a rare cat appears to make a spontaneous recovery with good supportive care is enough of an impetus to lead owners to try a number of alternative therapies that are touted mainly on the Internet.[1] Many of these therapies are expensive and come with a high emotional burden. It is important that all such treatments be adequately tested on an adequate number of cats with confirmed disease and in a randomized, placebo controlled and double blinded manner. The most promising therapies will probably involve drugs that specifically target viral proteins important in viral replication. Coronaviruses have large genomes with many potential target genes and hopefully safe and effective antiviral drugs will be developed. It is anticipated that such drugs might be developed as offshoots of research investigating emerging and highly fatal coronavirus infections of humans.

Conflict of interest statement

The author has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

References

Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., et al., 2009. Feline infectious peritonitis. ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery 11, 594–604.

Addie, D.D., McDonald, M., Audhuy, S., Burr, P., Hollins, J., Kovacic, R., Lutz, H., Luxton, Z., Mazar, S., Meli, M.L., 2012. Quarantine protects Falkland Islands (Malvinas) cats from feline coronavirus infection. Journal of Feline Medicine and Surgery 14, 171–176.

Amer, A., Siti Suri, A., Abdul Rahman, O., Mohd, H.B., Faruku, B., Saeed, S., Tengku Azmi, T.I., 2012. Isolation and molecular characterization of type I and type II feline coronavirus in Malaysia. Virology Journal 9, 278.

An, D.J., Jeoung, H.Y., Jeong, W., Park, J.Y., Lee, M.H., Park, B.K., 2011. Prevalence of Korean cats with natural feline coronavirus infections. Virology Journal 8, 455.

Bauer, B.S., Kerr, M.E., Sandmeyer, L.S., Grahn, B.H., 2013. Positive immunostaining for feline infectious peritonitis (FIP) in a Sphinx cat with cutaneous lesions and bilateral panuveitis. Veterinary Ophthalmology 16 (Suppl. 1), 160–163.

Can-Sahna, K., Soydal Ataseven, V., Pinar, D., Oğuzoğlu, T.C., 2007. The detection of feline coronaviruses in blood samples from cats by mRNA RT-PCR. Journal of Feline Medicine and Surgery 9, 369–372.

Castro-Prieto, A., Wachter, B., Sommer, S., 2011. Cheetah paradigm revisited: MHC diversity in the world's largest free-ranging population. Molecular Biology and Evolution 28, 1455–1468.

Ceciliani, F., Grossi, C., Giordano, A., Pocacqua, V., Paltrinieri, S., 2004. Decreased sialylation of the acute phase protein α1-acid glycoprotein in feline infectious peritonitis (FIP). Veterinary Immunology and Immunopathology 99, 229–236.

Chang, H.W., de Groot, R.J., Egberink, H.F., Rottier, P.J., 2010. Feline infectious peritonitis: Insights into feline coronavirus pathobiogenesis and epidemiology based on genetic analysis of the viral 3c gene. The Journal of General Virology 91, 415–420.

Chang, H.W., Egberink, H., Halpin, R., Spiro, D.J., Rottier, P.J., 2012. Spike protein fusion peptide and feline coronavirus virulence. Emerging Infectious Diseases 18, 1089–1095.

Court, M.H., Greenblatt, D.J., 2000. Molecular genetic basis for deficient acetaminophen glucuronidation by cats: UGT1A6 is a pseudogene, and evidence for reduced diversity of expressed hepatic UGT1A isoforms. Pharmacogenetics 10, 355–369.

Declercq, J., De Bosschere, H., Schwarzkopf, I., Declercq, L., 2008. Papular cutaneous lesions in a cat associated with feline infectious peritonitis. Veterinary Dermatology 19, 255–258.

Drechsler, Y., Alcaraz, A., Bossong, F.J., Collisson, E.W., Diniz, P.P., 2011. Feline coronavirus in multicat environments. The Veterinary Clinics of North America. Small Animal Practice 41, 1133–1169.

Dye, C., Helps, C.R., Siddell, S.G., 2008. Evaluation of real-time RT-PCR for the quantification of FCoV shedding in the faeces of domestic cats. Journal of Feline Medicine and Surgery 10, 167–174.

Fischer, Y., Ritz, S., Weber, K., Sauter-Louis, C., Hartmann, K., 2011. Randomized, placebo controlled study of the effect of propentofylline on survival time and quality of life of cats with feline infectious peritonitis. Journal of Veterinary Internal Medicine 25, 1270–1276.

Fischer, Y., Sauter-Louis, C., Hartmann, K., 2012. Diagnostic accuracy of the Rivalta test for feline infectious peritonitis. Veterinary Clinical Pathology 41, 558–567.

Fischer, Y., Weber, K., Sauter-Louis, C., Hartmann, K., 2013. The Rivalta's test as a diagnostic variable in feline effusions – evaluation of optimum reaction and storage conditions. Tierärztliche Praxis 41, 297–303.

Foley, J.E., Poland, A., Carlson, J., Pedersen, N.C., 1997. Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. Journal of the American Veterinary Medical Association 210, 1313–1318.

Gaffney, P.M., Kennedy, M., Terio, K., Gardner, I., Lothamer, C., Coleman, K., Munson, L., 2012. Detection of feline coronavirus in cheetah (*Acinonyx jubatus*) feces by reverse transcription-nested polymerase chain reaction in cheetahs with variable frequency of viral shedding. Journal of Zoo and Wildlife Medicine 43, 776–786.

Gamble, D.A., Lobbiani, A., Gramegna, M., Moore, L.E., Colucci, G., 1997. Development of a nested PCR assay for detection of feline infectious peritonitis virus in clinical specimens. Journal of Clinical Microbiology 35, 673–675.

Giori, L., Giordano, A., Giudice, C., Grieco, V., Paltrinieri, S., 2011. Performances of different diagnostic tests for feline infectious peritonitis in challenging clinical cases. The Journal of Small Animal Practice 52, 152–157.

Hartmann, K., Ritz, S., 2008. Treatment of cats with feline infectious peritonitis. Veterinary Immunology and Immunopathology 123, 172–175.

Hartmann, K., Binder, C., Hirschberger, J., Cole, D., Reinacher, M., Schroo, S., Frost, J., Egberink, H., Lutz, H., Hermanns, W., 2003. Comparison of different tests to diagnose feline infectious peritonitis. Journal of Veterinary Internal Medicine 17, 781–790.

Herrewegh, A.A., de Groot, R.J., Cepica, A., Egberink, H.F., Horzinek, M.C., Rottier, P.J., 1995. Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. Journal of Clinical Microbiology 33, 684–689.

Hornyák, A., Bálint, A., Farsang, A., 2012. Subgenomic mRNA of feline coronavirus by real time polymerase chain reaction based on primer-probe energy transfer (P-sg-QPCR). Journal of Virological Methods 181, 155–163.

Hseih, L.E., Lin, C.N., Su, B.L., Jan, T.R., Chen, C.M., Wang, C.H., Lin, D.S., Lin, C.T., Chueh, L.L., 2010. Synergistic antiviral effect of *Galanthus nivalis* agglutinin and nelfinavir against feline coronavirus. Antiviral Research 88, 25–30.

Ives, E.J., Vanhaesebrouck, A.E., Cian, F., 2013. Immunocytochemical demonstration of feline infectious peritonitis virus within cerebrospinal fluid macrophages. Journal of Feline Medicine and Surgery 15, 1149–1153.

Jeffery, U., Deitz, K., Hostetter, S., 2012. Positive predictive value of albumin:globulin ratio for feline infectious peritonitis in a mid-western referral hospital population. Journal of Feline Medicine and Surgery 14, 903–905.

Kennedy, M.A., Abd-Eldaim, M., Zika, S.E., Mankin, J.M., Kania, S.A., 2008. Evaluation of antibodies against feline coronavirus 7b protein for diagnosis of feline infectious peritonitis in cats. American Journal of Veterinary Research 69, 1179–1182.

Kim, Y., Mandadapu, S.R., Groutas, W.C., Chang, K.O., 2013. Potent inhibition of feline coronaviruses with peptidyl compounds targeting coronavirus 3C-like protease. Antiviral Research 97, 161–168.

Kipar, A., Meli, M.L., Baptiste, K.E., Bowker, L.J., Lutz, H., 2010. Sites of feline coronavirus persistence in healthy cats. The Journal of General Virology 91, 1698–1707.

Legendre, A.M., Bartges, J.W., 2009. Effect of polyprenyl immunostimulant on the survival times of three cats with the dry form of feline infectious peritonitis. Journal of Feline Medicine and Surgery 11, 624–626.

Lewis, K.M., O'Brien, R.T., 2010. Abdominal ultrasonographic findings associated with feline infectious peritonitis: A retrospective review of 16 cases. Journal of the American Animal Hospital Association 46, 152–160.

Li, X., Scott, F.W., 1994. Detection of feline coronaviruses in cell cultures and in fresh and fixed feline tissues using polymerase chain reaction. Veterinary Microbiology 42, 65–77.

Licitra, B.N., Millet, J.K., Regan, A.D., Hamilton, B.S., Rinaldi, V.D., Duhamel, G.E., Whittaker, G.R., 2013. Mutation in spike protein cleavage site and pathogenesis of feline coronavirus. Emerging Infectious Diseases 19, 1066–1073.

Litster, A.L., Pogranichniy, R., Lin, T.L., 2013. Diagnostic utility of a direct immunofluorescence test to detect feline coronavirus antigen in macrophages in effusive feline infectious peritonitis. The Veterinary Journal 198, 362–366.

Liu, I.J., Tsai, W.T., Hsieh, L.E., Chueh, L.L., 2013. Peptides corresponding to the predicted heptad repeat 2 domain of the feline coronavirus spike protein are potent inhibitors of viral infection. PLoS ONE 8, e82081.

Marioni-Henry, K., 2010. Feline spinal cord diseases. The Veterinary Clinics of North America. Small Animal Practice 40, 1011–1028.

Meli, M.L., Burr, P., Decaro, N., Graham, E., Jarrett, O., Lutz, H., McDonald, M., Addie, D.D., 2013. Samples with high virus load cause a trend toward lower signal in feline coronavirus antibody tests. Journal of Feline Medicine and Surgery 15, 295–299.

Michimae, Y., Mikami, S., Okimoto, K., Toyosawa, K., Matsumoto, I., Kouchi, M., Koujitani, T., Inoue, T., Seki, T., 2010. The first case of feline infectious peritonitis-like pyogranuloma in a ferret infected by coronavirus in Japan. Journal of Toxicologic Pathology 23, 99–101.

Murray, J., Kiupel, M., Maes, R.K., 2010. Ferret coronavirus-associated diseases. The Veterinary Clinics of North America. Exotic Animal Practice 13, 543–560.

Paltrinieri, S., Giordano, A., Tranquillo, V., Guazzetti, S., 2007. Critical assessment of the diagnostic value of feline α_1-acid glycoprotein for feline infectious peritonitis using the likelihood ratios approach. Journal of Veterinary Diagnostic Investigation 19, 266–272.

Paltrinieri, S., Marchini, I., Gelain, M.E., 2012. Flow cytometric detection of alpha-1-acid glycoprotein on feline circulating leucocytes. Australian Veterinary Journal 90, 291–296.

Pearks Wilkerson, A.J., Teeling, E.C., Troyer, J.L., Kahila Bar-Gal, G., Roelke, M., Marker, L., Pecon-Slattery, J., O'Brien, S.J., 2004. Coronavirus outbreak in cheetahs: Lessons for SARS. Current Biology 14, R227–R228.

Pedersen, N.C., 2009. A review of feline infectious peritonitis virus infection: 1963–2008. Journal of Feline Medicine and Surgery 11, 225–258.

Pedersen, N.C., Black, J.W., 1983. Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus. American Journal of Veterinary Research 44, 229–234.

Pedersen, N.C., Sato, R., Foley, J.E., Poland, A.M., 2004. Common virus infections in cats, before and after being placed in shelters, with emphasis on feline enteric coronavirus. Journal of Feline Medicine and Surgery 6, 83–88.

Pedersen, N.C., Allen, C.E., Lyons, L.A., 2008. Pathogenesis of feline enteric coronavirus infection. Journal of Feline Medicine and Surgery 10, 529–541.

Pedersen, N.C., Liu, H., Dodd, K.A., Pesavento, P.A., 2009. Significance of coronavirus mutants in feces and diseased tissues of cats suffering from feline infectious peritonitis. Viruses 1, 166–184.

Pedersen, N.C., Liu, H., Scarlett, J., Leutenegger, C.M., Golovko, L., Kennedy, H., Kamal, F.M., 2012. Feline infectious peritonitis: Role of the feline coronavirus 3c gene in intestinal tropism and pathogenicity based upon isolates from resident and adopted shelter cats. Virus Research 165, 17–28.

Pfefferle, S., Schöpf, J., Kögl, M., Friedel, C.C., Müller, M.A., Carbajo-Lozoya, J., Stellberger, T., von Dall'Armi, E., Herzog, P., Kallies, S., et al., 2011. The SARS-coronavirus-host interactome: Identification of cyclophilins as target for pan- coronavirus inhibitors. PLoS Pathogens 10, e1002331.

Porter, E., Tasker, S., Day, M.J., Harley, R., Kipar, A., Siddell, S.G., Helps, C.R., 2014. Amino acid changes in the spike protein of feline coronavirus correlate with systemic spread of virus from the intestine and not with feline infectious peritonitis. Veterinary Research 45, 49 [Epub ahead of print].

Provacia, L.B., Smits, S.L., Martina, B.E., Raj, V.S., Doel, P.V., Amerongen, G.V., Moorman-Roest, H., Osterhaus, A.D.M.E., Haagmans, B.L., 2011. Enteric coronavirus in ferrets, The Netherlands. Emerging Infectious Diseases 17, 1570–1571.

Raaben, M., Posthuma, C.C., Verheije, M.H., Te Lintelo, E.G., Kikkert, M., Drijfhout, J.W., Snijder, E.J., Rottier, P.J., de Haan, C.A., 2010. The ubiquitin-proteasome system plays an important role during various stages of the coronavirus infection cycle. Journal of Virology 84, 7869–7879.

Sabshin, S.J., Levy, J.K., Tupler, T., Tucker, S.J., Greiner, E.C., Leutenegger, C.M., 2012. Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. Journal of the American Veterinary Medical Association 241, 331–337.

Schneider, M., Ackermann, K., Stuart, M., Wex, C., Protzer, U., Schätzl, H.M., Gilch, S., 2012. Severe acute respiratory syndrome coronavirus replication is severely impaired by MG132 due to proteasome-independent inhibition of M-calpain. Journal of Virology 86, 10112–10122.

Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A., Alazawy, A., 2010. Diagnostic methods for feline coronavirus: A review. Veterinary Medicine International 2010, pii: 809480.

Simons, F.A., Vennema, H., Rofina, J.E., Pol, J.M., Horzinek, M.C., Rottier, P.J., Egberink, H.F., 2005. A mRNA PCR for the diagnosis of feline infectious peritonitis. Journal of Virological Methods 124, 111–116.

Soma, T., Wada, M., Taharaguchi, S., Tajima, T., 2013. Detection of ascitic feline coronavirus RNA from clinically suspected cats of feline infectious peritonitis. The Journal of Veterinary Medical Science 75, 1389–1392.

Stephenson, N., Swift, P., Moeller, R.B., Worth, S.J., Foley, J., 2013. Feline infectious peritonitis in a mountain lion (*Puma concolor*), California, USA. Journal of Wildlife Diseases 49, 408–412.

Takano, T., Ishihara, Y., Matsuoka, M., Yokota, S., Matsuoka-Kobayashi, Y., Doki, T., Hohdatsu, T., 2013a. Use of recombinant nucleocapsid proteins for serological diagnosis of feline coronavirus infection by three immunochromatographic tests. Journal of Virological Methods 196, 1–6.

Takano, T., Katoh, Y., Doki, T., Hohdatsu, T., 2013b. Effect of chloroquine on feline infectious peritonitis virus infection in vitro and in vivo. Antiviral Research 99, 100–107.

Tanaka, Y., Sato, Y., Sasaki, T., 2013. Suppression of coronavirus replication by cyclophilin inhibitors. Viruses 5, 1250–1260.

Taylor, S.S., Tappin, S.W., Dodkin, S.J., Papasouliotis, K., Casamian-Sorrosal, D., Tasker, S., 2010. Serum protein electrophoresis in 155 cats. Journal of Feline Medicine and Surgery 12, 643–653.

Terada, Y., Minami, S., Noguchi, K., Mahmoud, H.Y., Shimoda, H., Mochizuki, M., Une, Y., Maeda, K., 2014. Genetic characterization of coronaviruses from domestic ferrets, Japan. Emerging Infectious Diseases 20, 284–287.

Vogel, L., Van der Lubben, M., Te Lintelo, E.G., Bekker, C.P.J., Geerts, T., Schuijff, L.S., Grinwis, G.C.M., Egberink, H.F., Rottier, P.J.M., 2010. Pathogenic characteristics of persistent feline enteric coronavirus infection in cats. Veterinary Research 41, 71.

Wang, Y.T., Su, B.L., Hsieh, L.E., Chueh, L.L., 2013. An outbreak of feline infectious peritonitis in a Taiwanese shelter: Epidemiologic and molecular evidence for horizontal transmission of a novel type II feline coronavirus. Veterinary Research 44, 57.

Wiggans, K.T., Vernau, W., Lappin, M.R., Thomasy, S.M., Maggs, D.J., 2013. Diagnostic utility of aqueocentesis and aqueous humor analysis in dogs and cats with anterior uveitis. Veterinary Ophthalmology 17, 212–220.

Wise, A.G., Kiupel, M., Garner, M.M., Clark, A.K., Maes, R.K., 2010. Comparative sequence analysis of the distal one-third of the genomes of a systemic and an enteric ferret coronavirus. Virus Research 149, 42–50.

Zoia, A., Slater, L.A., Heller, J., Connolly, D.J., Church, D.B., 2009. A new approach to pleural effusion in cats: Markers for distinguishing transudates from exudates. Journal of Feline Medicine and Surgery 11, 847–855.