Lucy Has Some ‘Splaining to Do

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History and Presentation

Lucy was a 6-month-old female spayed domestic short hair that presented to Mississippi State University College of Veterinary Medicine Community Veterinary Services (MSU-CVM-CVS) on October 22, 2019. She had a history of inability to gain weight since her recent adoption, lethargy, vomiting, and anorexia in the two to three weeks prior. She was brought to MSU-CVM-CVS due to an inability to stand when placed on her feet and upon admittance, Lucy was laterally recumbent and showing signs of respiratory distress. A cursory physical exam was performed, and Lucy had bilateral nystagmus and pale, icteric mucus membranes. A blood glucose test indicated hypoglycemia (the lowest reading was 30 mg/dL) and Lucy was placed on a dextrose constant rate infusion in an attempt to stabilize her. An abdominal focused assessment with sonography for trauma showed free fluid in the abdomen. She was dehydrated and was give fluids intravenously. A complete blood count showed decreased packed cell volume, decreased platelet count, neutrophilia and lymphopenia. A biochemistry panel showed a hyperbilirubinemia, an albumin of 1.0, a mild increase in alanine transaminase (ALT), and electrolyte imbalances. Feline infectious peritonitis was suspected and euthanasia via barbiturates was elected due to her poor prognosis.

Introduction

In the large majority of the feline population, coronaviruses are endemic, with approximately 40% of cats worldwide having been exposed and up to 90% exposure in high density populations.[7] Feline coronavirus (FCoV) causes mild to moderate enteritis and fever in kittens and can be asymptomatic in adult cats.[4,7] Infection is usually transient and cleared by two weeks (70%), but up to 13% of cats may become persistently infected and chronically shed the virus in their feces.[7] In 5-10% of cats infected with FCoV, the virus mutates and becomes more

virulent.[7,8] There is a change in the viral tropism, causing the virus to switch from replicating in the enterocytes to macrophages/monocytes thereby allowing systemic spread.[4,7,8]

This change in tropism does not always result in the development of feline infectious peritonitis (FIP). The pathogenesis of FIP is the result of an inappropriate immune response to the mutated virus. There are two main hypersensitivity reactions seen in FIP, Type III and Type IV. Type III hypersensitivity reactions form immune complexes between the host's antibodies and the antigens. The immune complexes can become lodged in vessel walls which incites a local inflammatory response leading to vasculitis and vascular leakage. This response results in the effusive (or wet) form of FIP. Type IV hypersensitivity reactions occur when macrophages infiltrate, causing local and chronic inflammation.[8] This type of inflammation occupies space and can result in organ dysfunction and is the cause of the non-effusive (or dry) form of FIP.[3] As with many immune-mediated diseases, the pathogenesis likely involves a combination of hypersensitivity responses, dependent on the host immune system.

Predilections

Male cats and cats under 2 years old are at higher risk of developing FIP.[3,7] There is a small bimodal increase in risk for older cats due to a higher total exposure to FCoV over their lifetime. Breed predispositions include Abyssinian, Bengal, Birman, Ragdoll, and Rex breeds.[3,7] This varies dependent on region, suggesting an inherited genetic component in familial lines rather than entire breeds.[3,4,7]

The environment may have an effect on the risk of a cat developing FIP. Cats that develop FIP typically have some recent history of stress such as a surgery, adoption, or sickness. Stress causes immunosuppression, leading to an increase in viral replication and subsequently increased risk of viral mutation.[7] Cats from high density populations or multi-cat households are

at increased and repeated risk of exposure to FCoV thus increasing the risk of an improper immune response and infection with the more virulent form of FCoV.[4]

Pathophysiology

FCoV is grouped into two serotypes based on the spike (S) protein and its behavior.[3,8] Serotype 1 is much more prevalent and is the cause of 80-90% of naturally occurring cases of FCoV.[3,4,7,8] The second serotype of FCoV is a result of homologous recombination between the feline coronavirus and the canine alphacoronavirus, with the spike protein from the canine virus.[3,7,8] Each serotype uses different host cell receptor for entry, with Type 1 still unknown and Type 2 using feline aminopeptidase N (APN) as its target receptor. APN can be found in the brush border of small intestines, the renal microvilli, and myeloid origin cells such as macrophages.[3,7] Both serotypes can cause FIP.[3,8]

FCoV can be further divided into two biotypes: the feline enteric coronavirus (FeCV) and feline infectious peritonitis virus (FIPV).[3,8] Although the viral genes responsible for biotype conversion are not completely understood, several candidate genes have been identified. FeCV enters via the apical epithelial cells of the enterocytes from the distal duodenum to the cecum. The virus is transmitted fecal-orally, and infection is generally controlled by mucosal IgA and systemic IgG antibodies.[3] Occasionally, once the enterocytes infected the virus spreads to macrophages, allowing the systemic spread and the potential development of FIP.[3,4,8]

There are many speculations as to why FeCV changes biotypes to become FIPV. However, FeCV has a moderate to high rate of mutation despite its proofreading capabilities, leading to difficulties finding a mutation responsible for the change.[3,4,7] Mutations in the S protein have been found in the fusion peptide sequence and the furin cleavage motif. Both sites are closely related, and a mutation to the S protein may allow systemic spread of the virus as

opposed to enterocyte cell tropism. Systemic spread of S protein mutated FCoV has also been found in non-FIP FCoV infected cats and therefore is not necessarily the primary pathology that causes FIP.[4,7] A deletional mutation in the 3c protein, an accessory protein, causes the virus to lose the ability to replicate in the intestinal epithelial cells and instead allows the virus to enter macrophages.[4] Mutations of the S protein and 3c protein are often found together, but mutation of either protein allows FCoV to spread systemically. Other mutations of the membrane protein have been found but are of unknown importance.[4] Varied mutations in analyzed cases indicate no one mutation is identifiable as the sole cause of FIP.[2]

FIP has two major forms as previously mentioned: effusive and non-effusive. Both forms of the disease are nearly 100% fatal and cause non-specific signs early in the course of the disease. Common clinical signs include anorexia, lethargy, weight loss or failure to gain weight in kittens, and a waxing and waning fever that is not responsive to medications.[3,7]

The wet form is seen in up to 80% of FIP cases. Cats will develop effusion most commonly in the abdominal cavity. Effusion can also form in the thorax, the pericardium, and rarely, the scrotum. Clinical signs may result dependent on location.[3,7] The fluid is a modified transudate that is typically transparent, viscous, straw-yellow to serosanguinous, and protein rich.[4,7] It is considered a modified transudate due to its poor cellularity but acts as an exudate due to its highly proteinaceous makeup. The total protein concentration of the fluid is >35 g/L with >50% of the proteins being immunoglobulins. The low number of cells present in the fluid show a pyogranulomatous mixture – large amounts of macrophages, small to moderate numbers of neutrophils, and only a small fraction of lymphocytes.[7] Lesions in the wet form are consistent with a pyogranulomatous serositis and pleuritis with aggregates of macrophages, mild infiltrates of neutrophils, and less commonly lymphocytes. Damage is focused around small capillaries

where immune complexes are readily caught and bound in the vessel walls, such as the omentum and the serosal surfaces of organs.[3,7]

The non-effusive form has a more insidious onset and may only show clinical signs late in the disease process. Pyogranulomatous inflammation can affect a variety of organ systems, with clinical signs often dependent on the organ system affected.[3,4,7] Generally, the pyogranulomas that define the non-effusive form of FIP can be of variable size and may extend from the serosal surface of an organ into the parenchyma. Serosal lesions are perivascular aggregates of macrophages with small numbers of neutrophils, similar to the wet form. However, the lesions are surrounded by B lymphocytes and plasma cells that can extend into the surrounding tissue, differentiating it histopathologically.[3] Focal pyogranulomatous masses can be in many locations, most commonly the mesenteric lymph nodes and surrounding tissues.[5,7] Ocular or neurologic signs are possible with infection of the associated organ systems. Due to the chronicity of the dry form disease process, neurologic FIP may be difficult to diagnose until late stage. Neurologic forms carry a grave prognosis due the damage done to the central nervous tissues by pyogranulomas. Renomegaly along with renal disease signs may occur if kidney function is impaired. More rare presentations include a dermatological form causing multiple small, non-pruritic papules or nodules, and a diffuse pyogranulomatous pneumonia.[7]

Diagnostics

FIP can be a frustrating disease to diagnose due to the confusing and sometimes unrewarding nature of FIP and FCoV tests - no one specific test is pathognomonic for FIP. Instead, clinicians must focus on the signalment, clinical signs, and various test results to build a diagnosis.[4]

On a complete blood count (CBC), FIP cats can have a non-specific leukocytosis, neutrophilia with a left shift, or normocytic, normochromic anemia.[7] A more reliable parameter is lymphopenia, which can be seen in 55-77% of FIP cases. It is speculated that the FIPV effect on macrophages causes them to produce cytokines that results in lymphocyte apoptosis.[4,7] A biochemistry panel may be more helpful than a CBC in building a FIP diagnosis. In cases with the dry form, values may vary depending on what organ system is affected. A hyperbilirubinemia may be seen in up to 63% of cases without liver function impairment.[4,7] This is due to accumulation hemoglobin from microhemorrhages and red blood cell lysis from irregular vasculature instead of liver disease.[4] Acute phase protein production is upregulated during FIP infections and while interlukin-1, interlukin-6, and TNF-∂ are important mediators in the disease, they are nonspecific and not helpful in diagnosis.[4,7] Another acute phase protein, ∂ 1-acid glycoprotein (AAG) is often markedly increased, typically >1.5 mg/ml, and can make a good diagnostic standard.[7] AAG elevation is not specific for FIP but has a stronger positive predictive value compared to other acute phase proteins. Hyperglobunemia is seen in as many as 89% of cases, which is often paired with a low-normal or hypoalbunemia in approximately 65% of cases.[7] The increase in immunoglobulins will cause a decrease of albumin to globulin (A:G) ratio.[4,7] With a high index of suspicion, an A:G ratio of <0.4, it is very likely to be FIP. Vice versa, if a case has low suspicion due to lack of clinical signs and low prevalence, an A:G ratio of >0.8 is very unlikely to be FIP.[7]

If effusion is present, it can be evaluated to help diagnose FIP. The fluid can be used similarly to blood to look at the acute phase protein AAG and the A:G ratio with the same diagnostic ranges. Due to high protein levels and a quasi-exudative nature of the fluid, a Rivalta test can be used to confirm the effusion's quality.[7] The Rivalta test is an easy, quick, and

inexpensive way to qualify effusion, making it an important preliminary test for ruling *out* FIP due to the high negative predictive value.

Antibody tests are available, but they cannot distinguish between FCoV and FIP and only demonstrate exposure to FCoV with subsequent seroconversion.[4,7] While FIP infected cats may have high titers, there is a large overlap between non-FIP FCoV and FIP infected cats, making titers on an individual undiagnostic.[7] There are approximately 10% of FIP infected cats that also are seronegative on antibody tests, further confounding antibody test results.[4,7] An exception is using an antibody test in a neurologic form of FIP and if suspected, cerebrospinal fluid (CSF) titers of 640 or greater are indicative of FIP.[4]

Antigen PCR tests also generally have difficulty differentiating the two biotypes of FCoV due to the same genomic code. PCRs used to test for FIP attempt to use a specific mutation as the testing point to help differentiate between FeCV and FIPV such as the S protein mutations and the 7b ORF gene mutation. A variety of samples can be collected and used for PCRs, such as affected tissue, blood, effusion, CSF, aqueous humor, and feces. Feces is used to identify cats that are currently shedding FCoV and is otherwise unhelpful in the diagnosis of FIP.[7] PCR can be run on a CSF sample if the neurologic form is suspected but should not be used in other forms as it may miss systemic disease that has not progressed to a neurologic stage.[7] S protein PCR is best used on effusive fluids rather than blood samples.[2,65] The test has a 100% specificity, but a 6.5% sensitivity for serum or plasma samples versus a 65% sensitivity for effusive fluid samples.[2,4] The S protein rt-PCR is specific to Type 1 FCoV FIP and therefore may miss a Type 2 infection. The 7b gene PCR test is good for intrabdominal organ samples such as mesenteric lymph nodes, liver, spleen, kidneys, and omentum.[2]

Less common testing methods for FIP include messenger RNA quantification and serum protein electrophoresis (SPE).[4,7] Messenger RNA quantification uses the M protein of FCoV as its testing marker and the amount found directly correlates to the degree of viral replication. Since FeCV only replicates in enterocytes, presence of the M protein in high quantities in the samples (blood, effusion, non-enterocyte containing tissue samples) are indicative of the FIPV.[4] SPE is historically a testing method for FIP but it is no longer commonly used and shows polyclonal elevations.[4,7]

Histopathology is the gold standard for diagnosing FIP and the only confirmatory test for FIP is histopathology with immunostaining of the antigen in macrophages.[3,7] Immunohistochemistry can be used on affected tissue or effusive fluid. Effusion has a higher rate of false negatives due to the low cellularity properties and macrophages can be missed.[7] Histopathology reveals lesions previously mentioned above. Antemortem samples can be collected via ultrasound guided percutaneous needle-core biopsy, laparoscopy, or exploratory laparotomy.[7] These biopsy collection methods have varying degrees of invasiveness and a cat with FIP may not be stable enough to undergo more invasive surgeries. Due to the severity and progressive nature of FIP, most tissue samples are collected postmortem during necropsies and should be used to confirm the diagnosis of FIP.[4,7]

Treatment/Management Options

Treatment of FIP is focused on two main mechanisms: immune system modulation and decrease in viral replication. Corticosteroids have been used as a palliative treatment historically but do not necessarily change the outcome of the disease. Cytokines, such as feline and human recombinant interferon have been used but are not effective. Polyprenyl immunostimulant has

been used to stimulate the cell-mediated immune response to FIP and has some success as treatment in the dry form but has not been proven effective in the wet form of FIP.[4]

There is no commercially available antiviral drug labeled for use in treatment of FIP.[4,5,6,7] An antiviral currently being studied is GS44152. It is a 1'cyano-substituted adenine C-nucleoside ribose analogue which inhibits RNA synthesis.[1] GS44152 is a small molecule drug - it can easily enter cells and interact with the target molecules, directly interfering with the replication process of the virus. In the study by Dr. N.C. Pedersen and associates, naturally infected cats with non-neurologic forms of FIP were treated for a minimum of 12 weeks with 2 mg/kg subcutaneously every 24 hours. Cats that relapsed after the initial course went through a second course with an increased dose of 4 mg/kg SQ q24 for 12 weeks. Out of 26 cats, 18 needed only one course of treatment, five cats relapsed after the first course and underwent a second with a higher dose, and 2 cats had a second relapse but responded well to a third course of 4 mg/kg SQ q24 for 12 weeks. At the conclusion of the study, 25 out of 26 cats were long-term survivors with disease remission.[6] The most notable side effect of the majority of cats studied was injection site discomfort and skin reactions.[1,6] One cat had possible toxicity from the drug, with an elevation in blood urea nitrogen and symmetric dimethylarginine assay after week 8 of the second course of treatment with an increased dose. Treatment was immediately stopped, and values normalized after one month. The cat was disease free at the time of publication. Overall, the study showed that GS44152 was well tolerated by the cats and had promising results.[6]

FIP is a disease process where host-mediated immune responses, specifically systemic antibodies, can be much more harmful than helpful. Conventional methods of vaccination develop host immunocompetency to an antigen by priming the immune system and causing

production of antibodies to the antigen. In the case of FIP, conventional methods would not only be unsuccessful, but could propagate the undesired immune response. The paradoxical nature of a FIP vaccine would be to find a way to prevent viral infection yet also prevent enhancing the systemic immune response. Intranasal vaccines can promote mucosal IgA recognition and response to FCoV locally without triggering a systemic cascade.[8] A modified live intranasal vaccine using a temperature sensitive mutant of FCoV strain DF2-FIPV has been developed and shown to replicate in only the upper respiratory tract.[8,9] Unfortunately, this vaccine must be given before any exposure to the FCoV, which can be nearly impossible in endemic and high-density populations. As this vaccine is best administered after 16 weeks of age due to maternal antibody interference, the high likelihood of prior exposure decreases its utility.[8] Therefore, this vaccine is not recommended by the American Association of Feline Practitioners.[8,9] Attempts to vaccinate cats with an unattenuated field isolate of the canine coronavirus were unsuccessful.[8]

Case Outcome

Lucy was submitted for necropsy to confirm the diagnosis of FIP. She showed classic signs of FIP both ante- and postmortem. She had the clinical signs of fever, weight loss, lethargy, vomiting, anemia, icterus, respiratory, and neurologic signs at presentation which are consistent with the diagnosis. On necropsy, both forms of FIP were present: pyogranulomatous masses in the abdomen, an obstructive focal mass at the ileocolonic junction, lymphadenopathy of the mesenteric lymph nodes, proteinaceous effusion in the abdomen, thorax, and pericardium, and diffuse serositis and vasculitis.

Conclusion

Although a majority of cats are exposed to FCoV and have no to minimal disease, the 5-10% with the mutation to FIP have been a fervent topic of research for scientists and

veterinarians. The pathophysiology of the disease has yet to be elucidated and will likely be multi-factorial involving mutation and the host immune response. The promise of new antiviral medications on the horizon give hope for this fatal disease.

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