

WINN FIP SYMPOSIUM

PURRsuig FIP and WINNing



NOVEMBER 16 & 17, 2019

UNIVERSITY OF CALIFORNIA AT DAVIS, CALIFORNIA



Winn Feline Foundation has a long and outstanding tradition of bringing together the top experts in feline medical research for educational events that cover the most important and relevant information on the topic. This condensed summary from Winn's November 2019 symposium, PURRsuig FIP and WINNing, will help cat advocates everywhere understand that feline infectious peritonitis (**FIP**) is now a **treatable disease**.



FELINE CORONAVIRUS

Feline coronaviruses (FCoV) occur in two biotypes: feline enteric coronavirus (FECV), and feline infectious peritonitis virus (FIPV), which causes feline infectious peritonitis (FIP). FIP appears to be a relatively recent disease of cats—it was initially described in the late 1950s by Dr. Jean Holzworth at Angell Memorial Animal Center in Boston. Have coronaviruses only recently emerged in cats from another species? Did an earlier feline coronavirus mutate to more easily cause FIP? Is FIP a consequence of the post-World War II increase in keeping cats as household pets and the emergence of shelters, rescue groups, and catteries?

FELINE ENTERIC CORONAVIRUS (FECV)

Epizootiology and Pathogenesis¹

- FECV is an enveloped, single-stranded RNA virus that is associated with asymptomatic persistent enteric infections.
- It is found worldwide and is ubiquitous in domestic and wild Felidae.
- There are two serotypes: 80% to 95% are Serotype I (cat-like); 5% to 20% are Serotype II (dog-like).
- Serotype II has a higher incidence in Asia than in North America and Europe.
- Clinically inapparent infection of kittens can start after weaning (nine to ten weeks of age).
- Virus shedding in feces can persist for many weeks, months, or longer.
- Fecal-oral transmission is facilitated by shared litter boxes.
- The virus displays tropism for the mature apical epithelium of the lower intestine.
- Immunity develops slowly.
- Immunity is lost after shedding ceases, and recurrent infections are common.



FIP is not a rare form of FECV infection. It's not just that most cats get nothing and a few cats, because of some peculiarity, get FIP. That's what was thought for a long time. But when we finally were able to separate the two diseases, we realized they're different viruses. FIP viruses cause FIP, FECV causes enteric infections.

—Dr. Niels Pedersen

Passive Systemic and Local Immunity²

- Kittens can make IgM antibody and mount cell-mediated immune responses from birth.
- IgG and IgA antibodies are absorbed from colostrum during first few hours of life.
- Passive systemic immunity results from IgG and IgA from colostrum.
- Passive local immunity is derived from IgG and IgA antibodies in the milk.
- Passive systemic and local immunity protect kittens until their immune system matures.
- IgG and IgA production does not start until passive antibody protection is gone (six to eight weeks of age).
- Kittens' immune systems do not reach adult levels until 12 to 16 weeks of age.



Immunity to FECV Infection²

- Kittens born to FECV-exposed queens will have maternal immunity until nine weeks of age.
- Primary infection will evoke both a systemic and a local antibody response.
- IgA goes from blood transmitted across the intestinal epithelium into the mucous.
- There is no evidence of cell-mediated immunity or any changes in lymphocyte populations.
- The IgA antibody response slowly leads to cessation of virus shedding.
- Antibody titers wane as virus shedding ceases, and cats become susceptible to reinfection.
- Immunity to FECV infection is transient and recurrent infections are common.

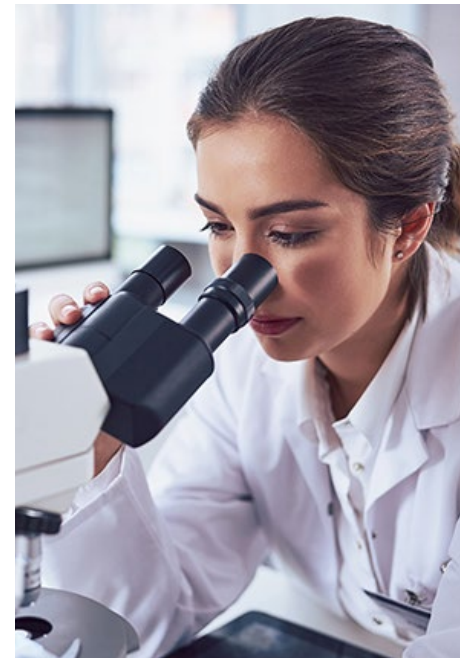
A pretty remarkable protection results from passive maternal antibody. That shows something about what can protect cats against FECV.

—Dr. Gregg Dean

FELINE INFECTIOUS PERITONITIS VIRUS (FIPV)

Origins of FIPV¹

- FIPV arises from FECV infection by mutation.
- FIPVs occur in regional lymphoid tissues of the lower intestine in 10% of FECV-infected cats.
- Mutations result from positive selection pressures favoring replication in macrophages.
- FIPVs acquire tropism for peritoneal macrophages and lose tropism for intestinal epithelium.
- Tropism change results from mutations in Spike (S, S1/S2) and accessory 3c genes.
- FIPV mutation is unique to each cat.
- FIPVs are strictly cell-associated, and local and systemic spread is in monocytes/macrophages.
- FIPV is confined to infected macrophages, which are not spread from cat to cat.
- There are rare cases of cat-to-cat spread (epidemic form) by Serotype II FIPVs.



FELINE INFECTIOUS PERITONITIS (FIP)

FIP Epizootiology^{1,3}

- FECV infection occurs in virtually all cats and kittens.
- FECV-to-FIPV biotype conversion occurs in 11% of cats.
- Only 1:10 to 1:30 cats with mutant viruses develop FIP.
- Worldwide, feline mortality among all cats due to FIP is thought to be 0.3% to 1.3%.
- FIP favors multi-cat environments—catteries, foster homes, rescue groups, shelters, and cats living in dense urban areas. If there is additional stress in these environments, the odds of FIP occurring increases.
- 95% of cases occur in cats less than seven years of age, 70% in cats less than 1.5 years of age, 50% in cats less than seven months of age.
- Pedigreed cats have three times greater incidence of FIP than random-bred cats.
- Males are slightly more susceptible.



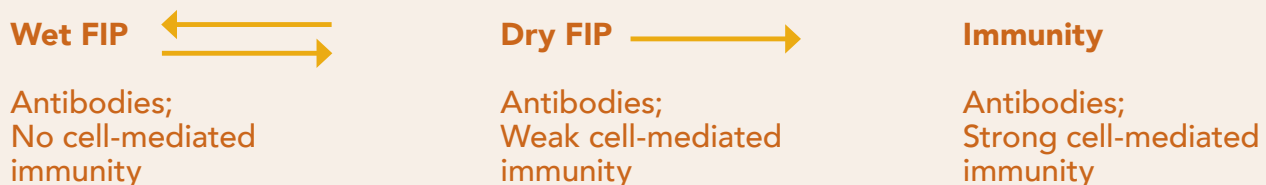
FIP Pathogenesis

- Macrophages are infected by immune complex virus through their Fc receptors.
- FIP is typical of other macrophage infections, such as tuberculosis, leprosy, and deep mycoses.
- FIP is mediated by cytokine responses of infected macrophages.
- Th1 (cell-mediated) cytokine responses are protective.
- Protective immunity is innate at onset and then becomes adaptive (active).
- Failure to establish protective immunity leads to a Th2 (inflammatory) response and disease.
- Affected cats have delayed apoptosis of infected macrophages, allowing for increased virus production.
- The incubation period from first replication in macrophages to clinical disease is days to months.
- The actual disease course varies from days to months and, rarely, a year or more.
- Once typical disease signs appear, historically, fewer than 5% of affected cats have survived to one year.

Two Disease Forms of FIP

- There are two major disease forms: effusive (wet, non-granulomatous, non-parenchymatous) and non-effusive (dry, granulomatous, parenchymatous).
- Wet and dry FIP resemble the lepromatous and tuberculoid forms of human leprosy, respectively.
- The two forms of FIP are dependent on the relative balance of cellular (Th1) and humoral (Th2) immune responses.
 - Wet FIP is characterized by a predominance of immediate hypersensitivity reactions (vasculitis) and high levels of virus replication in macrophages.
 - Dry FIP is characterized by a predominance of delayed hypersensitivity reactions and low levels of virus replication in macrophages.
- The disease form in a cat may change from wet FIP to dry FIP, or dry to wet, during the course of the illness.

What determines form of FIP?





DIAGNOSING FIP^{4,5}

In most cases a diagnosis of FIP can confidently be made by obtaining a complete history and performing a thorough exam of the patient, then using the following diagnostic tools to build a “diagnostic wall,” brick by brick. Various test results consistent with FIP can provide strong evidence for the diagnosis.

Signs and Symptoms

- High index of suspicion and playing the odds*
- Signalment: age, breed, origin (shelter/sanctuary or other dense multi-cat situations)
- Sudden loss of activity, lethargy, anorexia, weight loss
- Failure to thrive (smaller than normal, poor hair coat, thin)
- Cyclical (waxing/waning), antibiotic-unresponsive fever
- Recent stressful event (vaccinations, surgery, new home, relinquished to a shelter or foster home, other illnesses, etc.)
- Physical exam and presence of signs associated with FIP: jaundice, ascites (abdominal distension) or pleural fluid (dyspnea), uveitis or retinitis (may be unilateral), neurologic signs (ataxia, seizures, weakness), palpable abdominal masses

** The odds of making a diagnosis of FIP with minimal diagnostic testing depends on the clinician's level of experience with the disease. Although FIP is one of the most important infectious diseases of cats, the average veterinary practitioner may not see many cases unless they are working closely with shelters, rescue groups, and/or breeders, or their practice is feline exclusive. The more difficult cases are often seen by specialists or university hospitals.*

For any infectious disease, there needs to be four legs to the stool:

- A good diagnostic test
- A good therapeutic treatment
- A good vaccine
- A good environmental control plan

—Dr. Elizabeth Colleran

Basic Non-Definitive Tests

- CBC (anemia of chronic disease, leukocytosis, lymphopenia)
- Examination of ascites or pleural fluid (any effusion)
- Serum proteins (total protein-high, albumin, globulin-high, an A:G ratio of < 0.6 with the lowest A:G ratios tending to be in cats with wet FIP rather than dry)
- Bilirubin is elevated in 21% to 63% of FIP cases, often without marked elevation in hepatic enzyme activity
- Feline coronavirus antibody titer (cannot determine if antibodies are against FECV or FIPV, and only if titers are 1:3200 or greater)
- Tests for serum proteins associated with inflammation and immune response:
 - Serum protein fractionation
 - AGP (alpha-1-glycoprotein) markedly elevated (> 3g/l)
- Detect and analyze abdominal and/or thoracic effusions:
 - Yellow-tinged (due to bilirubin from RBC breakdown)
 - Mucinous (sticky or stringy)
 - Partial clots when not anti-coagulated
 - Cloudy (high RBC and WBC, fibrin tags)
 - Non-degenerative neutrophils, monocyte/macrophages, large foamy macrophages, lymphocytes
 - Protein 2-10+ g/dl
 - Rivalta test (should not replace a complete fluid analysis, but the test is inexpensive and easy to perform)⁶
- Imaging for evidence of effusion, and/or organ or central lymph node involvement:
 - Radiographs, ultrasound of abdomen and/or chest
 - MRI scans with contrast (neurological FIP)
 - Ophthalmoscopy



Diagnosis of FIP, not always an easy subject. I look back through my notes that I've had as an undergraduate and there wasn't a huge amount of information on FIP because it was a disease that was difficult to prove a diagnosis for. But we do need to have an index of suspicion that FIP is out there, that it is a possibility.

—Dr. Emi Barker



If you confirm the presence of FIP virus antigen or RNA within peritoneal-type macrophages within typical effusions or lesions, you have made a definitive diagnosis. However, this is compounded by all of the false negative test results that are made by IHC or PCR by laboratories using poor techniques or that have been given non-representative samples (i.e., samples containing either no or insufficient infected macrophages).

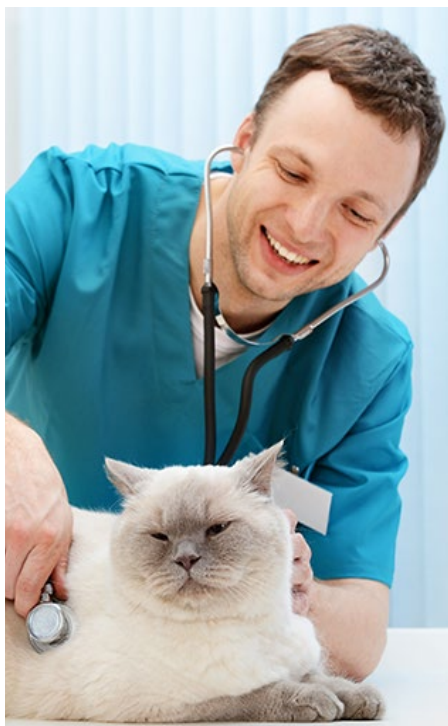
—Dr. Niels Pedersen

Definitive Tests**

This means FECV/FIPV RNA is identified in effusion or diseased tissues.

- Histopathologic or microscopic appearance of lesions is not pathognomonic unless combined with the rest of the clinical picture.
- Immunohistochemistry is positive for coronavirus antigen in macrophages within effusions of diseased tissues.
- Polymerase Chain Reaction (real time PCR) must have enough FIPV RNA in the effusion sample:
 - FCoV 7b RNA PCR is most sensitive; FIPV S mutation RNA test is less sensitive
 - Positive 7b RNA test is diagnostic even if the S mutation test is negative
 - PCR on blood is not highly sensitive^{7,8,9}

*** No test is definitive unless properly performed and truly positive. PCR and immunohistochemistry are falsely negative in about 30% of samples. False positives are uncommon. (Dr. Niels Pedersen)*



TREATING FIP

- There are only two ways cats diagnosed with FIP can be cured:
 - Find a way to stimulate a protective immune response.
 - Completely inhibit FIPV replication long enough to clear the virus from macrophages and/or to allow host immunity to develop.
- Symptomatic care (only to sustain life):
 - Nutrition: high-protein (animal meat), low-carbohydrate diet
 - Appetite stimulants
 - Fluid therapy support
 - Drain effusions only if necessary to prevent dyspnea or discomfort
 - Prednisolone (low dose) to decrease inflammation and improve appetite
 - Antibiotics are frequently given but have no significant effect on virus infections (doxycycline has been reported to have antiviral activity for several viruses)
 - Immunostimulants:
 - Feline interferon omega—no evidence of efficacy
 - Polyprenyl immunostimulant—there is some evidence of efficacy in 8% of dry FIP cases when used as a sole treatment^{10,11}
- Non-specific antiviral compounds: Several drugs and biologics inhibit viral replication by inhibiting normal cellular processes that are usurped by viruses to aid in their replication. However, effective viral inhibition cannot be achieved without significant cellular toxicity.
 - Itraconazole, cyclosporine, chloroquine, several antibiotics, and many plant extracts
- Antiviral drugs specifically targeting viral proteins (the treatment of choice):¹²
 - RNA transcription inhibitors (nucleoside analogs)—GS-441524¹³
 - 3CL viral protease inhibitors—GC-376^{14,15}

An accurate diagnosis and starting treatment as soon as possible are paramount to giving any cat with FIP the best chance to live and be cured.

*—Susan Gingrich,
Bria Fund founder*

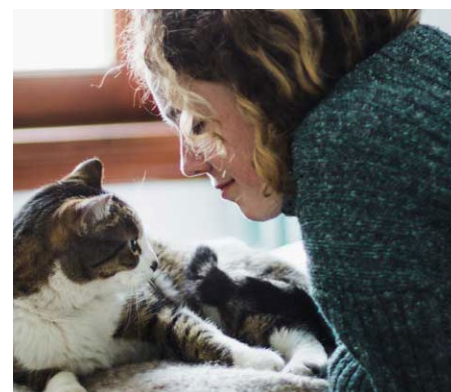


Current Non-FDA-Approved Antiviral Drug Status

- FIP is now considered a curable disease.
- Anecdotally, thousands of cats worldwide have been treated and possibly cured with antiviral compounds. Reversal of severe signs for both wet and dry forms of FIP (fever, loss of appetite, weakness, effusions/bloated abdomen, uveitis, neurological) can be seen within just days to weeks after starting antiviral treatment.
- The currently known drug with the most significant success for curing FIP is GS-441524, a nucleoside analog produced by Gilead Sciences, which is under patent and not available for veterinary use.
- Anivive Lifesciences, Inc. is working to gain FDA approval of GC-376 as a treatment for FIP.

Unresolved Concerns Regarding Non-FDA-Approved Antiviral Compounds

- Several companies in China have developed what they suggest are similar or the same compound/drug/products (these are unidentified products but they are reported to be nucleoside analogs) and are marketing these products worldwide as “dietary supplements” to treat FIP.
- The compounds have not received GRAS (generally recommended as safe) status. Under US FDA restrictions, the use of non-GRAS compounds in veterinary healthcare is illegal.
- Those treating cats with FIP are acquiring a version of GS-441524 through online or non-veterinary resources (such as members of the public referred through Facebook groups).
- Be sure to closely read the Terms of Service on a company’s website.
- No assurances are available as to bioactivity, safety, toxicity, and identity of the compounds being used in these non-FDA-approved substances.
- The course of treatment recommended can be expensive. Prices vary among companies and whether cost is related to the quality of the product remains unknown. Suppliers of GS-441524 should be chosen with care.
- No centralized data is being maintained or reviewed under scientific supervision.
- Veterinarians cannot prescribe or dispense these non-approved compounds. They can, though, choose to provide supportive and monitoring care for cats undergoing treatment.
- Establishing a good veterinarian-client-patient relationship is in the best interests of the patient.



PREVENTING FIP

Vaccination

- The reputation for failure of previous vaccines has slowed the process of vaccine development.
- Growing virus in cell culture is critical to vaccine development. Of the two serotypes, Type I is most common in North America but Type II is easier to grow and more common in Asia.¹⁶
- Vaccine development has also been hampered by the inability to grow FECV Serotype I, so the current vaccine is directed against the less common and less pathogenic Serotype II.
- Vaccine-induced antibody-dependent enhancement, in which the presence of specific antibodies can be beneficial to the virus, is a concern. This occurs when the vaccine induces a sub-neutralizing antibody response instead of a sterilizing response. One possible route to an effective vaccine is to reengineer the attenuated virus to induce stronger immunity.

While a single-dose vaccine that induces strong lifelong immunity is the most desirable, it may be more efficient to tailor a vaccine to an intended use, such as a strong, rapidly effective vaccine with short duration of immunity for shelter environments, where exposure is brief.

—Dr. Gregg Dean



- New high-throughput technologies are improving the chances of developing a vaccine by working backward from an understanding of the immune response to FIP and using that for a rational design.
- Mutant virus and cell line research must investigate and illuminate the role of interferon to successfully develop vaccines. This work is underway.
- In an effort to preserve the genetic diversity of various cat breeds, there may be more cats imported into North America from Asia, where Serotype II is more common.
- Respiratory transmission of FECV has been achieved in Serotype II, which may complicate vaccine development.
- Given enough time and money, if an effective vaccine is developed, perhaps such a vaccine should be administered only to kittens or cats most at risk, such as in shelters, rescue groups, sanctuaries, and pedigreed cat breeders.

It's like a roll of the dice if you're going to get FIP out of a coronavirus. And it's really different to roll the dice once than to roll it 10,000 or a million times. We are increasing the risk that it's going to turn into FIP.

—Dr. Kate Hurley



There's an immune response that seems to possibly eliminate the virus maybe for some period of time, but the immunity wanes and cats are susceptible again.

—Dr. Gregg Dean

Is there a genetic reason why 11% of cats infected with FIP virus don't eventually go on to develop FIP?

—Dr. Leslie Lyons

Virus Volume and Stress

- Stressful conditions have a tremendous impact on virus shedding.
- There are multiple mutations that cause disease from reservoirs of FECV in crowded conditions.
- Every virus shed is an experiment in potential mutation and exposure of cats to that mutation.
- Reducing the environmental viral load is a critical component in prevention.
- Stress appears to trigger disease in some cats. However, tools for accurately analyzing stress triggers may be somewhat lacking.
- Nutrition, lack of passive immunity for orphan kittens, and gastrointestinal dysbiosis are all worthy of investigating as possible stressors.
- Distinguishing the role of stress from the role of comorbid conditions is not yet possible.

Immunity

- Strong immune response and virus elimination may occur for only a period of time.
- FECV does NOT induce durable immunity. Immunity wanes and cats become susceptible again.
- Passive maternal antibodies are highly protective and may be useful in creating more durable immunity.
- Innate cellular immunity is required, which in turn requires growing FECV Serotype I in cell culture for vaccine development.

Genetic Resistance or Susceptibility

- 11% of cats develop the FIP biotype mutations, but only a fraction of those become sick. Identifying the genetic component for this is crucial.
- While pedigreed cats have increased susceptibility, it appears that all cats of a particular breed do not seem to be susceptible. Rather, particular lineages within a breed have high susceptibility.
- The reverse must then be true—there are lineages that have genetic resistance.
- There may be an interferon gamma gene that could be a marker for increased risk.
- Other markers may also exist, given the complexity of this disease.



Environmental Control

- According to a recent study,¹⁷ certain bentonite-based cat litters can decrease viral shedding and environmental contamination. However, the clinical significance has not yet been demonstrated.
- Early weaning of kittens is not desirable, as passive immunity from colostrum (IgG) and lactation (IgA) can effectively protect kittens from coronavirus infection.
- A coronavirus-free environment is not practical, as reinfection is nearly impossible to prevent.

MULTI-CAT ENVIRONMENTS

Shelters

- In well-run shelters today, FIP is not a common problem.
- A well-functioning shelter should see less than 1% FIP cases (fewer than ten cases per 1,000 cats).
- Shelters should balance intake with healthy outcomes by reduced crowding, thus decreasing virus shedding and exposure to infectious disease.
- One study showed that FECV shedding increased millions-fold in some cats after a week in the shelter.³
- Developing fostering programs, so bottle-raised kittens or queens with kittens do not come into the shelter but go straight into foster homes, lessens the odds of exposure to all infectious diseases, including FIP.
- Using double compartment enclosures with 8.5 square feet of floor space leads to a 50% decrease in exposure to upper respiratory infections (URIs).
- Placing portals between compartments decreases the incidence of respiratory disease by 90%.
- Group housing is not ideal for cats. If grouped, place no more than three to five cats in each group.¹⁸
- Keep the group stable—no in and out movement of cats. Don't mix short-stay and long-stay cats.
- If you want to be a cat sanctuary, be a sanctuary. Don't mix being a shelter and a sanctuary.
- "Don't kit-nap kittens" program: If a litter of kittens is found, encourage the public to leave them alone and see whether the queen returns—which can take up to 15 hours or more.¹⁹
- If kittens are not socialized, consider trap-neuter-return (TNR) to place them back in their cat colony.
- Early age spay and neuter of kittens at 1.5 pounds to increase their adoptability poses no increased health risk to them.

What is the best way to care for kittens in catteries, shelters, and rescues to minimize FIP losses?

—Dr. Niels Pedersen¹²



I'm not here to teach you about how to treat infectious diseases. I'm here to explain to you how to do surveillance, and how to prevent them.

—Dr. Gary Whittaker

We must find ways to keep cats out of shelters to prevent exposure and work with community partners to not bring in kittens to shelters. We need to raise up cats or increase the means to help adopt more cats out that are healthier, or in other situations to trap, neuter, and return cats back to their normal environments.

—Dr. Kate Hurley



Rescue Groups

- Shelters and other animal welfare groups need to offer tools and mentoring to rescue groups for infectious disease control.
- Rescues are trying to do too much and thus increase direct exposure to infectious diseases.
- A consistent TNR (trap-neuter-return) program usually leads to healthier cat colonies.

Catteries

- Elimination of FIP from a cattery is only possible by total elimination of FECV.
- It is not possible to test and eliminate FECV from a cattery because FECV is ubiquitous in cat environments.
- Pedigreed cats producing kittens with FIP should not be used for breeding, including those pedigreed cats who have been cured of FIP.



CONCLUSION

Our mission to end FIP is not finished. Research must continue in such areas as improving diagnostic methods, developing additional antiviral drugs, and especially strategies to prevent FIP infection in cats.

Winn Feline Foundation is at the forefront of funding FIP-related research.

Winn's Bria Fund for FIP Research is instrumental in providing grants MT13-006, W13-020, W15-010, W16-022, MTW17-020, W19-026 toward the development of the new antiviral drugs, GC-376 and GS-441524. Additional grants in recent years, such as MT16-014, W16-023, W16-024, W17-021, MTW17-022, W18-007, W18-010, W19-024, W19-025, and W19-027, are funding research into new FIP diagnostics, other FIP antiviral drugs, and vaccine development. Winn thanks our generous donors to the Bria Fund for supporting this critically needed research.

For a list of FIP projects funded by Winn's Bria Fund for FIP Research, see:

<https://www.briafundsupporters.com/studies>

<https://www.winnfelinefoundation.org/grants/grant-awards>

How to use social media to find the best information to help cat parents:

Winn Feline Foundation: www.winnfelinefoundation.org

Bria Fund for FIP Research Supporters: www.briafundsupporters.com

SOCK FIP: sockfip.org

Zen By Cat: www.zenbycat.org

Veterinary Information Network (VIN): www.vin.com

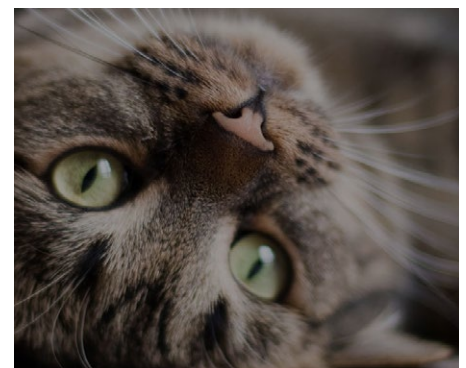
FIP Warriors Facebook Group for Cat Parents:
www.facebook.com/groups/158363205096283/

FIP Warriors Facebook Group for Veterinarians:
www.facebook.com/groups/2422765211310743/

FIPcatsSupport@group.io

This is a treatable disease. We will cure this disease. We basically have the cure now; it's just a matter of finding the exact right drug and the right combination (of drugs) and then making those available at an affordable price.

—Dr. Brian Murphy



Words used by attendees to describe this symposium: wow, hope, curable, winning, exciting, inspirational, networking, future, passion, fantastic, best, authoritative



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