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Posted Date: 23 January 2023

doi: 10.20944/preprints202301.0408.v1

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Abstract: After an incubation period of weeks to months up to 14% of cats infected with feline coronavirus (FCoV) develop feline infectious peritonitis (FIP): a potentially lethal pyogranulomatous perivasculitis. The aim of this study was to find out if stopping FCoV faecal shedding with antivirals prevents FIP. Guardians of cats from which FCoV had been eliminated at least 6 months earlier were contacted to find out the outcome of their cats: 27 households were identified containing 147 cats; 13 cats were treated for FIP and 109 others shed FCoV. A 4-7 day course of oral GS-441524 antiviral stopped faecal virus shedding. Follow-up was from 6 months to 3.5 years: 11 of 147 cats died, but none developed FIP. No FIP-recovered cat relapsed. Cats from 8 households recovered from chronic FCoV enteropathy. A previous field study of 820 FCoV-exposed cats was used as a retrospective control group: 37 of 820 cats developed FIP: the difference was statistically significant ($p = 0.0038$). Conclusions: early treatment of FCoV-infected cats with oral antivirals prevented FIP. Nevertheless, should FCoV be re-introduced into a household then FIP can result. Further work is required to establish the role of FCoV in the aetiology of feline inflammatory bowel disease.

Keywords: feline coronavirus; feline infectious peritonitis; FIP prevention; GS-441524; chronic enteritis; inflammatory bowel disease; IBD; antiviral; diarrhea; itraconazole

1. Introduction

Coronaviruses are epitheliotropic single-strand positive sense RNA viruses belonging to the order Nidovirales, genus Coronavirus, and family *Coronaviridae.* Feline coronavirus (FCoV) is a member of the subfamily alphacoronavirus. The FCoV species is further divided into two types: I and II, the first type being wholly feline, and type II being a recombinant hybrid between type I and canine coronavirus (CCoV) [1,2]. Type I FCoV is maintained in a group of cats by a cycle of infection, continuous virus shedding for some months in faeces, then cessation of virus shedding. Immunity is short lived so transient infection may be followed by re-infection by the same strain or a different coronavirus strain [3–5]. Re-infection and virus shedding at the limits of detection, and/or inhibition of polymerase chain reaction (PCR) tests by faecal inhibitors [6] can give an erroneous impression of intermittent virus shedding. Around 13% of type I FCoV infected cats becomes persistently infected, i.e. a carrier cat, [3] shedding the same strain of virus in the faeces continuously [4]. In carrier cats, the virus replicates in the large intestine, especially the colon [7].

Clinically, FCoV is a major cause of acute diarrhoea, usually in kittens, but it can affect cats of any age and can occasionally be fatal [8]. Chronic FCoV infection has been reported to cause chronic diarrhoea [3]. FCoV fulfils at least three of the five criteria which are required by the World Small Animal Veterinary Association International Gastrointestinal Standardization Group for a diagnosis of inflammatory bowel disease (IBD) [9] also called chronic enteropathy (CE). The clinical criteria that chronic FCoV enteritis fulfils are first, chronic (ie, over 3 weeks in duration) gastrointestinal signs (e.g, anorexia, vomiting, weight loss, diarrhea, hematochezia, mucoid feces); second, inability to

 doi:10.20944/preprints202301.0408.v1

document other causes (e.g. parasites, protozoa, bacteria) of gastroenterocolitis by thorough diagnostic evaluation; third, inadequate response to appropriately designed and implemented therapeutic trials (i.e., dietary, antibacterial, anthelmintic) [9–13]. Whether it fulfils the fourth criterion—histopathologic evidence of mucosal inflammation—is unknown; and the fifth criterion, a clinical response to anti-inflammatory or immunosuppressive agents, tends to be met only temporarily, if at all.

A minority of FCoV-infected cats develop a potentially lethal pyogranulomatous perivasculitis, feline infectious peritonitis (FIP) [14]. When FCoV is first detected in a household, mortality due to FIP can be as high as 14%, but in a retrospective study of 820 cats and kittens living in FCoV-endemic households overall FIP mortality was 4.5% in adults and up to 9.6% in kittens [15].

We reported previously that a 4-7 day course of Mutian Xraphconn pills stopped faecal shedding of FCoV [16]: (Mutian pills were subsequently shown to contain the adenosine nucleoside analogue GS-441524 [17]. We then considered whether this brief treatment prevented FIP: the outstanding question was whether the antiviral drug had simply eliminated virus from the gut, or whether virus persisted elsewhere in the body, allowing FIP to develop at a later date. In this paper we provide evidence that early treatment of FCoV infection does prevent FIP.

2. Materials and Methods

2.1. Cats

We searched the database of one of the authors (DDA) for households of cats from which FCoV had been eradicated and contacted the cat guardians to find out what happened to their cats. The criteria for including a household were first that all the FCoV-infected cats had negative faecal FCoV RT-qPCR tests after treatment. Previous research showed that the probability of dying of FIP was greatest in the first 6 months after the first exposure to FCoV [15]. Consequently, the second criterion for inclusion was that only households with at least 6 months follow-up were included so that adequate time was allowed for incubating FIP, if present, to emerge. Households with insufficient data or follow-up of less than 6 months were rejected. As shown in Table 1, 27 households containing 147 cats had sufficient follow-up to be included.

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Table 1. Mortality of 147 cats in 27 households from which FCoV was eradicated.

Hh	No. cats in hh	No. of FIP cats treated	No. non-FIP cats cleared of FCoV with 4-7d GS-441524	How long followed post GS-441524 in months	No. cats developed FIP	No. cats died	Time of death post antiviral in months	Comments
1	4	1	2	41	0	0		Itraconazole treatment had reduced viral load but failed to eliminate the infection, so cats were subsequently treated with Mutian pills. Faeces of all 4 cats still tested negative 11 mo post FCoV eradication and FCoV antibody titres reduced significantly in two cats (1280 to 80 and >1280 to 320) within 4 mo. FCoV antibody titres reduced insignificantly (>1280 to 1280) in a third cat but remained high in the FIP recovered cat for over 2 y.
2	17	0	13	40	0	2	10 mo 29 mo	13 of 17 cats were treated following the histopathologically confirmed death of one cat to FIP. FCoV antibody titre of 4 cats reduced to zero and that of a 5th cat reduced from 640 to 20. Two cats died in the 3 y follow-up: one at 10 mo and one at 29 mo after the household was cleared of FCoV; both cats had tumours: one developed autoimmune haemolytic anaemia following chemotherapy for lymphoma and the other died following a kidney infection related to being paraplegic and doubly incontinent for 5 y.
3	7	0	3	26	0	0		Chronic diarrhoea in one young cat resolved following treatment.
4	3	1	2	26	0	0		
5	4	0	2	27	0	0		One chronic FCoV diarrhoea kitten introduced from rescue shelter: all 4 cats got diarrhoea but only one other tested FCoV RT-PCR positive. Reduction of FCoV antibody titre from >1280 to 80 in 13 mo in the chronic diarrhoea recovered cat. FCoV titre was 40 at 5 mo post treatment then 0 at 10 mo post treatment in the other FCoV shedding cat; and 0 and 40 at 5 mo post FCoV eradication in the two non-shedding cats.
6	2	1	1	24	0	0		FCoV antibody titre reduced from 640 to 80.
7	5	1	3	30	0	0		FCoV titre reduced from >10,240 to 40: 80, and 160 in three cats within 6 mo. FCoV titre stayed at 640 for 12 mo in 4th cat although no faecal shedding was detected, reducing to 160 at 16 mo. FCoV titre reduced from >10,240 to 640 in 9 mo in the FIP recovered cat.
8	25	0	20	38	0	1	24 mo: sudden death.	One cat became FCoV seronegative (others not tested). One 10 y old cat died suddenly, having been fine in the morning and eaten his breakfast. No post mortem.
9	3	0	2	36	0	0		A kitten with diarrhoea was adopted from a shelter. Itraconazole treatment reduced viral load but failed to eradicate the infection. The cats were subsequently treated with Mutian pills and chronic diarrhoea resolved in all three cats, but one cat continues to regurgitate regularly.
10	20	0	20	34	0	1	30 mo: HCM	16 adults and four 4 mo old kittens cleared of FCoV following the death of two cats to FIP. One death due to hypertrophic cardiomyopathy (HCM) 30 mo after the household was cleared of FCoV.

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11	2	1	1	26	0	1	14 mo: throat tumours	The cat treated for FIP developed throat tumours and died under GA 14 mo after FIP treatment. The in contact cat was alive and well at 26 mo.
12	3	1	0	23	0	0		Only the FIP patient (biopsy and ascites FCoV RT-PCR confirmed) required antiviral treatment: the faeces of the two in contact cats were negative.
13	2	1	1	21	0	0		
14	2	1	1	19	0	0		
15	3	0	2	19	0	1	21 mo: acute kidney failure	The index case was an 11 y old Maine Coon cat which presented with a history of what was believed to be exocrine pancreatic insufficiency: she recovered completely following elimination of FCoV. One sudden death at 21 mo of a 4 y old Maine Coon with acute kidney failure.
16	13	0	13	18	0	1	Sudden death 4 wk post kittening	Breeding household. The single death since FCoV was eradicated was a sudden death 4 wks post kittening: the cat went into seizure and was euthanased at an emergency vet. No abnormalities detected on post mortem. 23 kittens were born since FCoV was cleared: no FIP occurred in any of them.
17	3	1	1	18	0	1	10 mo: cancer	Resolution of chronic gastrointestinal signs, described as inflammatory bowel disease, following FCoV eradication. The cat who died of cancer wasn't the cat who was treated for FIP.
18	3	0	2	16	0	0		Chronic FCoV diarrhoea cat resolved with GS-441524 treatment and still well 16mo later.
19	2	0	2	15	0	0		Resolution of chronic diarrhoea. The FCoV antibody titre of both cats reduced to 0.
20	2	1	1	10	0	0		
21	4	1	3	15	0	0		
22	2	1	1	15	0	0		
23	4	0	2	13	0	2	9 mo and 10 mo	At 9 mo post FCoV eradication a very old cat was euthanased and at 10 mo a young cat was killed on the road. Two new kittens were introduced 12 mo after FCoV eradication: both tested FCoV antibody negative after being in the household over one month showing that they had not been exposed to virus.
24	2	1	1	11	0	0		
25	1	0	1	8	0	0		Uveitis erroneously suspected to be due to FIP.
26	1	0	1	9	0	0		The treated cat was in contact with an FIP cat who died: his guardian wishes to purchase a new kitten.
27	8	0	8	6	0	1	3 mo: blood clot	Prior to clearing FCoV, an 8 wk old kitten died of histopathologically confirmed necrosuppurative enterocolitis with crypt and gland abscesses: the lesions stained positive for FCoV. One cat died of a blood clot 3 mo after FCoV was eradicated. 5 litters were born post FCoV eradication: this breeder spot checks kittens: they have been negative. Two new FCoV- infected kittens have been introduced into the household and cleared of infection since the original FCoV eradication.
	147	**13**	**109**		**0**	**11**	**Totals**	

No. = number. Hh = household. mo = months. wk = week(s). y = year(s).

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2.2. FCoV RT-qPCR

FCoV reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed at the University of Glasgow Veterinary Diagnostic Laboratory, Scotland, as previously described with the addition of a control for faecal PCR inhibitors [16,18]; at the Zoologix Laboratory, California, USA, and at the Veterinarmedizinisches Labor, University of Zurich, Switzerland.

2.3. FCoV Antibody Titre

FCoV antibody titre was measured at IDEXX Laboratories, Wetherby, England and, as previously described [19], at the University of Glasgow Veterinary Diagnostic Laboratory, Scotland.

2.4. Determination of the Risk of FIP Having Ceased

The gold standard of proof that FCoV was eliminated from a cat's body entirely was reduction of his or her FCoV antibody titre to zero. A significant reduction in antibody titre was also taken to indicate that there was no longer viral antigen present to stimulate the immune response: in other words, that the cat was no longer infected with FCoV and that the risk for FIP had passed (unless the cat would be re-infected in future, obviously).

It would not have been ethical to ask pet guardians to have their cats blood tested for the purposes of establishing their FCoV antibody titre, although we did request testing leftover blood should the cat require a blood test for some unrelated reason. The second criterion for determining that a cat had truly recovered from FCoV infection was the survival of the cat without developing FIP for a minimum of 6 months from the date of the virus being cleared. In our previous study of 820 cats in 73 households most FIP occurred within 6 months of the cat's first known exposure to FCoV; 73% (27 of 37) of FIP deaths occurred within 18 months and by 36 months the probability of the remaining cats not developing FIP was 95% [15]. In a study of 400 kittens from households with endemic FCoV infection, all FIP kitten deaths occurred before one year of age [19].

2.5. Statistical Analysis

As it would have been unethical to establish a placebo group, the data from a previous study of 73 households containing 820 cats was used as retrospective control group [15]. The 2 cohorts were similar: a mix of ordinary pet households and a few cat breeders.

Statistical significance of results was calculated using an online Fisher's exact test: https://www.socscistatistics.com/tests/fisher/default2.aspx. A p value of <0.01 was considered highly significant and a p value of <0.05 was considered significant.

3. Results

3.1. FCoV Elimination

FCoV was eradicated from 27 households containing a total of 147 cats (see Table 1.). Household size ranged from 1 to 25 cats. Their ages ranged from 8 weeks to 18 years, with 56 cats being under 2 years old and 36 of those were up to 12 months of age.

The most common reason for treating FCoV-infected non-FIP cats with antiviral drugs was to prevent re-infection of a patient being treated for actual (n = 13) or suspected (n = 2) FIP. In six households (3, 5, 9, 15, 19 and 27) cats were treated for FCoV-associated chronic enteropathy (CE). Five cat breeders wished to eradicate coronavirus following FIP deaths (households 8, 10, 16, 18) and an outbreak of fatal FCoV enteritis of kittens (Household 27). The remaining two households eradicated FCoV from their cats to prevent virus transmission to uninfected cats.

The cats with FIP treated with oral anti-virals stopped shedding FCoV in their faeces. Virus-shedding non-FIP cats (n = 109) were treated with 4 to 7 days of oral GS-441524 made by Bova Specials UK, Ltd, London, England (n = 3); Mutian Xraphconn, Mutian Biotechnology Co., Ltd., Nantong, China (n = 98); or Panda, https://maxpawhealth.com, USA (n = 8). Previous studies showed that a

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dose of one pill of Mutian X 100/kg given for a minimum of 4 days would stop FCoV shedding [16]. The Mutian 100 pill packaging claimed to contain 5mg of active antiviral (subsequently shown to be GS-441524 [17]. However, independent analysis of a Mutian 200 pill showed that it contained 18mg of GS-441524, not 10mg (Nick Bova, personal communication) and a Mutian 50 pill contained 7.2mg, not 2.5mg (Dominik Mirowski, personal communication). Consequently, makes other than Mutian were administered at a dose of 10mg/kg. Usually one course of treatment sufficed; if not, treatment was repeated until faeces tested negative for FCoV RNA by RT-qPCR. Twenty-five (17%) of the 147 cats did not shed FCoV and did not require antiviral treatment.

3.2. FCoV Antibody Titre Reduction

Reduction of FCoV antibody titre to zero was considered proof of recovery from FCoV infection. A substantial reduction of the cats' FCoV antibody titres occurred in 7 households (1, 2, 5, 6, 7, 8, 19) containing 59 cats. FCoV antibody titres returned to zero in 9 cats, proving that they have eliminated FCoV, and there was a significant reduction in FCoV antibody titre in a further 9 cats. In an individual household not all of the cats became seronegative within the short time of our follow-up: cats which had recovered from FIP often remained seropositive for over one year post recovery even though they had stopped shedding virus (we saw this in cats in Households 1 and 7). Two new kittens introduced into Household 23 remained seronegative one month after introduction showing that they had not been exposed to FCoV: this was deemed an indirect proof of FCoV eradication from that household. These 2 kittens were not included in the survival statistics.

3.3. Survival of Cats after FCoV Elimination

In the present study, we followed up 147 cats for at least 6 months (range 6-41 months) from the time of FCoV eradication. Of these, 136 cats were known to have survived and 11 cats died, but no cat developed FIP (nor did any FIP treated cat relapse). In the retrospective control group: 37 of 820 cats died of FIP (not including index FIP cases) over the whole period of observation and most FIP deaths occurred within the first 6 months from the first detection of FCoV/FIP in the household [15]. The Fisher exact test statistic value of the difference between 37 of 820 and 0 of 147 was 0.0038 which was highly significant at $p < 0.01$.

3.4. Survival of Cats for at Least 18 months after FCoV Elimination

In the previous study most FIP occurred within the first 6 months. FIP did occur after that time, but the risk of not developing FIP was 95% at 18 months [15]. Consequently, a subset of data from cats in the present study whose survival outcomes were known for at least 18 months after FCoV elimination was analysed separately. This subset of data is shown in the first 17 households of Table 1: 8 cats died, but no cat died of FIP. The causes of death are shown in Table 1.

By 18 months from first FCoV diagnosis, 27 of 572 FCoV-exposed cats in the retrospective control group had died of FIP [15] whereas in the present study, 109 non-FIP cats were exposed to FCoV infection, and none developed FIP. The difference between the 572 cats followed previously and the 109 in the present study is significant: $p = 0.0138$ ($p < 0.05$).

3.5. Comparison of Non-FIP Mortality between the Two Cohorts of Cats

In the present study 11 of 147 (7.5%) cats died of non-FIP related conditions and in the retrospective control group 87 of 820 (10.6%) cats died of causes other than FIP: although it appeared that fewer cats died in the present study, the difference was not statistically significant ($p > 0.05$).

3.6. Resolution of FCoV-Associated Chronic Enteritis in 8 Households

The cats in Households 3, 5, 9, 15, 19 and 27 were intentionally treated to eradicate FCoV in order to cure various chronic gastrointestinal (GI) signs that had been diagnosed as idiopathic inflammatory bowel disease (IBD), chronic enteropathy (CE) or exocrine pancreatic insufficiency. CE signs resolved in all cats, except that chronic regurgitation in one cat in Household 9 did not improve.

 doi:10.20944/preprints202301.0408.v1

Household 27 had lost kittens with histopathologically confirmed necrosuppurative enterocolitis with crypt and gland abscess lesions staining positive for FCoV (Veterinary Medical Diagnostic Laboratory, Colombia, Missouri, USA). FCoV eradication from this cattery prevented further kitten deaths.

The guardians of the 6 households above knew that their cats' CE signs were due to FCoV, but for 2 others (Households 17 and 18) the short course of GS-441524 curing their cats was unexpected. Some guardians of FIP cats also reported resolution of GI signs when FIP was treated (data not shown). Seven other CE recovered cats were from households which were excluded because follow-up was under 6 months.

4. Discussion

While it was previously shown that a short course of an antiviral drug would stop FCoV shedding in the faeces, it was unknown whether this effect was temporary, or if virus could linger elsewhere in the body allowing FIP to develop at a later stage [16]. Given that it would not have been ethical to request repeat blood tests from pet cats to establish whether or not the virus had been eliminated from the body (i.e. to demonstrate a return of the FCoV antibody titre to undetectable levels), a second method was to observe the cats for a period of time after which the risk of FIP would be deemed negligible. Because it would also have been unethical to give placebos to pet cats to establish a control group, the data from a previous study, performed before the advent of anti-coronavirus drugs, was used as a retrospective control group. We believe we have demonstrated that eliminating early FCoV infection is an effective method to prevent FIP.

A possible criticism of our study is that necropsies were not carried out on most of the cats which died. However, the guardians and veterinary surgeons of these cats had experience of FIP and would have been very alert to the possibility of it appearing and able to recognise it had it occurred. FIP was suspected in one cat in Household 7 when an in-contact cat of the FIP survivor presented with tenderness over the lumbar spine. For this reason his blood was tested and a significant reduction in his FCoV antibody titre provided reassurance that he did not have FIP, and he recovered uneventfully with symptomatic treatment. Making antibodies costs the body energy, and the globulins themselves thicken the blood, therefore the body stops making antibodies as soon as it deems it safe to do so, but keeps memory T cells with the blueprint so it can produce antibodies again if need be.

One concern could have been whether we had inadvertently chosen a population of cats which were already immune to FCoV infection, since the main risk for FIP is following first exposure to FCoV infection [15]. However, this was not the case as 56 cats (38%) were less than two years of age, and around half of FIP cases occur in cats under 2 years of age. Furthermore, in 2 households following the introduction of a pedigree kitten, it was the older incumbent cats, aged 3 and 7 years, rather than the kittens, who developed FIP.

We expected that in our group of treated cats there might be some that were incubating undetected FIP, for whom a short course of antiviral would not be sufficient, since most cats require around 7-8 weeks [20] or even 12 weeks of treatment [21]. However, that was not the case, possibly because of the small size of the cohort of cats.

Most households were small (fewer than 10 cats) and eradication of FCoV from the house was straightforward. However, virus elimination from larger households was more challenging, because FCoV is highly contagious, and if even one cat did not eliminate infection it might rapidly re-infect the other cats with whom it shares litter trays. Indirect virus transmission on cat litter dust fomites is also a risk. One cat breeder with a very large household who eradicated FCoV from her cats, unfortunately re-introduced the virus by bringing in a large number of FCoV-infected pedigree kittens. As expected re-infected cats began to shed virus again and further FIP cases occurred (data not shown). The breeder reported that re-introduction of FIP into her cattery was especially serious in kittens, presumably because they had no maternally derived antibody protection. This household is a reminder that once FCoV is eradicated from a household, strict precautions must be put in place to prevent its re-introduction. Within the households that failed to meet inclusion criteria, this

household was the only one to experience FIP other than in their original case. Four households also introduced infected kittens or cats post-FCoV eradication, but they were quarantined and treated before being allowed to share litter trays with uninfected cats and no virus transmission occurred.

Another criticism of this work is that eliminating the virus from the body artificially may interfere with the development of natural immunity. That may be the case, but should be weighed against the fact that FIP was prevented and re-exposure to FCoV infection can be avoided if virus is eradicated from the entire household of cats.

In many countries GS-441524 is available directly to the public: there is a risk that if FCoV elimination or FIP treatment is not performed at a high enough dose, or for a long enough duration, then virus resistance to the drug could ensue: it is essential that a post-treatment faecal RT-PCR test should be performed to ensure that virus elimination has been effected. In our experience, injectable GS-441524 does not consistently eliminate FCoV from the gut: one cat treated for FIP with GS-441524 injections for 12 weeks still shed virus in his faeces at least two years later: it is from such carrier cats as this that virus mutants resistant to GS-441524 could emerge. In a recent study of 26 cats which experienced relapses, 23 were treated using injectable rather than oral GS-441524 [22] but unfortunately no control group of cats without relapses was presented.

The elimination of FCoV cured some cats in the study that were suffering from gastrointestinal conditions. The presence of FCoV in a diarrhoea profile for infectious disease tends to be overlooked because the virus is highly prevalent in purebred cats and multicat environments such as cat shelters, but the recovery of several cats in our small cohort shows that a response to an oral (not systemic) anti-coronavirus drug should now be included amongst the therapeutic trials performed in suspect feline IBD cases.

5. Conclusions

In conclusion, cats that were infected with FCoV and did not have FIP, were treated with a short course of an antiviral drug. The treatment was safe, cleared the virus from the gastrointestinal tract, and prevented the future development of FIP. The treatment also cured a number of cats that were suffering from chronic gastrointestinal disease.

Author Contributions: Conceptualization, D.D.A. and O.J.; methodology, D.D.A. and O.J.; validation, D.D.A., F.B. and O.J.; formal analysis, D.D.A., F.B. and O.J.; investigation, F.B., B.C., J.C-R., S.C., M.F., S.H., E.J., C.J., S.L., and D.D.A.; data curation, D.D.A., F.B., B.C., J.C-R., S.C., M.F., S.H., E.J., C.J., and S.L.; writing—original draft preparation, D.D.A.; writing—review and editing, F.B., O.J.; project administration, D.D.A.; funding acquisition, J.C-R., D.D.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the cats' guardians and the subscribers and donors to www.catvirus.com: we also thank subscribers for funding D.D.A. We are grateful to MDPI for waiving publication fees on this paper.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Written informed consent was obtained from the cat guardians.

Acknowledgments: We are extremely grateful to the following for permission to use their cats' data: Katarzyna Browarna, Eilidh Durkin, Candice Prowting, Mariko Parslow, Lynn Rollins, Anni McAuliffe of Everafter Ragdolls, Susan Marshall, Tanya Osborne, Elena Scott-Forbes, Kristen Williamson. We give our deepest thanks to the subscribers and donors to www.catvirus.com for funding DDA and making this research possible.

Conflicts of Interest: The authors declare no conflict of interest. D.D.A. is a former employee of the Veterinary Diagnostics Laboratory at the University of Glasgow and has received honoraria for webinars from Biogal, Israel, who make the FCoV Immunocomb antibody test, although neither of these constitutes a conflict of interest unless this paper is being understood to be advocating FCoV RT-PCR and antibody testing.

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