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Oral Mutian®X stopped faecal feline coronavirus shedding by naturally infected cats

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ABSTRACT

Feline coronavirus (FCoV) is common among cats living indoors in groups. In about 10% of infected cats, a potentially lethal disease, feline infectious peritonitis (FIP) occurs. Virus transmission is faecal-oral. Mutian® Xraphconn (Mutian X) is a product marketed to treat cats with FIP but is also being used to stop virus shedding, although no clear guidelines exist for its use for this purpose. The aim of this study was to establish the minimum dose and treatment duration required to ensure viral clearance from the faeces of asymptomatic virus-shedding cats. In five multicat households, 29 cats naturally infected with FCoV and actively shedding virus in the faeces were given Mutian X pills. Virus shedding was monitored using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) controlled for faecal inhibitors to ensure sensitivity. Mutian X given orally cleared the virus in 29 cats; although four cats required a repeated course to finally stop virus shedding. A dose of 4 mg/kg q24 h for four days was found to be the optimal treatment protocol: 2 mg/kg cleared only 80% of cats. Post-treatment using a sensitive RT-qPCR test was essential to ensure that virus clearance had been achieved, since failure to clear even one cat can result in re-infection of the others. Records of virus shedding by cats before treatment provided a retrospective control: significantly more cats stopped shedding virus after Mutian X than recovered from infection during the control period ($p < .00001$). This is the first report of the successful elimination of faecal FCoV shedding in chronically infected cats.

1. Introduction

Feline coronavirus (FCoV) is a positive strand RNA virus ubiquitous in multi-cat environments such as breeding catteries, rescue shelters, cat sanctuaries and large cats in zoos, but rare in stray and feral cats. (Addie et al., 2012; Cave et al., 2004). Two FCoV genotypes are currently known: FCoV type I (FCoV-I), which is predominant in the field, (Addie et al., 2003; Hohdatsu et al., 1992; Li et al., 2019) and FCoV type II (FCoV-II), which derives from recombination between FCoV-I and canine coronavirus (Decaro and Buonavoglia, 2008; Herrewegh et al., 1998; Terada et al., 2014).

FCoV is primarily a pathogen of the gastrointestinal tract of domestic cats, replicating in the intestines and is spread by faecal-oral transmission from cats that are either persistently or transiently infected (Addie and Jarrett, 2001; Addie et al., 2003). FCoV infections are often subclinical, but may result in a number of problematic conditions such as acute and chronic diarrhoea, stunting of kittens or transient upper respiratory signs in newly infected kittens and cats, and faecal incontinence in persistently infected carrier cats (Addie and Jarrett, 1992; Kipar et al., 1998). FCoV RNA was found in five of 14 cats with chronic caecocolic disease, although it was unclear if FCoV was the cause of the lesions (Hahn et al., 2017).

Some cats that acquire the infection, typically 7–14% (Addie et al., 1995) develop feline infectious peritonitis (FIP), until recently considered to be an almost uniformly fatal disease. However, in a major advance, Pedersen et al. (2019) found recently that FIP can be cured by the parenteral administration of GS-441524, a novel antiviral nucleoside analogue. While this drug has yet to be licensed for veterinary use,

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several products, some specifically described as GS-441524, and others with proprietary names, are being sold through the internet to cat guardians who treat their own cats suffering from FIP.

FCoV persists in multicat households by infection and re-infection of healthy cats by the same, or another, strain of virus and by the development of a persistently infected carrier status in 13% of FCoV-infected cats which act as reservoirs of infection in a feline population without developing FIP (Addie et al., 1995; Addie and Jarrett, 2001; Addie et al., 2003; Herrewegh et al., 1997). There is an urgent need of methods to prevent cats in high density environments such as breeding and rescue catteries becoming infected with FCoV. An FIP vaccine is licensed in some countries, but the first dose is administered at 16 weeks (Gerber et al., 1990) which is too late to protect kittens, that are usually infected when maternally derived antibody wanes at between five and seven weeks of age (Addie and Jarrett, 1992; Pedersen et al., 1981). At present, therefore, the major way to prevent FIP is to prevent exposure to the virus by quarantine and rigorous hygiene. FCoV transmission is faecal-oral; sharing litter trays with a FCoV shedder and fomite transmission are the major risk factors for uninfected cats. In dry indoor environments, FCoV can survive up to seven weeks in fomites (Scott, 1988).

Another method of control would be to stop the excretion of virus in the faeces of infected cats by antiviral therapy. Some cat guardians have considered whether the drugs shown to cure FIP might also stop asymptomatic FCoV-infected cats shedding virus, thereby preventing them acting as a source of infection and providing a means to establish households of cats that are free of the virus. Mutian® X (Nantong Mutian Biotechnology Co. Ltd. China) is one of those products, it is formulated in capsules containing nicotinamide mononucleotide, Crocin I, S-Adenosylmethionine, Silymarin and Mutian X, which is a novel synthetic adenosine analogue (patent pending in China), exhibiting broad-spectrum activity against RNA viruses (Tony Xue, CEO of Nantong, personal communication). Nucleoside analogues function by replacing adenosine, thus terminating replication of the RNA virus genome. Mutian X is efficacious by the oral route unlike previously described anti-coronavirus drugs, which must be given by painful injection, often causing severe inflammatory responses (Pedersen et al., 2019), which is of concern for the development of feline injection site sarcoma.

Although recombinant feline interferon omega (FeIFNω: Virbagen Omega, Virbac, France) was previously shown to reduce FCoV shedding, this is the first report to document an anti-viral that stopped the excretion of FCoV in the faeces of naturally infected cats.

2. Materials and methods

2.1. Cats

FCoV testing was implemented because the households had experienced FIP among their cats. A summary of the cats and the reasons for screening for FCoV shedding is shown in Table 1.

Thirty-four of 50 cats in five multicat households (A-E) were naturally infected with FCoV and were treated with Mutian X. Results from five cats from Household E could not be included because the intervals between faecal tests left the possibility that the cats might have spontaneously stopped shedding virus, rather than Mutian X having stopped virus shedding: thus they were excluded from both treatment and control groups (Table 1). Therefore, there remained data of 29 cats treated with Mutian X.

Household C was a Ragdoll breeding cattery with a history of endemic FCoV for at least four years despite testing for FCoV antibodies and shedding, and rigorous hygiene. In 2015 this cattery experienced eight FIP deaths. Keeping cats in small groups or singly to attempt to prevent FCoV transmission resulted in clearance of virus in eight of 24 cats. Prior to the observational study we report here, the cattery owner (SC) discovered she could reduce coronavirus shedding in some cats using Mutian X tablets: we worked with her to optimise dose and duration of treatment for stopping virus shedding.

2.2. Faecal sample origin and preparation

Faecal samples were mailed to the Veterinary Diagnostic Laboratory (VDL) at the University of Glasgow, UK. The samples were mailed using Royal Mail or FedEx: they were not on ice, nor in RNA-preserving buffer. Cat guardians were advised to try to submit faecal samples with no cat litter attached, since cat litter can inhibit the PCR reaction.

2.3. FCoV reverse-transcriptase quantitative polymerase chain reaction

FCoV RNA was detected by semi-quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) as previously described (Dunbar et al., 2019), except that to control for false negative results GAPDH was replaced by a control for PCR inhibitors in faecal samples, the exact nature of this control cannot be revealed because the laboratory deems it a commercial secret. Testing was performed in duplicate. Those performing the FCoV RT-qPCR tests were blinded to which treatments the cats were receiving. Threshold cycle (C_T) number was used as the measure of viral load. The lower the C_T, the more virus present in the sample. Samples with no signal at C_T 40 were considered negative.

2.4. Treatment protocols

Cat guardians purchased Mutian X online. Mutian X was available in either tablet format, containing 4 mg of the anti-viral Mutian X ingredient, or capsules (Mutian X 200) containing 10 mg. The exact nature of Mutian X is a commercial secret, prior to Chinese patent being granted: the manufacturer describes it as an adenosine nucleoside analogue (Tony Xue, personal communication).

Cat guardians were using 4 mg/kg q24 hours per os for seven days as per advice from social media groups for stopping FCoV shedding. The owner of Household C (SC) was experimenting with different doses and protocols with the aim of minimising dose and duration of treatment to stop faecal virus shedding: we report the outcome of using 2 mg/kg and 4 mg/kg doses. The owners of Household E (FB and BC) set up a camera and alarm system which enabled the recording of the exact number of hours post onset of treatment that a faecal sample was passed, and to ensure the samples were not contaminated or degraded.

Prior to the use of Mutian X, cat guardians were attempting to prevent FCoV transmission within their households by quarantining FIP cats (Households A and D), keeping cats in as small groups as possible given their facilities (Households C and E), and using Dr. Elsey Cat Attract cat litter (Addie et al., 2019) or other bentonite-based cat litter (Households A and E respectively). Some households also tried to reduce FCoV shedding using probiotics, recombinant FeIFNω, and a 21 day course of itraconazole (Households A and D) which has been shown to have in vitro activity against Type I FCoV (Takano et al., 2019). These various treatments were grouped into a single control period for each Household (Figs. 1–3), since numbers of cats for each of these parameters were too small to be statistically significant, and also because combinations of prevention strategies were used.

2.5. Statistical analysis

This was an observational study so that there was no opportunity to conduct a placebo-controlled trial. However, FCoV shedding records prior to treatment with Mutian X, where available, were used as a retrospective control period. Cats acted as their own controls, which meant that variables such as housing, breed, and age, which affect FCoV shedding, were constant.

Fisher's exact test (two-tailed) and correlation coefficient were carried out using the statistics package in Excel (Microsoft Office 2007).

Table 1
Details of the five households of cats which were cleared of feline coronavirus using Mutian X.

Cats	Breed	FCoV positive	FCoV negative	Reason for faecal FCoV RT-qPCR testing
A1–4	British Shorthair pet cat siblings	2	2	Initially all four cats shed virus. Cat A1 had effusive FIP diagnosed by a positive FCoV RT-qPCR test on ascites (Herrewegh et al., 1995; Longstaff et al., 2017; Lorusso et al., 2019). Cat A1 ceased virus shedding following treatment with FeIFNω, itraconazole, and GC376 protease inhibitor injections (Pedersen et al., 2018). Cat A1 was kept isolated from the other three cats and they were tested for FCoV shedding prior to re-uniting them, in order not to re-infect Cat A1. Cat A2 stopped shedding virus subsequent to seven injections of GC376 given because FIP was suspected, but his condition was a urinary tract infection which resolved with antibiotics. Thus Mutian X was only required for Cats A3 and A4. Dr. Elsey Cat Attract cat litter (Addie et al., 2019) was used and Cat A2 remained uninfected despite continued sharing of litter trays with cats A3 and A4. All four cats tested FCoV negative five months after their first negative faecal tests.
B1 – B2	Turkish Van, indoor pet cat	1	1	Cat B1 was in contact with Cat B2 who was treated for FIP using Mutian II injections and Mutian X tablets prior to this study. Cat B1 was shedding FCoV.
C1–25	Ragdoll breeding cattery	17	8	Cats were routinely tested because this was a breeding cattery wishing to eliminate FCoV, and therefore FIP. Eight FIP deaths had occurred prior to testing beginning. At the start of the Mutian X treatment, 14 of 24 cats were shedding FCoV; one FCoV shedding cat (C10) was introduced during the study period (the breeder having confidence in her ability to stop Cat C10 shedding virus using Mutian X). Cats were kept in small groups of up to three cats. Sawdust based cat litter was used. Two of ten negative cats became infected during the study period, bringing the total treated to 17.
D1–2	Siberian pet cats	1	1	Cat D2 had effusive FIP diagnosed by a positive FCoV RT-qPCR test on ascites but was not shedding virus in the faeces. D1 was the housemate cat found to be shedding FCoV and was treated to avoid re-infecting cat D2. A 21 day itraconazole course had failed to stop Cat D1 shedding virus.
E1–17	Domestic shorthair rescue cats	13	4	One cat died of histopathologically suspected FIP prior to this study (immunohistochemistry was not performed to confirm diagnosis). FIP was suspected in a second cat (E14) and GS-441524 treatment was begun; but he had multicentric lymphoma, so the anti-viral drug was stopped. Cats were divided into three groups of up to eight cats. ten cats became infected during the study period despite clumping bentonite-based cat litter being used. Results of five infected cats (E6, E8, E10, E11 and E13), could not be counted as having stopped shedding due to Mutian X because of the absence of a faecal sample immediately prior to Mutian X treatment: it is therefore possible that they had ceased shedding virus prior to onset of treatment. However, it is also possible that they had stopped shedding virus within 24 hours of treatment.
Total		**34**[a]	**16**	

FCoV pos = FCoV being shed in faeces during time of the Mutian treatment part of the study.
FCoV neg = FCoV not being shed in faeces during time of the Mutian treatment part of the study.
[a] Five positive cats in Household E had to be excluded (see the 'Reason For Faecal RT-PCR' testing column), leaving 29 cats in the study.

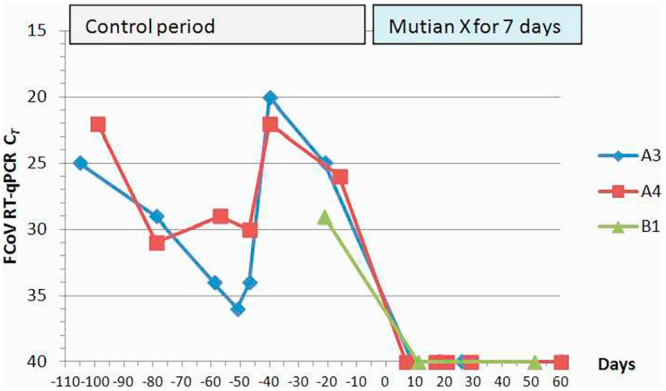

Fig. 1. FCoV shedding in cats treated with 4 mg/kg Mutian X for 7 days in Households A and B.
In this graph, the records of faecal FCoV RNA detection—RT-qPCR C_T—is shown on the y axis and the time in days on the x axis for three cats A3, A4 and B1. Each time point indicates a faecal test. This graph is normalised to time zero for test period for Mutian X start.
Mutian X was given for seven days from Days 0 to Cats A3, A4, and B1 after which their faecal samples remained negative for 155, 157 and 51 days (x axis only shows up to 60 days). The graph shows that no intermittent virus shedding or re-infection occurred.

3. Results

The results are summarised in Table 2. Prior to this study, the owner of Household C had attempted various Mutian X treatment regimes in her cats. A single dose treatment was attempted on two cats (C10 and C12) and while virus load reduced, it failed to clear infection and virus load increased again in Cat C10. Cat C1 was given a single injection of Mutian three days prior to oral treatment which had reduced her virus shedding from C_T 20 to 32. Thus a single dose was ineffective at clearing infection and may have risked the emergence of resistant viruses.

3.1. Results of Mutian X at a dose of 2 mg/kg

Ten cats received a full course at 2 mg/kg and two other cats began at 2 mg/kg then were increased to 4 mg/kg. Eight of ten cats stopped shedding FCoV using Mutian X tablets at a dose of 2 mg/kg SID (Fig. 2A) for seven or four days. Two probable FCoV carrier cats (C1 and C2) that had shed virus at levels of C_T 18–20 (C1) and C_T 35 (C2) for over a year prior to treatment were treated for seven days at a dose of 2 mg/kg after which their faeces were negative, C1 and C2 remained negative when tested five and seven days later.

The remaining eight cats were treated for four days as shown in Fig. 2A and Table 2. Daily samples were available for five cats; two (C3 and C5) cleared the virus within 24 hours and three under 72 h (C4, C6 and C7). The other cats were not tested daily, so an accurate time of cessation of virus shedding could not be determined.

Cats C8 and C10 failed to totally clear the virus using the 2 mg/kg dose for four days; therefore they were treated again at 4 mg/kg, after which they became negative, as shown in Fig. 2B and Table 2. Cat C6 was in the same group as C8 and C10, and began shedding virus again within nine days, requiring a second course of treatment (Figs. 2A and B).

Two more cats (E1 and E2) started treatment at 2 mg/kg SID but were changed to the higher dose within 48 hours due to the results from Household C (Fig. 3): these cats were therefore included in the 4 mg/kg rather than the 2 mg/kg category.

In summary, a Mutian X dose of 2 mg/kg was considered inadequate: it had cleared the virus in eight cats and reduced, but not abrogated, virus shedding in two cats (Fig. 2A). Thereafter, the dose

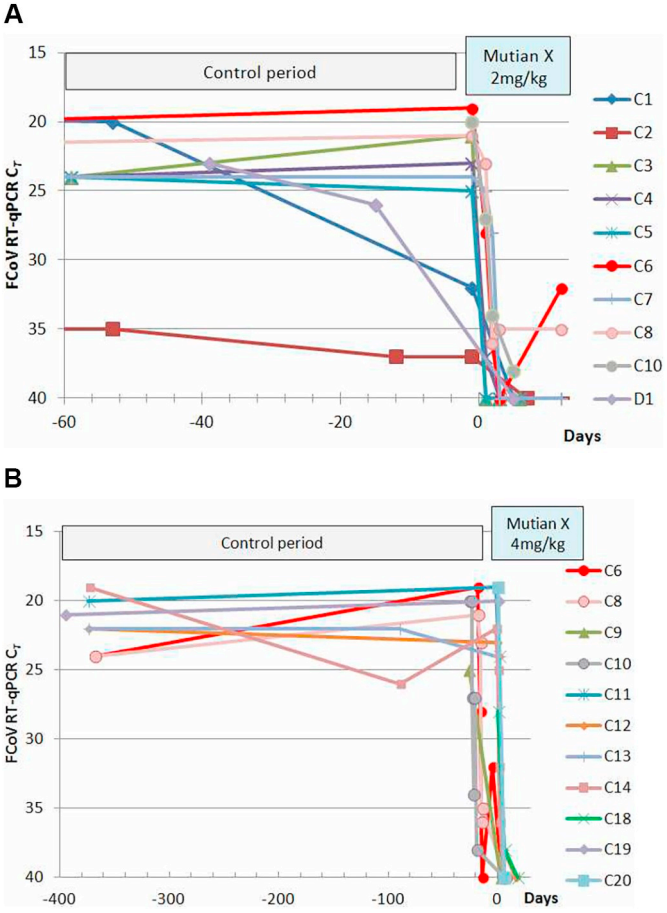

Fig. 2. Faecal FCoV shedding before and after treatment with Mutian X at a dose of 2 mg/kg and 4 mg/kg q24 h in Households C and D.
Faecal FCoV RNA detection—RT-qPCR C_T—is shown on the y axis and the time in days on the x axis for ten cats treated with Mutian X at an oral dose of 2 mg/kg SID (Fig. 2A), and for eleven cats at a dose of 4 mg/kg SID (Fig. 2B). Each time point indicates a faecal test. These graphs are normalised to time zero for the start of the Mutian X treatment and cut off at Day 60 and 400 prior to testing: the exact extent of the no treatment control period is indicated in Table 2.
Two cats (C8 and C10) failed to clear the infection at 2 mg/kg dose (Fig. 2A) and were treated again at 4 mg/kg (Fig. 2B). Cat C6 was kept in a group with Cats C8 and C10, became re-infected, and required re-treatment, which is why this cat also features in both figures.

was increased to 4 mg/kg.

3.2. Results of Mutian X at a dose of 4 mg/kg

A dose of 4 mg/kg reliably cleared virus within seven days in 21 of 22 (95%) cats (two cats from Household A, one from Household B, ten of eleven from Household C and eight from Household E) (Figs.2B and 3). Cat C18 was the only cat who failed to clear the virus after a six day course of 4 mg/kg dose, but she vomited some of the capsules and she required a repeat course for three more days after which she was negative.

3.3. Summary: Mutian X cleared 29 cats of FCoV infection

Ten cats received a full course of Mutian X at 2 mg/kg: eight cats (80%) cleared infection, but Cats C8 and C10 did not, and Cat C6 who was housed with them, began shedding virus again. Therefore at the end of that part of the study seven cats were clear of virus, and three required to be re-treated at 4 mg/kg.

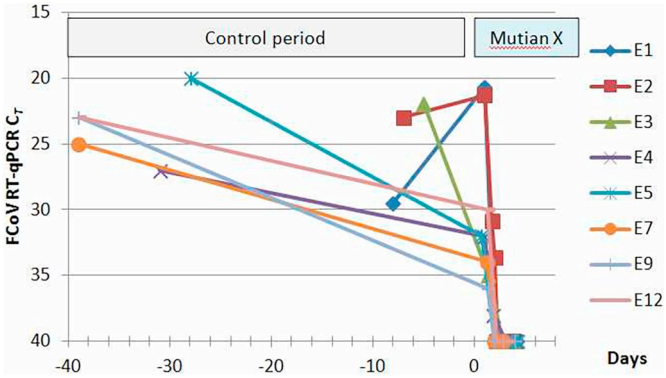

Fig. 3. Faecal FCoV shedding before and after treatment with Mutian X in Household E.
Faecal FCoV RNA detection—RT-qPCR C_T—is shown on the y axis and the time in days on the x axis for eight cats in Household E treated with Mutian X at an oral dose of 4 mg/kg SID for four days (Cats E1 and E2 were started on a dose of 2 mg/kg, but increased to 4 mg/kg). Each time point indicates a faecal test. Cat results are normalised to time zero for the start of the Mutian X treatment.

Twenty-two cats received a course of Mutian X at a dose of 4 mg/kg and 21 cats (95%) became negative. Cat C18, who had vomited some of the capsules, required re-treatment, after which she too, became negative. Thus all 29 cats were cleared of infection, but four cats had required a second course of treatment.

3.4. Cats shedding more virus did not require a longer course of Mutian X

To determine whether cats that were shedding a higher amount of virus required more days of anti-viral treatment than cats with a lower virus quantity, starting FCoV RNA C_T was plotted against days to become negative. Serial accurately timed daily faecal samples were available for 14 cats (Fig. 4) and all 14 cleared virus within 75 h of starting treatment.

No correlation between starting FCoV RNA C_T and time to become negative was found ($R = 0.0048$.

3.5. Comparison of Mutian X vs cessation of virus shedding control periods

Pre-treatment virus shedding records were used as a retrospective control in this observational study: FCoV virus shedding results prior to Mutian X administration were available for 25 treated cats (shown in Table 2). Two additional cats in Household C spontaneously stopped shedding virus prior to the start of treatment period (data not shown): one cat was kept in isolation and one was allowed outdoors. Therefore two of 27 (7.4%) cats stopped shedding virus during the control period: the difference between the number of cats stopping shedding virus during the control period and subsequent to Mutian X at 4 mg/kg was statistically significant ($p < .00001$).

3.6. Cats that had cleared virus using Mutian X did not spontaneously begin shedding virus again

Repeat negative samples were available from six cats dosed at 2 mg/kg and 12 cats dosed at 4 mg/kg (Table 2). Cats dosed at 2 mg/kg and 4 mg/kg remained negative for at least three to 18 days and one to 157 days respectively from their first negative result, showing that the cats remained negative post-treatment and did not start spontaneously shedding virus again. FCoV antibody titres were available for Cats A3 and A4 five months post-treatment: they had reduced from 1280 to 80 and from over 1280 to 320 respectively.

4. Discussion

Mutian X pills stopped faecal FCoV shedding in 29 naturally infected cats; however, four of the 29 cats required a second course of treatment before virus was eliminated.

Since successful treatment of FIP using anti-viral drugs was first reported (Pedersen et al., 2018; Pedersen et al., 2019) cat guardians have been able to source various anti-FCoV drugs, including Mutian X, via social media. Our view is that it is preferable that if they propose to use such medications, even though the products are unlicensed, they should do so under proper veterinary guidance, rather than following advice from social media group moderators, however knowledgeable. Professor Pedersen has provided a lucid view of the current situation in relation to the treatment of cats with FIP with 'black market' drugs, and the subsequent dilemma for veterinary surgeons. (https://ccah.vetmed.ucdavis.edu/sites/g/files/dgvnsk4586/files/inline-files/Black%20market%20production%20and%20sale%20of%20GS_0.pdf).

To paraphrase Professor Niels Pedersen, we would prefer that these drugs be approved, licensed, and made available in the normal manner, rather than being sold on the black market, but we are willing to advise veterinary surgeons and their clients. Advice from those with FIP experience is likely to reduce unnecessary use of anti-coronavirus drugs: in two of the five households reported here FIP therapy levels of anti-viral treatment was begun in two cats which did not have FIP, and ceased when a proper diagnosis was obtained: anti-viral treatment has been stopped in nine other mis-diagnosed cats (DA personal observation). We present an observational study of cat guardians who were using Mutian X to stop their cats from shedding virus, and have defined a protocol which optimises and minimises its use. Furthermore, we hope that by stopping cats shedding FCoV, especially in purebred cats, the prevalence of FIP will reduce, which will further reduce the use of unlicensed drugs.

A four day course of Mutian X at a dose of 2 mg/kg cleared coronavirus in eight cats, but failed to clear infection in Cats C8 and C10. Some opinion leaders have expressed concern that use of Mutian X to stop virus shedding in cats without FIP will lead to drug resistance and failure of the drug to treat FIP, although the ability of Mutian X to treat FIP has not actually yet been documented, so far as we are aware. We ask those holding such views to consider the following: first that this drug is already being used for the purpose of virus elimination by cat guardians and surely it is better that a proper protocol has been developed, rather than they are left to do trial and error themselves. Second, we have found that an intensive four day course stopped virus shedding while cats being treated for FIP have higher virus loads (Kipar et al., 2006) and require a longer course of treatment, up to 12 weeks (Pedersen et al., 2019), which surely is more likely to allow resistant virus strains to develop? Indeed, virus resistance to a nucleoside analogue was documented in a cat being treated for FIP (Pedersen et al., 2019). Anti-virals are used prophylactically in other viral infections: there is precedent for this approach.

We found no evidence for the development of drug resistance in asymptomatic cats at a dose of 4 mg/kg. In our view treating breeding queens for four days to stop FCoV shedding is less likely to induce drug resistance than treating at least one in ten of their kittens for FIP, requiring 12 weeks of medication. However, treatment of virus shedding cats must be done properly: one concern is that cat guardians may be tempted to reduce cost and use an insufficient dose or duration of treatment (SC and DDA, personal observations), which might result in selection of viruses which are resistant to Mutian X. Another concern is that a blunderbuss approach may be taken: treating all the cats in a household without first confirming that they are virus shedders.

No correlation was found between FCoV RNA C_T prior to treatment and time to become negative, therefore cats shedding more virus did not require a longer treatment course than cats shedding a lower amount of virus and a four day course is likely to suffice, rather than seven days.

Table 2
Duration of FCoV shedding during control period and during Mutian X at 2 mg/kg and 4 mg/kg.

Cats	Control	Duration of virus shedding (days)	Number cats stopped shedding during control period	Dose and duration of Mutian X	Number cats stopped shedding FCoV on Mutian X	Duration of virus shedding (days)	Known duration of absence of FCoV shedding post Mutian X (days)
A3	Itraconazole 10 mg/kg	>105	0/2 (A3 reduced while on	4 mg/kg q24	2/2	<7	155
A4	21 days; FeIFNω	>99	itraconazole then increased	hours for			157
	100,000 units q24 hrs per os		when stopped)	7 days.			
B1		Unknown		4 mg/kg q24 hours for 7 days.	1/1	< 7	51
C1	No treatment	>327	0/2	2 mg/kg q24	2/2	<5	5
C2		>337		hours for 7 days		<7	7
C3	No treatment	>59	0/3	2 mg/kg q24 for	3/3	1	5
C4		>59		4 days		3	3
C5		>59				1	5
C6	No treatment	>368	0/4	2 mg/kg q24 hours	2/4	4	Re-infected & re-treated
C7		>106		for 4 days		3	18
C8		>368				>12	Re-treated
C10		>1				>5	Re-treated
C8	These two cats failed to clear virus on 2 mg/kg and were re-treated at 4 mg/kg			4 mg/kg	3/3	<4	Not re-tested
C10	Housed with C8 and C10, re-infected and re-treated at 4 mg/kg					<5	Not re-tested
C6						See above	Not re-tested
C11	No treatment	>374	0/3	4 mg/kg[a]	3/3	<5[a]	11
C12		>374					Not re-tested
C13		>374				<5	Not re-tested
C9	No treatment	Unknown	0/1	4 mg/kg	2/2	<3	Not re-tested
C14		>373				<6	Not re-tested
C18	No treatment	Unknown		4 mg/kg for		>6[b]	Not re-tested
C19		>395		6 days		<11	Not re-tested
C20		Unknown	0/1	(C18)	2/3[b]	<11	Not re-tested
D1	Itraconazole 10 mg/kg	>39	0/1	2 mg/kg	1/1	<5	Not re-tested
	21 days						
E1	No treatment	>8	0/3	4 mg/kg	3/3	2	42
E2		>7				2.25	50
E3		>5				2.75	47
E4	No treatment	>31	0/5	4 mg/kg	5/5	3	31
E5		>28				2	30
E7		>39				2	1
E9		>39				2	2
E12		>39				2.21	2

> at least.
< up to.
This table shows the duration of 29 FCoV shedding cats either not treated, or treated with itraconazole, or FeIFNω and probiotics, compared with duration of virus shedding on Mutian X treatment. Cats are organised according to their groups in their homes. Cats C6, C8 and C10 are listed twice. Although none of 25 cats for which pre-treatment records were available stopped shedding virus during the control period, this table does not show the virus shedding of untreated cats, two of which became negative in Household C prior to the study.
[a] Cat C12 was treated for one day, missed a day, one day of 2 mg/kg then 2 days of 4 mg/kg - low positive at C_T 38. Then 3 days of 4 mg/kg then negative.
[b] Cat C18 faecal sample was C_T 38 at Day 6 and so the cat was re-treated for 3 days and subsequently became negative. This cat had vomited some of the capsules.

Since the study was observational rather than prospective, there was no formal placebo group. The lack of a placebo group in this study was problematic: it could be argued that the cats spontaneously stopped shedding FCoV, co-incidental with the onset of Mutian X therapy. Cats do spontaneously stop shedding FCoV some months post-infection: FCoV-I is shed in the faeces for months to years (Addie et al., 1995; Addie and Jarrett, 2001; Addie et al., 2003). Little is known about FCoV-II shedding, in one experimental infection of laboratory cats virus was shed for up to 15 days (Stoddart et al., 1988); and no persistently FCoV-II infected cat has been reported (Addie and Jarrett, 2001; Addie et al., 2003). That said, it is unlikely that 29 cats would all cease virus shedding co-incidentally within days of beginning Mutian X treatment, we did retrospectively compare the results prior to Mutian X, when virus eradication was attempted by quarantine and hygiene, and/or itraconazole, and FeIFNω with and without probiotics.

Another argument might be that the cats stopped shedding virus as a delayed result of the treatments given to some cats prior to Mutian X: this argument could not apply to Households B, C, and E, where the cats did not receive any other treatments. However, in Household A, virus load did decrease in two cats on probiotics (Fortiflora, Purina Pro Plan, USA) and FeIFNω, but remained at a constant level in the third cat. Therefore, a combination of interferon and probiotics may have reduced FCoV shedding. FeIFNω has previously been shown to reduce, but not stop, FCoV shedding (Gil et al., 2013). Probiotics are routinely used to treat children with viral diarrhoea (Szajewska et al., 2019), but the interaction of the gut microbiome and FCoV has not been extensively explored (Meazzi et al., 2019): further investigation into the possibility that probiotics might reduce coronavirus load is warranted.

Cats in Households A and D also received itraconazole which has been shown to have in-vitro activity against FCoV-I (Takano et al., 2019). However, a 21 day course of treatment failed to clear these three cats of FCoV, although viral RNA quantity did decrease in Cat A3 while she was on itraconazole, but bounced back as soon as it was stopped.

Another possibility was that the Mutian X could be excreted in the faeces and be inhibiting the FCoV RT-qPCR assay, but this was unlikely because, as shown in Figs. 2 and 3, FCoV RNA was being detected in cats during Mutian X treatment, and also because faecal samples remained negative after the cats had ceased Mutian X.

While a four-day course of Mutian X tablets at 4 mg/kg appeared to be effective in stopping FCoV shedding, daily monitoring of faecal FCoV

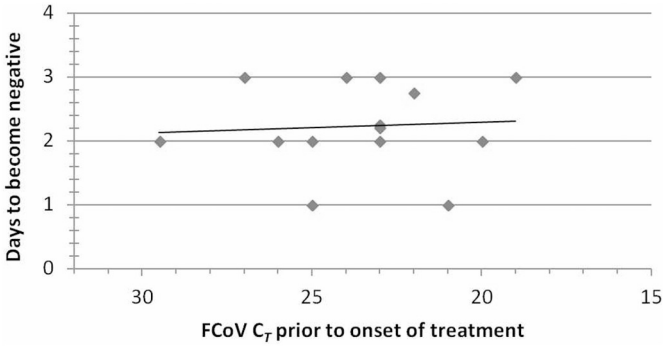

Fig. 4. Lower FCoV RNA C_T did not correlate with requirement for a longer duration of treatment.
The aim of this scatter plot was to determine whether cats that were shedding a higher amount of virus required more days of anti-viral treatment than cats with a lower virus quantity. Daily monitoring of faeces was available for 14 cats (there are 4 results at C_T 23, two are overlaid which gives an appearance of 13 samples). Although there was a slight incline in the trendline, there was no correlation between starting FCoV RNA C_T and days to a negative result ($R = 0.0048$).

shedding using a laboratory which rapidly reports virus quantity is necessary to tailor treatment duration according to the circumstances of each individual cat. The virus is highly contagious, and if cats are sharing litter trays, they will be rapidly re-infected, as was the experience in Households C and E, with large numbers of cats, even though they were kept in small groups. In Household C, re-infection of cat C6 occurred in less than nine days: she was housed with one cat who had failed to clear the virus on a 2 mg/kg dose of Mutian X; the cat litter used was sawdust based. It could be argued that Cat C6 was shedding virus intermittently, but C6 was housed in a group who had failed to clear virus at the 2 mg/kg dose, thus was far more likely to have been re-infected than latently infected.

Intermittent virus shedding was not seen in any cat in this trial, nor in previous studies of sequential tests on hundreds of cats using the modern, sensitive FCoV RT-qPCR technology (Addie, unpublished observation), and controlling for false negative results due to faecal PCR inhibitors (Dye et al., 2008). In our experience, cats either shed virus continuously or they stop shedding virus: the appearance of intermittent virus shedding could always be traced back to exposure to the faeces of an infected cat; or where virus quantity was so low that it was on the borderline of the ability of the assay to detect it, or by the presence of excessive cat litter on the sample: sodium bentonite based cat litters bind organic material.

No side effects of Mutian X were observed by us other than one cat having initially vomited some capsules, which might have been an adverse reaction, although that cat didn't vomit subsequent capsules. However, that does not mean that Mutian X is without side effects: elevation of liver enzymes has been seen in cats with FIP that are being treated for many weeks (Addie, unpublished observation).

Use of a laboratory that has an adequately sensitive FCoV RT-qPCR test is absolutely essential for the detection and eradication of FCoV from a household. False negative results will doom the effort to failure. Six of eight samples sent to one highly reputable university veterinary laboratory were falsely reported as negative (data not shown). A major problem identified during our study was that the delay in reporting FCoV RT-qPCR results—usually of at least one week from the date of sample submission—delayed separation of negative and positive cats, and allowed re-infection of cats that had cleared infection.

5. Conclusion

This study showed that the oral administration of Mutian X tablets, at a dose of 4 mg/kg for four days, stopped shedding of FCoV from naturally infected cats. This treatment would be a useful adjunct for establishing FCoV-free households of cats, in addition to current measures of hygiene, housing cats in small groups and the use of virus-inhibiting cat litter (Addie et al., 2019). Further observations of cats which stopped shedding virus should determine whether Mutian X can prevent the development of FIP. Also, if the serum anti-FCoV titres of treated cats decline over the coming year, it will indicate that the virus has been cleared systemically, as well as from the gastrointestinal tract.

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Declaration of Competing Interest

There is no conflict of interest of any authors in relation to the submission.

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