Persistent feline coronavirus infection in a cat with cardiac and gastrointestinal signs

Background Feline coronavirus infection causes feline infectious peritonitis in a subset of cats, but can also result in persistent infection. The tissue reservoirs of feline coronavirus and the role of viral persistence in pathogenesis are poorly understood.
Aims This study aimed to identify sites of feline coronavirus persistence in a naturally infected cat, identify disease correlates and characterise within-host viral evolution.
Methods The study followed a 5-year-old Bengal cat for 6 years and collected non-invasive samples, including faeces and conjunctival, oropharyngeal and saliva swabs. At 11-years-old, the patient was euthanised as a result of respiratory distress, and tissue samples were collected. The authors used hybridisation capture and next-generation sequencing methodologies focused on the feline coronavirus S gene, along with RNA in-situ hybridisation.
Results During the study, the patient was diagnosed with inflammatory bowel disease, alimentary small cell lymphoma, chronic rhinitis and mitral valve regurgitation. Feline coronavirus was detected in the nasal cavity, intestine, faeces and conjunctiva in 2017, and in the intestine, faeces and heart in 2022. Sequence analysis showed that the virus adapted to tissue reservoirs over time.
Conclusions This study identifies potential feline coronavirus reservoirs. The relationship of persistent feline coronavirus infection to chronic conditions warrants further investigation.
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Feline coronavirus is a common and highly transmissible infection of cats. Consistent with the first clinical report (Holzworth, 1963), a recent phylogeographic study indicated that feline coronavirus type 1 (by far the most prevalent of the two viral genotypes) emerged in the United States between the 1950s and the 1970s, followed by rapid spread worldwide (Lauzi et al, 2020). It is now endemic everywhere that cats are housed in groups. Transmission is generally considered to be faecal-oral, leading to infection of enterocytes and causing mild and self-limiting gastroenteritis. While enterocytes appear to be the primary target of infection, a monocyte-associated viraemia is common, even in healthy animals (Gunn-Moore, 1998; Can Sahna, 2007). In a small subset of cats, feline coronavirus gains the ability to productively infect monocytes and tissue macrophages, triggering systemic vasculitis. This form of feline coronavirus infection, known as feline infectious peritonitis, is historically fatal; however, recent advances in the development of antivirals have led to rapid advances in the treatment of this otherwise lethal disease (Coggins et al, 2023).

The prevalence of feline coronavirus has been estimated at 75–95% in multi-cat households (Pedersen, 1995) and approaching 100% in high density shelter or breeder situations. Feline coronavirus infection has several clinical outcomes (*Figure 1*) (Thayer et al, 2022), with the colon being identified as a primary site of feline coronavirus persistence. This allows carrier cats to serve as a source of new infections (Herrewegh et al, 1997; Kipar et al, 2010). To date, genomic analysis of the persistent gastrointestinal reservoir has received little attention.

Feline infectious peritonitis arises in a small subset of infected cats (<5%; Thayer et al, 2022), and these viruses have been more extensively analysed genomically. Overall, a combination of both host and viral factors are thought to lead to the development of feline infectious peritonitis within feline coronavirus-infected cats. Viral mu-

tations associated with the transition to feline infectious peritonitis have been documented in multiple areas of the viral genome (such as open reading frame 3 and 7), but new genomic analysis now suggests that mutations in the viral spike (S) protein is key to this transition (Zehr et al, 2023). The S protein is responsible for viral entry into the host cell (Jaimes et al, 2020). Along with the spike 'M1058L' substitution (Barker and Tasker, 2020), mutations in an important viral activation motif known as the S1/S2 cleavage site have been linked to feline infectious peritonitis pathogenesis and systemic spread (Licitra et al, 2013; André et al, 2019; 2020; Healey et al, 2022) and are a focus of this study. Notably, even if a cat does not develop feline infectious peritonitis, the S gene is under high evolutionary selective pressure (Herrewegh et al, 1997; Desmarets et al, 2016; Zehr et al, 2023).

The study of viral pathogenesis during persistent infections is essential to understand how evolution can result in viral variants having a range of disease profiles (Griffin, 2022), including the acute presentation of feline infectious peritonitis. This study sought to identify viral reservoirs outside of the colon, note potential correlations with the development of chronic feline diseases and characterise within-host evolution in an area of the genome under high selective pressure. Whether feline coronavirus infection is associated with the development of chronic feline diseases remains an important question for future study.

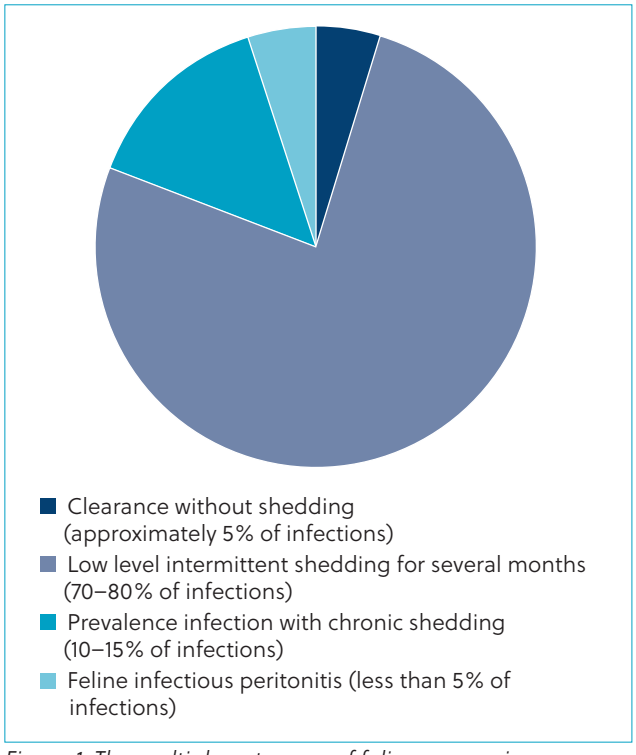

Figure 1. The multiple outcomes of feline coronavirus infection on domestic cats.

Methods

Sample collection

A 5-year-old neutered male Bengal cat was enrolled in 2017 as a healthy cat in a long-term feline coronavirus project (cat ID #576). Over this time period, the cat was clinically diagnosed with inflammatory bowel disease and chronic rhinitis, with biopsy and histopathology performed in 2017. He was also diagnosed with a low-grade heart murmur secondary to mitral valve regurgitation. From 2017 to 2022, the authors collected various non-invasive samples, including faeces and conjunctival, oropharyngeal, and saliva swabs (*Table 1*). In 2017, samples such as blood, and nasal and intestinal biopsies were taken during routine vet visits. The faecal, swab and blood samples were analysed immediately after collection, then frozen at -80°C until further use. The tissues resulting from the biopsies were preserved in formalin-fixed paraffin-embedded blocks by the Animal Health Diagnostic Center, Cornell University. The samples taken in 2019 and 2020 were stored in DNA/RNAshield (Zymo Research) and submitted to the Metacats project (http://metasub.org/metacats). In 2022, the cat was euthanised at 11 years of age because of respiratory distress. A necropsy was performed by the Cornell University College of Veterinary Medicine pathology service. Eighteen tissues and biofluids were collected at necropsy and were deposited in the Cornell Veterinary Biobank (https://www.vet.cornell.edu/departments/centers/cornell-veterinary-biobank) under accession number 29801. The tissues were flash-frozen in liquid nitrogen and stored at -80°C until further use, and the biofluids were frozen at -80°C immediately after collection.

RNA-based in-situ hybridisation

A probe targeting the RdRp gene of feline coronavirus was used to detect viral RNA (RNAscope 2.5 VS Probe V-FIPV-ORF1a1b, Advanced Cell Diagnostics) using in-situ hybridisation (Sweet et al, 2022). The in-situ hybridisation process was carried out by the Animal Health Diagnostic Center using the automated staining platform Ventana Discovery Ultra (Roche Tissue Diagnostics) and the Discovery kits for mRNA Sample Prep, mRNA Red Probe Amplification and mRNA Red Detection and the Ventana Hematoxylin and Ventana Bluing Reagents for counterstaining. Detailed methods for RNA in-situ hybridisation were performed, as published by Sweet et al (2022).

Feline coronavirus sequence analysis

Viral sequencing was performed by hybrid capture methodology (Houldcroft et al, 2017; Gaudin and Desnues, 2018) and libraries were sequenced on the iSeq 100 platform (Illumina). Detailed methodologies of RNA extraction and sequencing can be found in Olarte-Castillo et al (2023).

Phylogenetic analysis

The obtained nucleotide sequences of the S1/S2 region (furin cleavage site) for all the positive samples were aligned using the Clustal Omega algorithm (Sievers et al, 2011). An unrooted neighbour-joining tree was constructed using the nucleotide sequence of the seven variants obtained using Geneious Prime 2022.1.1.

Results

At the time of enrolment in the authors' feline coronavirus study in 2017, the patient weighed 4.7 kg and had a body condition score of 5/9. Before enrolment, he had been diagnosed with feline asthma. Over the study period, the patient's diagnosis list was expanded to include inflammatory bowel disease and chronic rhinitis, based on

Table 1. Samples from cat #576 collected and analysed in this study

Sample type	Collection date	Feline coronavirus screening result
Nasal biopsy	04/28/17	Positive
Intestinal biopsy	04/28/17	Positive
White blood cells	11/17/17	Negative
Conjunctival swab	11/17/17	Positive
Oropharyngeal swab	11/20/17	Negative
Faeces	11/20/17	Positive
Faeces	09/05/19	Negative
Faeces	2020	Negative
Saliva swab	2020	Negative
Brain	01/06/22	Negative
Lung	01/06/22	Negative
Urine	01/06/22	Negative
Faeces	01/06/22	Negative
Stomach content	01/06/22	Negative
Kidney	01/06/22	Negative
Intestine - ileum	01/06/22	Negative
Intestine - duodenum	01/06/22	Negative
Intestine - jejunum	01/06/22	Positive
Spleen	01/06/22	Negative
Heart ventricle	01/06/22	Positive
Liver	01/06/22	Negative
Pancreas	01/06/22	Negative
Lymph node	01/06/22	Negative
Eye, cornea	01/06/22	Negative
Mucosal tissue, nasal cavity	01/06/22	Negative
Plasma, EDTA	01/06/22	Negative
Serum	01/06/22	Negative

biopsies and histopathology. He was also diagnosed with a low-grade heart murmur secondary to mitral valve regurgitation. The patient's upper respiratory signs were managed with doxycycline (25 mg by mouth once daily during periods when the animal was experiencing flares). In total, 27 samples were screened for the presence of feline coronavirus RNA, of which seven were positive (*Table 1*).

To explore the tissue distribution of feline coronavirus, samples collected at necropsy were examined histologically using RNA in-situ hybridisation. Using this technique, feline coronavirus RNA was detected in isolated cells in the heart and in epithelial cells of the small intestine (jejunum) (*Figure 2*).

From six of the seven positive samples, a 120-nucleotide segment of the S gene was obtained and sequenced. This region includes the core residues of the FCoV-1 spike S1/S2 cleavage site which has been associated with feline infectious peritonitis progression (Licitra et al, 2013). It is also a region that may be associated with systemic spread and is known to be under high selective pressure (Zehr et al, 2023). In the sequences obtained, both variants obtained from faeces were identical despite the 6-year difference in collection date, and the variants from the heart only differed in one nucleotide, which resulted in a different residue of the S1/S2 cleavage site (*Figure 3*). The amino acid sequences of the S1/S2 cleavage site of feline coronavirus obtained from the conjunctiva, intestine and nasal tissue in 2017 were all different (*Figure 3*). The S1/S2 cleavage site of the feline coronavirus from the conjunctiva sample taken in 2017 has two additional residues (*Figure 3*, underlined). Sequences from the faeces and intestine were consistent with a typical feline enteric coronavirus-like virus; however, tissue samples indicated a down-regulated furin cleavage site (via point mutation, coloured red in *Figure 3*) with the conjunctival sample predicted to up-regulate the furin cleavage site (via an insertion, indicted with bold underline in *Figure 3*).

In 2017, nasal biopsies showed evidence of moderate, diffuse, acute neutrophilic rhinitis with neutrophilic and suppurative exudate. There was no evidence of neoplasia in the examined sections. These findings demonstrate an inflammatory process composed of chronic plasma cells and lymphocytes, as well as a more acute neutrophilic component. In the intestinal biopsies taken in 2017, there was evidence of mild-to-moderate, diffuse, chronic lymphoplasmacytic enteritis with intraepithelial lymphocytes and rare nests in the duodenum, consistent with an inflammatory process. In the necropsy report performed in 2022, the immunohistochemical stains reveal a majority of T-lymphocytes infiltrating the villi epithelium and lamina propria in the jejunum. These features are indicative of small-cell, T-lymphocyte, epitheliotropic alimentary lymphoma.

Discussion

The authors demonstrated that feline coronavirus variants detected in the faeces of the same individual over 5 years were identical in their spike S1/S2 sequence. This supports the idea that persistent viral shedding by an individual is the result of persistent feline coronavirus infection from an initial infection, rather than re-infection with a distinct variant or strain.

Interestingly, associated pathologies related to the tissues in which feline coronavirus was found (intestine, nasal cavity, heart) were reported for this cat and included a long history of inflammatory bowel disease and heart murmur. The cat also experienced chronic rhinitis, and although feline coronavirus RNA was found in the nasal biopsy taken in 2017, it was not found in the mucosal tissue of the nasal cavity taken in 2022 (*Table 1*). Therefore, it cannot be determined whether the virus remained in the nasal cavity over time. In humans, persistent infection with different viruses has also been related to chronic symptoms that linger after the patient's recovery from the acute infection. For example, cardiomyopathies have been related to persistent infection with enterovirus (Kühl et al, 2005) and SARS-CoV-2 (Omidi et al, 2021). Weakness, fatigue, memory loss and ataxia have also been linked to persistent viral infection with West Nile virus and post-

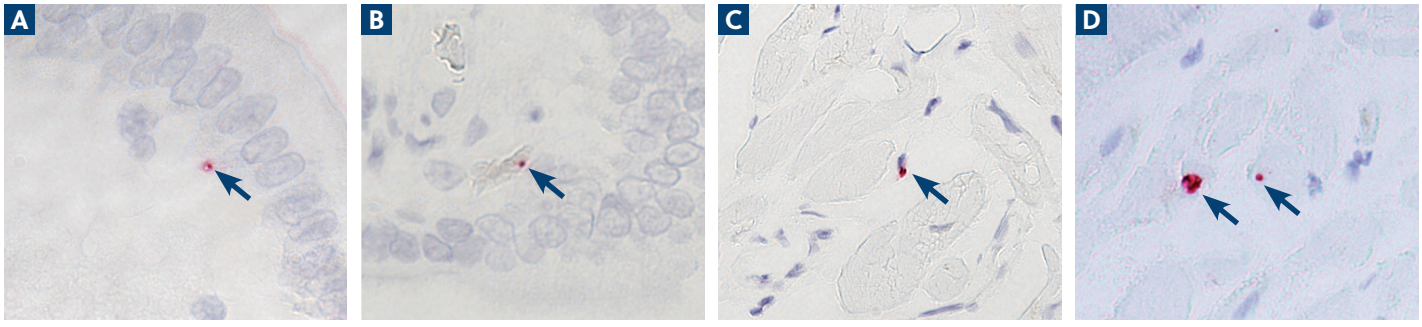

Figure 2. Detection of feline coronavirus RNA (in magenta, indicated by arrows) by in-situ hybridisation in (A, B) intestine and (C, D) heart of cat #576 in 2022. All photos were taken at x600 magnification.

acute sequelae of coronavirus infection, or 'long COVID' (Halpin et al, 2021). Although further studies are needed to assess if feline coronavirus can cause tissue-specific pathologies by establishing a viral reservoir in different tissues (eg inflammatory bowel disease, heart murmur, rhinitis), several studies have linked feline coronavirus infection with certain chronic ailments of cats. For example, myocarditis has been previously reported in a cat with feline infectious peritonitis (Ernandes et al, 2019) with pronounced viral antigen in the heart detected by immunohistochemistry, along with a report of feline infectious peritonitis-linked dilated cardiomyopathy (Yoshida et al, 2016). There are also reports of feline coronavirus in the heart and muscle tissue in a sub-clinical cat (Kiss et al, 2000) and one with epicarditis (Araujo et al, 2020). In addition, Malbon et al (2019) noted the involvement of both the liver and heart in feline coronavirus pathogenesis. However, no direct assessment of feline coronavirus infection of heart tissue was evaluated, by either polymerase chain reaction or histology. Feline coronavirus infection has been detected in a cat with rhinitis (André et al, 2020) and in persistently infected cats with chronic diarrhoea (Addie and Jarrett, 2001). It is also important to note that one report revealed that administering the non-Food and Drug Administration approved anti-viral drug GS-441524 to cats infected with feline coronavirus and experiencing chronic enteropathy or inflammatory bowel disease resulted in the elimination of viral shedding in faeces and the resolution of chronic gastrointestinal symptoms in all cats involved in the study (n=7) (Addie et al, 2023).

The author's studies show that cardiac tissue can harbour a virus with an S1/S2 mutation typically associated with feline infectious peritonitis, but this cat did not present as a cat with feline infectious peritonitis. This suggests that viruses with modified cleavage sites do not necessarily result in progression to feline infectious peritonitis, and that mutated viruses need to be present in distinct tissues for disease development. Samples from organs that filter interstitial fluid collected from soft tissues and return it to the vascular system (eg lymph nodes), and that may have more defined a role in feline infectious peritonitis progression may be more diagnostic, even though these samples were negative in this persistently infected cat that was not clinically diagnosed with feline infectious peritonitis. The mutated S1/S2 regions in the nasal and heart tissue may reflect the virus in a quiescent and non-transmissible state, compared to the conjunctival and faecal samples where shedding is likely to be occurring.

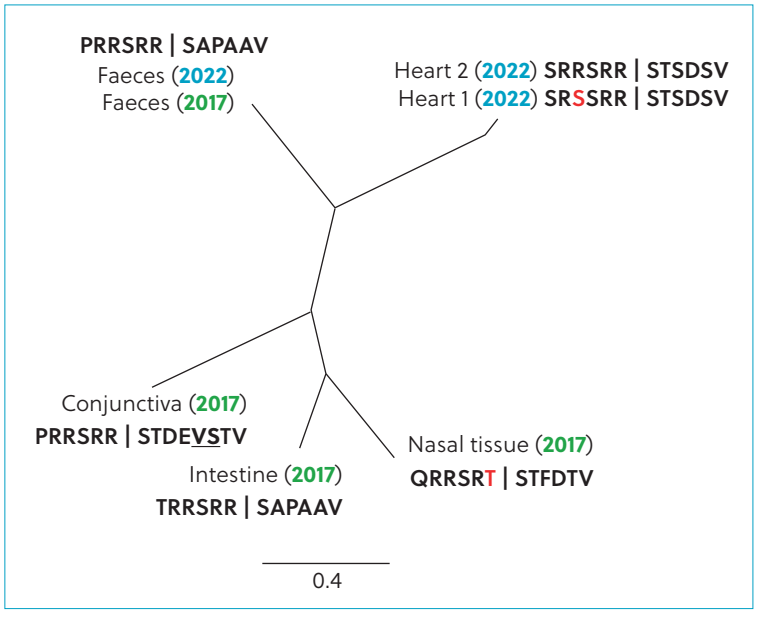

Figure 3. Unrooted phylogenetic tree of the S1/S2 region (120nt) of seven variants of feline coronavirus obtained from cat #576 in 2017 (in green) and 2022 (in blue). Each sample name includes the tissue in which the virus was detected and in parenthesis the year of collection. Next to or below each name are the amino acid sequences of the S1/S2 cleavage site in which a vertical line indicates the site in which cleavage occurs. Additional residues in the S1/S2 cleavage site are underlined. The length of the branches is proportional to the number of nucleotide changes between the sequences.

Conclusions

This study is unique in that it follows a persistently infected animal over the course of 6 years, identifying potential feline coronavirus reservoirs in the nasal cavity, conjunctiva, heart and intestine. Sequence analysis provided evidence that the virus evolved within the patient over time to adapt to different tissue reservoirs. CA

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KEY POINTS

- Feline coronavirus can persist in cats over long time periods without causing clinical feline infectious peritonitis.
- The virus can spread to the heart tissue, with sequences predictive of spike protein down-regulation.
- The virus may be shed from the conjunctiva, with sequences predictive of spike protein up-regulation.
- The virus evolves over time, and in different tissues.
- Virus evolution in persistently infected animals may impact disease presentation and virus transmission.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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