An outbreak of a pathogenic canine coronavirus type 2 in captive snow leopards *(Panthera uncia)* in the U.S, with severe gastrointestinal signs

Ximena A. Olarte-Castillo [1] and Gary R. Whittaker [1,2]

1. Department of Microbiology & Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA
2. Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA.

Abstract

The species *alphacoronavirus-1* comprises a set of diverse viruses of cats, dogs, and pigs, and is highly recombinogenic. Within this species, canine coronavirus type 2 (CCoV-2) can infect multiple species of canids, causing a range of clinical outcomes. CCoV-2 is genetically related to feline coronavirus type 1 (FCoV-1) and type 2 (FCoV-2), with FCoV-2 being a recombinant genotype of FCoV-1 and CCoV-2. Recently, a novel FCoV (FCoV-23) resulting from recombination with a highly pathogenic (pantropic) CCoV-2 (pCCoV-2) has been identified as the cause of a widespread outbreak among stray/feral cats in Cyprus. To understand the origin of recombinant variants it is crucial to identify hosts that can be infected with viruses in the species *alphacoronavirus-1*. Experimental evidence indicates that domesticated cats likely play a central role in the emergence of recombinant variants, as they can also be infected with CCoV-2. Wild felids are genetically closely related to domestic cats and may also be susceptible to FCoV and CCoV infection; however, there have been no reports of natural infection with CCoV in domesticated or wild felids. In this study, we retrospectively investigated a localized outbreak of severe enteritis in snow leopards (*Panthera uncia)* housed in a zoological institute in the U.S. Molecular screening and whole genome sequencing revealed the shedding of CCoV-2 in the feces of the three sick leopards. Phylogenetic analyses of the spike gene revealed it is genetically related to pathogenic variants of CCoV-2 identified in domesticated dogs in the U.S., and to pCCoV-2 CB/05 circulating in Europe. This study provides the first genetic evidence of CCoV-2 infection in a wild felid and highlights the necessity of conducting surveillance of both FCoV and CCoV in domesticated and wild felids.

Introduction

Canine coronavirus type 2 (CCoV-2, *Alphacoronavirus*, *Coronaviridae* family) is a common virus of the domesticated dog (*Canis lupus familiaris (1)*). Although normally considered to cause mild enteric disease, many pathogenic variants have been reported in domesticated dogs worldwide (2-5). Pathogenic variants can cause gastrointestinal signs, including enteritis and diarrhea (3). Pathogenic variants of CCoV-2 that cause fatal systemic disease have also been described and are known as 'pantropic' CCoVs (pCCoVs (6)). The first pCCoV-2 variant (known as CB/05) was first detected in Europe in 2005 (6), and pCCoV-2 CB/05-like variants have since been reported in several other European countries (7-9). Knowledge of CCoV-2 in the U.S. is limited, but pathogenic non-systemic variants have been reported as the cause of fatal neonatal enteritis in domesticated dogs (3). CCoV-2 can also infect wild species of canids (i.e. other members of the Canidae family (10)), including widespread urban species like the red fox *(Vulpes vulpes* (11)*)* and the raccoon dog (*Nyctereutes procyonoides (12)*). CCoV-2 can also infect wild species outside the Canidae family, including mustelids like the Chinese ferret-badger (*Melogale moschata (13)*), ursids (bears) like the giant panda *(Ailuropoda melanoleuca (14)),* and hyenids (hyenas), like the spotted hyena *(Crocuta crocuta (15)).* As with domesticated dogs, CCoV-2 infection can result in subclinical signs or severe disease, including acute diarrhea (11, 16) and even neurological signs (14). A deadly outbreak of neurological CCoV-2 in captive giant pandas (14) highlights the potentially significant impact of CCoV-2 on endangered species. The recent detection of CCoV-2 infection in humans with respiratory symptoms has emphasized the need to investigate the host range and mechanisms for cross-species transmission of CCoV-2 (17, 18).

CCoV-2 belongs to the *alphacoronavirus-1* species (19) together with canine coronavirus type 1 (CCoV-1), feline coronavirus type 1 and 2 (FCoV-1 and FCoV-2, respectively), transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus (PRCV). Numerous recombinant variants among members of the *Alphacoronavirus*-1 species have been

documented (20). Variants of CCoV-2 that have recombined with TGEV circulate in Europe and are referred to as CCoV-2b (21, 22). Divergent variants resulting from the recombination between CCoV-1 and -2, known as CCoV-2c, have been detected in outbreaks in domesticated dogs as early as 1976 in the U.S. (23), and recently in the UK (24). Lastly, FCoV-2 is a recombinant genotype of FCoV-1 that acquired a region of its genome from CCoV-2 (25). Recently, a novel FCoV-2-like recombinant variant, FCoV-23, has spread among Cyprus's feral/stray cat population, resulting in a 40-fold increase in reported FCoV-related deaths on the island (26). This outbreak shows that novel recombinant variants of FCoV-1 and CCoV-2 can emerge and rapidly spread in specific populations, posing a serious threat to animal and human health.

Host species susceptible to infection with several alphacoronaviruses, may promote recombination during co-infection events. CCoV-2, FCoV-2, TGEV, and PRCV use the aminopeptidase (APN) protein (also known as CD13) for host cell entry (27, 28). *In vitro* studies have shown that the APN of the domesticated cat (feline APN, fAPN), can be used for cell entry by FCoV-2, CCoV-2, TGEV, and PRCV (27-29). A recent study revealed that the APN of carnivores, including domesticated and wild canids and felids, is highly conserved (>92.7% pairwise amino acid similarity (15)). This suggests that, like the domesticated cat, wild felids may be susceptible to infection with FCoVs and other alphacoronaviruses. Although infection or exposure to FCoVs has been reported in some species of wild felids (30), to our knowledge, natural infection with CCoVs has not been documented in felids (including domesticated cats). To determine if felids (wild or domesticated) can serve as mixing vessels for alphacoronaviruses, it is necessary to investigate whether FCoV and CCoV naturally infect and circulate in felids.

The snow leopard (*Panthera uncia*) is a wild felid found throughout the mountains of Central and Western Asia (31). Currently, this species is classified as vulnerable in the International Union for Conservation of Nature (IUCN) Red List (32). Due to their secretive nature, there is limited

information on infectious diseases that may affect this species (33). However, reports from captive animals have shown that, like other wild felids (30, 34), the snow leopard is susceptible to infectious diseases common in domesticated cats, including feline panleukopenia virus (35) and FCoV (36). Lethal infectious diseases common in domesticated dogs, like canine distemper, have also been reported in the snow leopard (35, 37). Although free-ranging leopards may not be greatly impacted by infectious diseases due to their solitary lifestyle in isolated areas (33), the expansion of livestock herding into these remote areas (38) may increase their risk of exposure to infectious diseases from domesticated animals and livestock. Thus, understanding the impact of common infectious diseases of domesticated cats and dogs on snow leopards could aid conservation efforts for wild populations.

Here, we report a retrospective molecular epidemiological investigation of a localized outbreak of acute diarrhea in captive snow leopards in a zoological institution in the U.S. in 2012. Pancoronavirus screening detected a CCoV-2 in the fecal samples of the three sick leopards. Genetic analysis of the whole genome did not find any recombination breakpoints, confirming it is a CCoV-2 and not an FCoV-2. Phylogenetic analyses indicated the detected CCoV-2 is closely related to pathogenic variants reported in the U.S and pCCoV-2 reported in Europe. Viruses shed from the animals showed the highest amino acid sequence similarity (95.6%) in the spike (S) protein to pCCoV-2 CB/05. This study provides the first genetic evidence of CCoV-2 infection in wild felids resulting in severe gastrointestinal signs. These results highlight the importance of felids (wild and domestic) as mixing vessels for the emergence of recombinant alphacoronaviruses.

Methods

Sample collection

At the end of January 2012, three snow leopards at a zoological institution in the U.S. started exhibiting signs of severe gastroenteritis. Two of the individuals, a five-year-old male (PU293) and a three-year-old female (PU294), experienced intractable diarrhea and vomiting, and the

third one, a ten-year-old female (PU296), experienced diarrhea and anorexia. Feces were collected from the three individuals, and an additional vomit sample was taken from PU294. After collection, samples were frozen at -80°C until further use. After treatment with fluids and parenteral antibiotics, all three snow leopards recovered.

Coronavirus screening and whole genome sequencing

Approximately 40mg of feces were used for RNA extraction using the Monarch® Total RNA Miniprep Kit (New England Biolabs, NEB) according to manufacturer's instructions, including the suggested DNaseI in-column step. Before the extraction, the samples were homogenized on a Bead Blaster 24 (Benchmark) bioruptor using 600 µl of lysis buffer and 500mg of ~2.3mm Zirconia/Silica beads (Biospec Products) for the vomit and ~0.1 mm zirconia/silica beads (Biospec Products) for the fecal samples. cDNA was synthesized using the LunaScript®RT SuperMix Kit. Screening for coronavirus was carried out using previously described pancoronavirus primers targeting a conserved region of the RNA-dependent RNA-polymerase (RdRp) gene (39). The resulting PCR products were sequenced in a MinION Mk1B (Oxford Nanopore Technologies, ONT) using the Native Barcoding Kit 24 V 14 (ONT) and a Flongle Flow Cell (R10.4.1, ONT). The sequence of the whole genome was obtained using the previously described primers covering the 3'-end of the genome (6) and a new set covering the rest of the genome, including the ORFs 1a and 1b (Supplementary Table 1), using the Q5 High-Fidelity 2X Master Mix (NEB). The resulting PCR products were sequenced as mentioned above for the screening but using a Flow Cell R10.4.1 (ONT). The resulting sequences were duplex basecalled using Dorado v0.5.1.

Genetic analyses

The three whole-genome sequences obtained in this study (28,511 nt) were aligned with 103 additional whole-genomes, including 35 TGEV, 29 FCoV-1, 1 CCoV-1, 3 FCoV-2, and 34 CCoV-2. This and other alignments in this study were performed using the MUSCLE algorithm (40). This alignment was used to detect recombination breakpoints in the obtained sequences

using the Recombination Detection Program (RDP (41)) method in RDP-5 (42). An additional alignment of the whole genome sequences obtained in this study and CCoV-2 S378/1978/USA (KC175341), pCCoV-2 CB/05/2005/Italy (K981644), FCoV-1 RM/2002/USA (FJ938051), and FCoV-2 NTU156/2007/Taiwan (GQ152141), was used to generate a similarity plot using SimPlot 5.1 (43).

Three additional alignments of different genomic regions were performed. One included the complete open reading frame (ORF) 1a (ORF1a, 11,902 nt) of 33 CCoV-2, 4 FCoV-1, 3 FCoV-2, 1 TGEV, and 1 CCoV-1. The second and third included a partial region of the S1 domain (the N-terminal domain, NTD, 212 nt), and the S2 domain of the S gene (224 nt), respectively. These two alignments included 70 CCoV-2 and 4 FCoV-2 sequences. These two alignments included the only available sequences of CCoV-2 variants circulating in domesticated dogs in the U.S. between 2009 and 2013 (3). Each of these alignments was used to compute the best-fitting nucleotide substitution model and to construct a maximum likelihood (ML) phylogenetic tree using MEGA 11 (44). Statistical support of the tree branches was estimated by performing a bootstrap with 1,000 replicates. The complete amino acid sequence of the S protein of the three CCoV-2s obtained in the study was aligned with 2 TGEV, 5 FCoV-2, and 58 CCoV-2 to calculate the pairwise percentage similarity in Geneious Prime 2023.2.1.

Results

From the four samples screened for CoV RNA, the feces from the three snow leopards (PU293, PU294, PU296) were positive, and the vomit of PU294 was negative. A phylogenetic tree of the complete OORF1a (11,902 nt) placed the three coronaviruses detected in the CCoV-1/CCoV-2 group and not in the FCoV-1/FCoV-2 group (Figure 1).

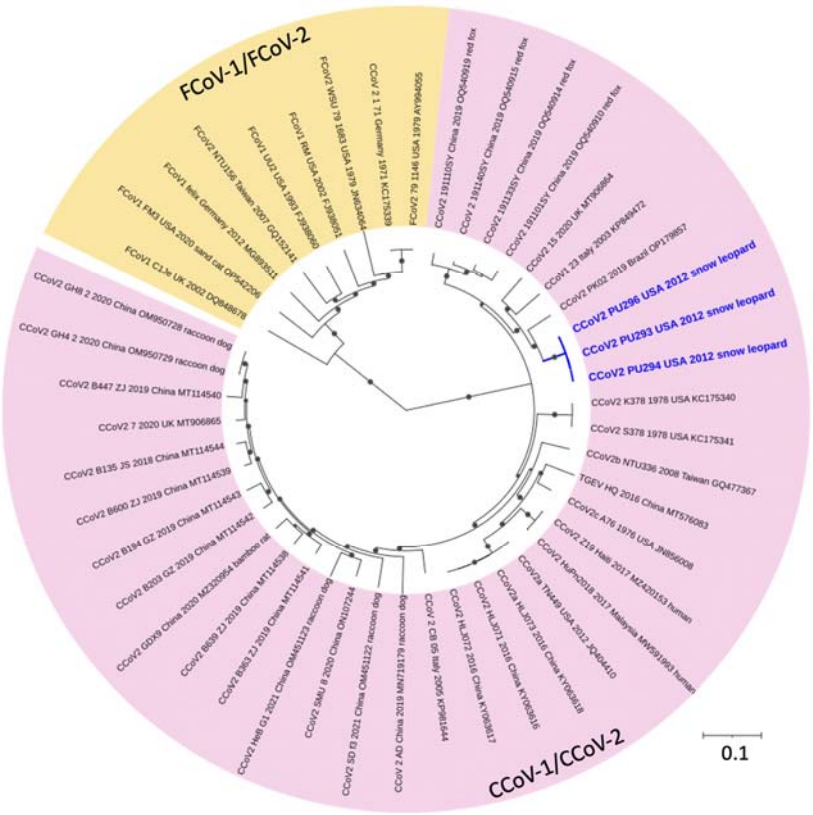

Figure 1. Maximum likelihood phylogenetic tree of the complete ORF1a (11,902nt) of selected FCoVs and CCoVs. Two groups can be observed, one including FCoV-1 and FCoV-2 (in yellow) and another one including CCoV-1 and CCoV-2 (in pink). In dark blue are the three sequences obtained in this study, which group together within the CCoV-1/CCoV-2 group. For all variants included, the sequence name includes the type of CoV (FCoV-1, -2, CCoV-1, -2), year and place of collection, and accession number. For variants recovered from wild animals, the species' common name is after the accession number. Dots in the branches indicate a bootstrap value higher than 0.6. The estimated best-fitted nucleotide substitution model was HKY+I+G.

To explore if the CoVs detected in the feces were recombinant (i.e. FCoV-2) or not (i.e. CCoV-2), the whole genome sequences of the three variants were obtained. The three whole genomes were 28,511nt long and 99.9% similar. They contained 11 ORFs (ORF1a-ORF1b-S-ORF3a-ORF3b-ORF3c-Envelope (E)-Matrix (M)-Nucleocapsid (N)-ORF7a-ORF7b). No deletions, insertions, or recombination breakpoints were detected in the three genomes compared to other FCoVs and CCoVs. A genetic comparison of the whole genome of PU294 and an FCoV-2

(NTU156/2007/Taiwan), an FCoV-1 (RM/2002/USA), and pCCoV-2 CB/05/2005/Italy show that along the whole genome, PU294 is consistently more similar to CCoV-2 and not to FCoV-1 (Figure 2). Only in the recombinant portion of FCoV-2 is PU294 similar to FCoV-2 (Figure 2).

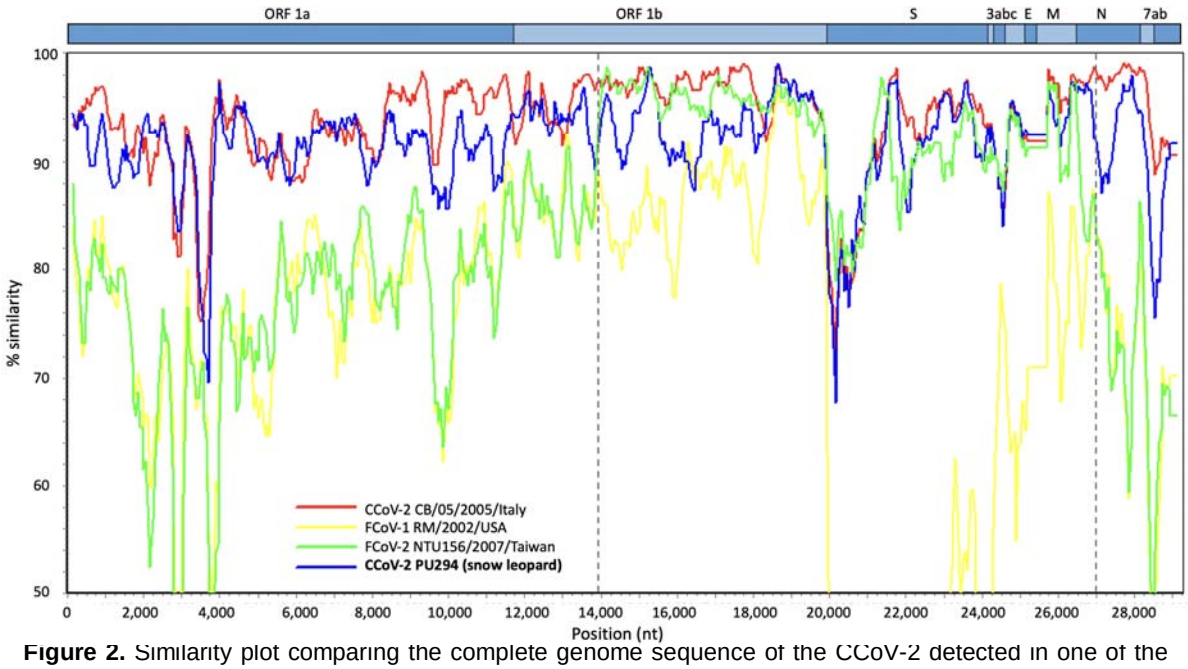

Figure 2. Similarity plot comparing the complete genome sequence of the CCoV-2 detected in one of the snow leopards (PU294) with other canine and feline coronaviruses. A graphical representation of the genome above the chart shows the location of each gene. The genome sequence of CCoV-2 S378/1978/USA (KC175341) was used as a reference. In red is pCCoV-2 CB/05/2005/Italy (K981644), in yellow is FCoV-1 RM/2002/USA (FJ938051), in green is FCoV-2 NTU156/2007/Taiwan (GQ152141), and in dark blue is one of the CCoV-2 detected in a snow leopard (PU294). Dashed vertical lines indicate the recombination breakpoint of FCoV-2 NTU156/2007/Taiwan (in green). Within this region, FCoV-2 is more similar to CCoV-2 (in dark blue) than FCoV-1 (in yellow). In the rest of the genome, FCoV-2 is more similar to FCoV-1 (in yellow). The graph was constructed using the Kimura 2 parameter distance model with a window of 300bp and a 50bp step.

Two additional phylogenetic trees of a partial region of the S1 (Figure 3A) and the S2 (Figure 3B) domains of the S gene, were constructed to compare the sequences obtained from the snow leopards and other CCoV-2 circulating in the U.S. during the period when the outbreak in the snow leopards occurred. In these phylogenies, the three sequences from the snow leopards

obtained in 2012 grouped together within a group containing other CCoV-2 from domestic dogs in the U.S obtained from 2009 to 2013 (in dark pink in Figure 3), as well as the pCCoV-2 variants CB/05/2005/Italy and 450/2007/Italy (in green in Figure 3) in the phylogeny of the S2 domain (Figure 3B), and NA/09/2009/Greece in the phylogeny of the S1 domain (Figure 3A). The pairwise similarity of the amino acid sequence of the complete S protein of the three sequences obtained in this study was 99.8% similar. These phylogenies also included CCoV-2 variants from different wild species (in light pink in Figure 3), including red foxes, raccoon dogs, Chinese ferret badgers, giant pandas, from China and spotted hyenas, and silver-backed jackals from Tanzania. For the CCoV-2 reported in wild animals, at least 4 groups are circulating in China. When compared to 65 additional sequences of CCoV-2, FCoV-2, and TGEV, the highest percentage amino acid similarity of the S protein of the CCoV-2 obtained in the three snow leopards was with pCCoV-2 CB/05/2005/Italy (95.6%), followed by pCCoV-2 450/2007/Italy (95.5%).

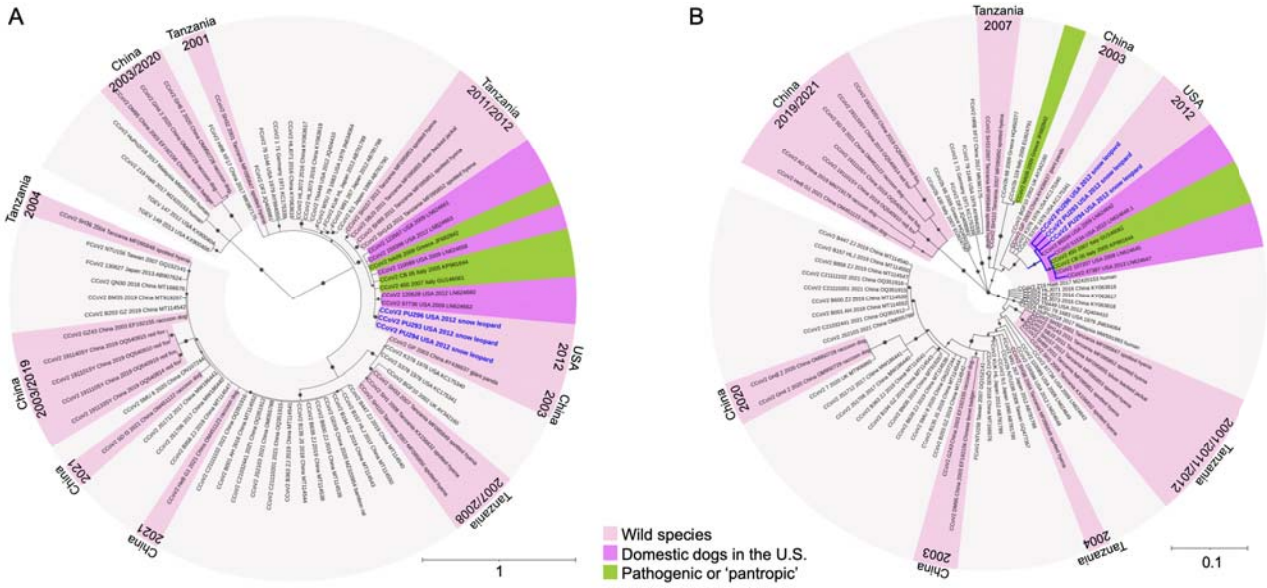

Figure 3. Maximum likelihood phylogenetic tree of a partial region of (A) the S1 domain (212nt) and (B) the S2 domain (224 nt) of S of selected FCoV-2 and CCoV-2. The three sequences obtained in this study are in dark blue. In light pink are the CCoV-2 recovered from wild species, and the place of collection and year are

shown at the tip of each group. In both trees, the three sequences obtained in this study group together with other sequences of CCoV-2 from domestic dogs collected in the U.S. in 2009, 2010, 2012, and 2013 (in dark pink) and two to three pathogenic or 'pantropic' CCoV-2 variants (in green). For all variants in the tree, the sequence name includes the type of CoV (FCoV-2, CCoV-2), year and place of collection, and accession number. The species' common name for variants recovered from wild animals is after the accession number. Dots in the branches indicate a bootstrap value higher than 0.6. The estimated best-fitted nucleotide substitution model for both alignments was GTR+I+G.

Discussion

Recombination is a significant mechanism that shapes the genetic diversity of coronaviruses (45, 46). Various recombinant variants of CCoV, including CCoV-2b (recombinants with TGEV (22)) and CCoV-2c (recombinants with CCoV-1 (23)), have been reported in domesticated dogs (21). Recombinant alphacoronaviruses also circulate in domesticated cats, including FCoV-2 (recombinants of CCoV-2 with FCoV-1 (25)). The diverse recombinant variants of FCoV-2 reported to date display different recombination breakpoints (25, 47, 48)**,** indicating multiple recombination events between FCoV-1 and CCoV-2. The recent deadly outbreak in feral/stray cats in Cyprus caused by the novel recombinant FCoV-23 (26) has further emphasized the need to identify new recombinant variants and understand the hosts and ecological conditions that promote recombination. Viruses must co-infect the same host cell to exchange their genetic material (i.e., recombine). Therefore, identifying hosts susceptible to infection with different alphacoronaviruses is an initial step toward understanding their role in the emergence of recombinant variants.

Experimental evidence suggests that domesticated cats likely play a key role in the origin of recombinant variants of alphacoronaviruses. Early experimental infection studies have indicated that domesticated cats can be infected with CCoV-2, with variable levels of viral shedding in feces (49). Additional *in vitro* studies have shown that fAPN supports the infection of several alphacoronaviruses, including FCoV-2, CCoV-2, TGEV, PRCV, and even the human CoV

(HCoV) 229-E (27-29). Furthermore, persistent infection of FCoV-1 is common in domesticated cats (1), which may increase the chances of co-infection with other alphacoronaviruses. A recent study revealed that the APN gene is highly conserved in carnivores (15), with a percentage nucleotide similarity of 97.6% and 99.2% in the Felidae and Canidae family, respectively. Given this high genetic similarity, the susceptibility of wild felids to infection with diverse alphacoronaviruses should be assessed. In this study, we conducted a retrospective genetic analysis of a localized outbreak of severe diarrhea and vomiting in three captive snow leopards. Analysis of whole genome sequences (Figure 2) and phylogenetic data (Figure 1) revealed the presence of CCoV-2 in the feces of the three sick snow leopards. These results support the idea that, as experimentally shown in domesticated cats (49), other felids can also be infected with CCoV-2. A previous study in zoological institution in the U.S showed that captive snow leopards can also be infected with and shed FCoV in their feces (36). Thus, it is necessary to assess if both FCoVs and CCoVs naturally circulate in wild and domesticated felids to understand their role in the origin of recombinant alphacoronaviruses.

Although CCoV commonly causes subclinical to mild gastrointestinal signs, specific mutant or recombinant variants can be highly pathogenic. The S protein of CoVs binds to the host cell receptor and triggers the fusion with the host cell membrane (50). Thus, it is an essential determinant of cellular tropism and pathogenicity. The phylogenetic trees of the partial S1 and S2 domains (Figure 3), placed the three variants obtained in this study together with other variants of CCoV-2 detected in domesticated dogs in the U.S. between 2009 and 2013 (in dark pink in Figure 3), from which only partial genomic sequences are available (3). These CCoV-2 variants were associated with severe enteritis in pups (3), consistent with the severe gastrointestinal signs observed in the three snow leopards. However, the genetic comparison of the amino acid sequence of the S protein showed that the highest overall similarity (>95.5%) was with two pCCoV-2 variants (CB/05/2005/Italy and 450/2007/Italy). The phylogenetic trees of

the partial S1 and S2 domains (Figure 3) also showed that the three CCoV-2 obtained in this study were closely related to these pCCoV-2 variants (CB/05/2005/Italy and 450/2007/Italy, in green in Figure 3). pCCoV-2 has been reported in several European countries and is linked to fatal outbreaks in pups in which viral systemic infection was detected (i.e., CCoV-2 RNA was detected in lungs, spleen, liver, kidneys, mesenteric lymph node, and brain). In contrast, although the CCoV-2 variants in the U.S. were also fatal, the virus was confined to the intestines (3). Further studies are necessary to comprehend the molecular mechanisms behind the extended tropism of pCCoV-2 compared to closely related strains with restricted tropism reported in the U.S., including the ones in this study. For this, surveillance and whole-genome sequencing of CCoV-2 circulating in domesticated dogs in the U.S. is essential. Interestingly, the S gene of FCoV-23 is highly similar (>97% pairwise nucleotide similarity) to other pCCoV-2, NA/09/2009/Greece (26). Thus, the deadly outbreak in cats in Cyprus could be the result of the emergence of a recombinant FCoV that obtained the S from a highly pathogenic CCoV-2. The results of this study suggest that pathogenic variants of CCoV-2 circulating in domesticated dogs can infect a felid species, the snow leopard, causing severe gastrointestinal signs. If these CCoV-2 can infect felids, recombination with FCoV-1 could occur, and the exchange of S can result in recombinant variants with distinct tropism, pathogenicity, and host range, as shown in the ongoing outbreak in Cyprus (51).

Studying the potential impact of infectious diseases on free-ranging populations of snow leopards is challenging due to their secretive nature (33). A previous study found FCoV infection and shedding in healthy snow leopards in two zoological institutions in the U.S. (36). In contrast, we report that CCoV-2 infection can result in severe gastrointestinal signs and viral shedding in feces. Due to their solitary lifestyle, snow leopards may acquire pathogens from other carnivore species (wild or domestic (33)). China is estimated to be home to more than 50% of the world's snow leopard population, making it a crucial figure for the conservation of this species (31).

China's economic growth is causing human encroachment on the habitat of snow leopards (31, 38) which may lead to their increased exposure to pathogens of domesticated and wild urban species. This study shows that at least four groups of CCoV-2 are circulating in wild species in China (Figure 3). Of epidemiological interest are the CCoV-2s circulating in common wild urban species, including raccoon dogs, in which a high prevalence of CCoV-2 was detected in Wuhan, China, at the beginning of the SARS-CoV-2 pandemic (12). These abundant species may interact with other wild and domesticated animals in urban areas, potentially increasing the risk of cross-species virus transmission and recombination. This is especially concerning given the high prevalence of CCoV-2 in domesticated dogs in mainland China (52). An additional risk for snow leopards and other wild species are the highly pathogenic variants reported in wild carnivores related to deadly outbreaks in China (14), including the recent outbreak of a divergent CCoV-2, which has been linked to the death of over 39,000 red foxes at a rescue center in the Guizhou province in China (11). Likewise, co-infection with CCoV-1 and CCoV-2 was reported in red foxes and raccoon dogs sampled in China in 2005 (53), which may promote the emergence of novel recombinant variants in these abundant wild species. Surveillance of both FCoV and CCoV in domesticated and wild carnivores in China is essential to understanding the role of wild species in the origin and spread of recombinant variants and the risk of cross-species transmission to vulnerable species, like the snow leopard.

References

1. Ettinger S, Feldman, EC, Cote E. 2024. Ettinger's Textbook of Veterinary Internal Medicine - eBook: Ettinger's Textbook of Veterinary Internal Medicine - eBook. Elsevier Health Sciences.
2. Naylor Matthew J, Walia Charanjiv S, McOrist S, Lehrbach Philip R, Deane Elizabeth M, Harrison Gavan A. 2002. Molecular Characterization Confirms the Presence of a Divergent Strain of Canine Coronavirus (UWSMN-1) in Australia. Journal of Clinical Microbiology 40:3518-3522.
3. Licitra BN, Whittaker GR, Dubovi EJ, Duhamel GE. 2014. Genotypic characterization of canine coronaviruses associated with fatal canine neonatal enteritis in the United States. J Clin Microbiol 52:4230-8.
4. Buonavoglia C, Decaro N, Martella V, Elia G, Campolo M, Desario C, Castagnaro M, Tempesta M. 2006. Canine coronavirus highly pathogenic for dogs. Emerg Infect Dis 12:492-4.
5. Escutenaire S, Isaksson M, Renström LH, Klingeborn B, Buonavoglia C, Berg M, Belák S, Thorén P. 2007. Characterization of divergent and atypical canine coronaviruses from Sweden. Arch Virol 152:1507-14.
6. Decaro N, Martella V, Elia G, Campolo M, Desario C, Cirone F, Tempesta M, Buonavoglia C. 2007. Molecular characterisation of the virulent canine coronavirus CB/05 strain. Virus Res 125:54-60.
7. Decaro N, Mari V, von Reitzenstein M, Lucente MS, Cirone F, Elia G, Martella V, King VL, Di Bello A, Varello K, Zhang S, Caramelli M, Buonavoglia C. 2012. A pantropic canine coronavirus genetically related to the prototype isolate CB/05. Vet Microbiol 159:239-44.
8. Decaro N, Cordonnier N, Demeter Z, Egberink H, Elia G, Grellet A, Le Poder S, Mari V, Martella V, Ntafis V, von Reitzenstein M, Rottier PJ, Rusvai M, Shields S, Xylouri E, Xu Z, Buonavoglia C. 2013. European surveillance for pantropic canine coronavirus. J Clin Microbiol 51:83-8.
9. Ntafis V, Xylouri E, Mari V, Papanastassopoulou M, Papaioannou N, Thomas A, Buonavoglia C, Decaro N. 2012. Molecular characterization of a canine coronavirus NA/09 strain detected in a dog's organs. Arch Virol 157:171-5.
10. Whittaker G, Stout A. [Review] Coronaviruses in Wild Canids: A review of the literature. Qeios doi:10.32388/5UZPYI.
11. Liu Y, Deng Y, Niu S, Zhu N, Song J, Zhang X, Su W, Nie W, Lu R, Irwin DM, Gao GF, Wang W, Wang Q, Tan W, Zhang S. 2023. Discovery and identification of a novel canine coronavirus causing a diarrhea outbreak in Vulpes. Sci Bull (Beijing) 68:2598-2606.
12. Wang W, Tian J-H, Chen X, Hu R-X, Lin X-D, Pei Y-Y, Lv J-X, Zheng J-J, Dai F-H, Song Z-G, Chen Y-M, Zhang Y-Z. 2022. Coronaviruses in wild animals sampled in and around Wuhan at the beginning of COVID-19 emergence. Virus Evolution 8.
13. Dong BQ, Liu W, Fan XH, Vijaykrishna D, Tang XC, Gao F, Li LF, Li GJ, Zhang JX, Yang LQ, Poon LL, Zhang SY, Peiris JS, Smith GJ, Chen H, Guan Y. 2007. Detection of a novel and highly divergent coronavirus from asian leopard cats and Chinese ferret badgers in Southern China. J Virol 81:6920-6.
14. Gao FS, Hu GX, Xia XZ, Gao YW, Bai YD, Zou XH. 2009. Isolation and identification of a canine coronavirus strain from giant pandas (Ailuropoda melanoleuca). J Vet Sci 10:261-3.
15. Olarte-Castillo XA, Dos Remedios JF, Heeger F, Hofer H, Karl S, Greenwood AD, East ML. 2021. The virus-host interface: Molecular interactions of Alphacoronavirus-1 variants from wild and domestic hosts with mammalian aminopeptidase N. Mol Ecol 30:2607-2625.
16. Yang S, He Y, Chen X, Kalim U, Wang Y, Yang S, Qi H, Cheng H, Lu X, Wang X, Shen Q, Zhang W. 2021. Viral Metagenomics Reveals Diverse Viruses in the Feces Samples of Raccoon Dogs. Front Vet Sci 8:693564.
17. Vlasova AN, Diaz A, Damtie D, Xiu L, Toh T-H, Lee JS-Y, Saif LJ, Gray GC. 2021. Novel Canine Coronavirus Isolated from a Hospitalized Patient With Pneumonia in East Malaysia. Clinical Infectious Diseases 74:446-454.
18. Lednicky JA, Tagliamonte MS, White SK, Blohm GM, Alam MM, Iovine NM, Salemi M, Mavian C, Morris JG. 2022. Isolation of a Novel Recombinant Canine Coronavirus From a Visitor to Haiti: Further Evidence of Transmission of Coronaviruses of Zoonotic Origin to Humans. Clin Infect Dis 75:e1184-e1187.

19. Whittaker GR, Andre NM, Millet JK. 2018. Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the Alphacoronavirus 1 Species. mSphere 3.
20. Le Poder S. 2011. Feline and canine coronaviruses: common genetic and pathobiological features. Adv Virol 2011:609465.
21. Decaro N, Mari V, Elia G, Addie DD, Camero M, Lucente MS, Martella V, Buonavoglia C. 2010. Recombinant canine coronaviruses in dogs, Europe. Emerg Infect Dis 16:41-7.
22. Decaro N, Mari V, Campolo M, Lorusso A, Camero M, Elia G, Martella V, Cordioli P, Enjuanes L, Buonavoglia C. 2009. Recombinant canine coronaviruses related to transmissible gastroenteritis virus of Swine are circulating in dogs. J Virol 83:1532-7.
23. Regan AD, Millet JK, Tse LP, Chillag Z, Rinaldi VD, Licitra BN, Dubovi EJ, Town CD, Whittaker GR. 2012. Characterization of a recombinant canine coronavirus with a distinct receptor-binding (S1) domain. Virology 430:90-9.
24. Radford AD, Singleton DA, Jewell C, Appleton C, Rowlingson B, Hale AC, Cuartero CT, Newton R, Sánchez-Vizcaíno F, Greenberg D, Brant B, Bentley EG, Stewart JP, Smith S, Haldenby S, Noble PM, Pinchbeck GL. 2021. Outbreak of Severe Vomiting in Dogs Associated with a Canine Enteric Coronavirus, United Kingdom. Emerg Infect Dis 27:517-528.
25. Herrewegh AA, Smeenk I, Horzinek MC, Rottier PJ, de Groot RJ. 1998. Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. J Virol 72:4508-14.
26. Attipa C, Warr AS, Epaminondas D, O'Shea M, Fletcher S, Malbon A, Lyraki M, Hammond R, Hardas A, Zanti A, Loukaidou S, Gentil M, Gunne-Moore D, Mazeri S, Tait-Burkard C. 2023. Emergence and spread of feline infectious peritonitis due to a highly pathogenic canine/feline recombinant coronavirus. bioRxiv doi:10.1101/2023.11.08.566182:2023.11.08.566182.
27. Tresnan DB, Holmes KV. 1998. Feline aminopeptidase N is a receptor for all group I coronaviruses. Adv Exp Med Biol 440:69-75.
28. Tresnan DB, Levis R, Holmes KV. 1996. Feline aminopeptidase N serves as a receptor for feline, canine, porcine, and human coronaviruses in serogroup I. J Virol 70:8669-74.
29. Tusell SM, Holmes KV. 2006. Molecular interactions of group 1 coronaviruses with feline APN. Adv Exp Med Biol 581:289-91.
30. Stout AE, André NM, Whittaker GR. 2021. FELINE CORONAVIRUS AND FELINE INFECTIOUS PERITONITIS IN NONDOMESTIC FELID SPECIES. J Zoo Wildl Med 52:14-27.
31. Alexander JS, Zhang C, Shi K, Riordan P. 2016. A spotlight on snow leopard conservation in China. Integr Zool 11:308-21.
32. McCarthy T, Mallon, D., Jackson, R., Zahler, P., McCarthy, K. 2017. Panthera uncia. . The IUCN Red List of Threatened Species 2017 e.T22732A50664030.
33. Ostrowski S, Gilbert M. 2016. Chapter 9 - Diseases of Free-Ranging Snow Leopards and Primary Prey Species, p 97-112. *In* McCarthy T, Mallon D (ed), Snow Leopards doi:https://doi.org/10.1016/B978-0-12-802213-9.00009-2. Academic Press.
34. Olarte-Castillo XA, Goodman LB, Whittaker GR. 2024. Molecular detection using hybridization capture and next-generation sequencing reveals cross-species transmission of feline coronavirus type-1 between a domestic cat and a captive wild felid. bioRxiv doi:10.1101/2024.01.02.573944:2024.01.02.573944.
35. Fix AS, Riordan DP, Hill HT, Gill MA, Evans MB. 1989. Feline Panleukopenia Virus and Subsequent Canine Distemper Virus Infection in Two Snow Leopards (Panthera uncia). Journal of Zoo and Wildlife Medicine 20:273-281.
36. Kennedy M, Citino S, McNabb AH, Moffatt AS, Gertz K, Kania S. 2002. Detection of feline coronavirus in captive Felidae in the USA. J Vet Diagn Invest 14:520-2.
37. Chinnadurai SK, Kinsel MJ, Adkesson MJ, Terio K. 2017. CANINE DISTEMPER IN A VACCINATED SNOW LEOPARD (PANTHERA UNCIA). J Zoo Wildl Med 48:1200-1203.
38. Alexander JS, Gopalaswamy AM, Shi K, Hughes J, Riordan P. 2016. Patterns of Snow Leopard Site Use in an Increasingly Human-Dominated Landscape. PLoS One 11:e0155309.
39. Watanabe S, Masangkay JS, Nagata N, Morikawa S, Mizutani T, Fukushi S, Alviola P, Omatsu T, Ueda N, Iha K, Taniguchi S, Fujii H, Tsuda S, Endoh M, Kato K, Tohya Y, Kyuwa S, Yoshikawa Y, Akashi H. 2010. Bat coronaviruses and experimental infection of bats, the Philippines. Emerg Infect Dis 16:1217-23.

40. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-7.
41. Martin D, Rybicki E. 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics 16:562-3.
42. Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B. 2021. RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evol 7:veaa087.
43. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 73:152-60.
44. Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38:3022-3027.
45. Wells HL, Bonavita CM, Navarrete-Macias I, Vilchez B, Rasmussen AL, Anthony SJ. 2023. The coronavirus recombination pathway. Cell Host Microbe 31:874-889.
46. Licitra BN, Duhamel GE, Whittaker GR. 2014. Canine enteric coronaviruses: emerging viral pathogens with distinct recombinant spike proteins. Viruses 6:3363-76.
47. Lin CN, Chang RY, Su BL, Chueh LL. 2013. Full genome analysis of a novel type II feline coronavirus NTU156. Virus Genes 46:316-22.
48. Wang YT, Su BL, Hsieh LE, Chueh LL. 2013. An outbreak of feline infectious peritonitis in a Taiwanese shelter: epidemiologic and molecular evidence for horizontal transmission of a novel type II feline coronavirus. Vet Res 44:57.
49. McArdle F, Bennett M, Gaskell RM, Tennant B, Kelly DF, Gaskell CJ. 1992. Induction and enhancement of feline infectious peritonitis by canine coronavirus. Am J Vet Res 53:1500-6.
50. Millet JK, Whittaker GR. 2015. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res 202:120-34.
51. Attipa C, Gunn-Moore D, Mazeri S, Epaminondas D, Lyraki M, Hardas A, Loukaidou S, Gentil M. 2023. Concerning feline infectious peritonitis outbreak in Cyprus. Veterinary Record 192:449-450.
52. Dong B, Zhang X, Bai J, Zhang G, Li C, Lin W. 2022. Epidemiological investigation of canine coronavirus infection in Chinese domestic dogs: A systematic review and data synthesis. Preventive Veterinary Medicine 209:105792.
53. Ma G-g, Lu C-p. 2005. [Two genotypes of Canine coronavirus simultaneously detected in the fecal samples of healthy foxes and raccoon dogs]. Wei sheng wu xue bao = Acta microbiologica Sinica 45:305-308.