Pangenome analysis of coronaviruses derived from major of canine and feline

Running title: Pangenome of canine and feline COVs

Hai Ha Pham Thi[1*], Thanh Luan Nguyen[2*]

[1] NTT High-Tech Institute, Nguyen Tat Thanh University, 298-300A Nguyen Tat Thanh Street, Ward 13, District 4, Ho Chi Minh City 700000, Vietnam. E-mail: nicolepham19@gmail.com

[2]Department of Veterinary Medicine, Institute of Applied Science, Ho Chi Minh City University of Technology - HUTECH, 475A Dien Bien Phu Street, Ward 25, Binh Thanh District, Ho Chi Minh City 700000, Vietnam. E-mail: nt.luan@hutech.edu.vn

[*]**Correspondence:**

Thanh Luan Nguyen, Department of Veterinary Medicine, Institute of Applied Science, Ho Chi Minh City University of Technology - HUTECH, E-mail address: nt.luan@hutech.edu.vn

Summary

Coronaviruses (CoVs) are a well-known cause of severe enteric, respiratory, and systemic disease in a wide range of animals and in humans. To understand the route of disease origin and viral transmission in companion animals, a comparative pan-genomic analysis of coronavirus sequences originating from major felines and canines were conducted. The average nucleotide identity (ANI) is a rapid procedure for assessing the very close antigenic relationship between feline CoV (FCoVs) and canine CoV (CCoVs) and ANI-based phylogenetic tree that clustered CoVs according to their respective host species. While pan-genomic analysis demarcated strains clearly. The distribution of the clinical isolates all across the categories in the hierarchical phylogenetic model enabled the visualization of their original ecological niche rather than their isolation source, as infections are extremely rare events and evolutionary dead-ends. In polymorphism analysis, we found seven accessory gene clusters common to the FCoV/CCoV category clade, including pantropic strains, that perform functions supporting their pathogenicity. In addition, the gene presence/absence among FCoVs and CCoVs would provide very valuable information on species-specific control measures against CoV disease, such as the selection of good markers for differentiating new species from common and/or pantropic isolates. Also, the virulent FCoV strains were grouped with human CoV strains NL63 and 229E confirming hypotheses stating that cats are highly susceptible to HCoVs, while dogs have low susceptibility to the virus. In conclusion, the combined analysis allows for better phylogenetic resolution and the implication of virus origins, recombination, and virus–host interaction, as well as biomarkers.

Keywords: Domestic animal, coronavirus, pan-genome, phylogenomics, pan-RT-PCR

1. Introduction

Coronaviruses (CoVs) are a large family of enveloped viruses. These non-segmented, single-stranded, positive-sense RNA viruses are the well-known causes of severe respiratory, enteric, and systemic infections in a wide range of hosts, including canines, felines, murines, equines, and humans. In general, *Coronavirinae* is divided into four genera: *Alpha*-, *Beta*-, *Gamma*-, and *Deltacoronavirus* (Woo et al., 2012). *Alpha*- and *Betacoronavirus* usually infect mammals, while *Gamma*- and *Deltacoronavirus* usually infect birds and fish. The coronaviruses of relevant veterinary species are especially considered, as the exact source of the current outbreak of coronavirus disease 2019 (COVID-19, caused by SARS-CoV-2) might have originated from an animal source and/ or be transmitted via domestic animals. Feline- and canine-derived coronaviruses are widespread among dog and cat populations. Canine coronavirus (CCoV), which can cause mild diarrhea (Decaro and Buonavoglia, 2008, 2011), and feline coronavirus (FCoV), which can cause the fatal disease known as feline infectious peritonitis (FIP) (Pedersen et al., 2014), are both *Alphacoronaviruses*. *Betacoronavirus* have been assigned to four distinct lineages (A, B, C, D), and six known coronaviruses capable of infecting humans (HCoVs) and causing mild illness similar to the common cold and gastrointestinal tract infection, and respiratory disease, have been distributed to both *Alphacoronavirus* (HCoV-229E and HCoV-NL63) and *Betacoronavirus* (HCoV-OC43 and HCoV-HKU1 belong to lineage A, SARS-CoV and SARS-CoV-2 to lineage B, and MERS-CoV to lineage C). CCoV and FCoV are distinct from SARS-CoV and SARS-CoV-2. However, these animals are clearly susceptible to this family of viruses (OIE, 2020; Shi et al., 2020). There is also currently limited evidence that companion animals

can be infected with SARS-CoV-2, and there is no evidence that canines or felines can be a source of infection to other animals or to humans. In addition, the worldwide report of coronaviruses genome sequences isolated from different animal species has attracted the attention of scientific society. One means of deriving meaningful and useful information from a large genomic dataset of coronaviruses is through comparative pan-genomic analysis. This approach can help identify the core genome of host-dependent isolates and extract accessory genomic features shared by a subset of these viruses or that are unique to hosts. Whereas core genomic features are required for the virus to be functional, accessory features are candidates for providing insights into the drivers of the unique capacities of the virus, explaining adaptive evolution in its reservoir host. Here, we describe the pan-genomes of the coronaviruses isolated from some veterinary animals, including CCoVs and FCoVs, based on these available complete genome data to assess the possible functions of these core/accessory features and to discover approaches for detection and treatment.

2. Comparative genome analysis of CoVs

2.1 Average nucleotide identity analysis of FCoVs and CCoVs

The average nucleotide identity (ANI) calculation based on the complete genome of some previously determined animal species sequences (Supplementary Table 1) revealed that FCoV strains had 85.26–100% similarity, CCoV strains had 84.45–100% similarity, murine and rat CoV strains had 89.88–100% similarity, mink CoV strains had 91.15–100% similarity, equine CoV strains had 98.74–100% similarity, and human CoV (HCoV) strains had 61.03–100% similarity (Fig. 1). The ANI-based phylogenetic tree clustered CoV strains according to their respective host species. Whole-genome ANI

between FCoV and CCoV strains showed 80.34–88.72% similarity, with the exception of type II CCoV strain KC175339 (ANI value of 85.65–99.89% when compared to FCoV genomes). This strain shared high similarity (> 99%) with FCoV (serotype II) strains DQ010921, DQ286389, JQ408980, GQ152141, and JN634064, suggesting the potential of cross-species jump between cats and dogs. In addition, this result shows that ANI analysis can be conducted quickly to assess the very close antigenic relationship between FCoVs and CCoVs and its potential for interspecies transmission (McArdle et al., 1992; Horzinek et al., 1982).

The high similarity between specific CCoV and FCoV strains also might reflect interspecies recombination in different parts of the viral genome. In fact, various sequence analysis studies (e.g. Escutenaire et al., 2007; Naylor et al., 2002; Herrewegh et al., 1998) have targeted the gene encoding the spike on the viral surface and encoding the membrane or integral membrane protein for understanding interspecies recombinations between FCoV and CCoV strains.

In addition, numerous markers have been proposed for the detection of FCoVs and CCoVs. However, there is major controversy regarding the existence of genetic markers for differentiating between their interspecies and intraspecies pathotypes (Terada et al., 2014; Hora et al., 2016). Based on significant antigenic differences between strains, CCoV and FCoV strains are generally distinguished into low-virulence (predominant strains causing enteritis disease, or type I) and high-virulence strains (typical strains causing multisystemic disease in a small percentage of animals, or type II). The genetic relation of low-virulence CCoV strains is more closely related to FCoV strains (termed FCoV-like CCoV strains) than typical CCoV strains. In some exceptions, some FCoV-

like CCoV strains are more virulent than typical CCoV strains, and cause severe hemorrhagic diarrhea (Benetka et al., 2006). In contrast to FCoVs, both type I and II CCoVs are commonly detected simultaneously in the same host (36.9–76.8% of dogs with diarrhea) (Terada et al., 2014), thus allowing genetic recombination to occur and increase CCoV infection severity in dogs and the emergence of CCoV variants (Pratelli et al., 2004; Escutenaire et al., 2007). Thus, accurate genotyping of field FCoVs and CCoVs is undoubtedly important.

2.2. Inside the pan-genome of CCoVs and FCoVs

Pan-genome analysis describes the set of all sequence entities (open reading frames [ORFs], genes, etc.) belonging to the viral genomes of interest. In the present study, pan-genome analyses of the 104 CoV genomes were performed using EDGAR v2.2 (Blom et al., 2016), and a total of 43 orthologous groups were identified, which constituted the CoV pan-genome. Fig. 2 shows that the size of the pan-genome inferred from the genome sequences of 12 CCoVs and 58 FCoVs was constituted by 15 and 19 orthologous groups, respectively. The power law coefficient within Heap's Law function was 0.146 for CCoVs and 0.147 for FCoVs (between 0 and 1), corresponding to the open pan-genome model (Tettelin et al., 2008) (Fig. 2C and 2D, respectively).

In contrast to ANI-based phylogenetic analysis (Fig. 1 and S1), pan-genome analysis revealed different partitioning of the examined CoV strains based on the comparative gene content of 43 orthologous groups (Fig. 3). The hierarchical clustering of seven different host CoV strains resulted in seven distinct, host-independent clusters. Remarkably, most feline-, canine-, and mink-derived strains belonged to cluster 1 and were distinguished from the equine, murine, and human strains (cluster 2). The feline and

canine cluster 1 was divided into sub-clusters consisting of feline–canine, and feline, feline–canine–human (HCoV_NL63: Accession no. MK334045 originated from China). This analysis is consistent with the finding that the recombinant CCoV strain HLJ-073 (accession no. KY063618) could replicate effectively in both canine lymphocytes and human THP-1 cells (Chen et al., 2019). In China, strain HLJ-073 had different biological characteristics from other reported CCoVs, and the hierarchical clustering can be useful for screening the special CCoV strains. In contrast, the murine strains in cluster 2 were divided into sub-clusters of murine, murine–equine–human, and murine–rat, and these sub-clusters are more closely related to new strains of HCoV (SARS-CoV-2) in cluster 2. In the analysis with additional HCoV strains HKU1, OC43, NL63, and 229E, virulent FCoV strains were grouped with HCoV strains NL63 and 229E (Fig. S2). Our results may support previous hypothesis stating that cats are highly susceptible to HCoVs while dogs have low susceptibility to the virus (Shi et al., 2020). However, increasing infection of highly virulent CCoVs that has been documented in puppies without apparent coinfections (e.g. Escutenaire et al., 2007; Decaro et al., 2007; Zicola et al., 2012) via recombination may be transmitted from their natural reservoir to a susceptible host, including humans, in different ways (e.g., in the case of CCoV strain HLJ-073, accession no. KY063618), as cats and dogs are in close contact with humans.

The comparison of hierarchical clustering and the phylogenetic tree in Fig. 3 shows some degree of conservation in the grouping of the viral strains. In the field of phylodynamics, the phylogenetic tree showed low evolution within the viral genome, i.e., point mutations associated with disease transmission or severity, compared to the hierarchical clustering tree (based on complete viral genomes), which describes major

viral genetic divergence and population dynamics (genotypes and sub-genotypes), pathogenesis, and vectors associated with virus transmission among populations (Faria et al., 2017, 2018). This pan-genomic analysis clearly demarcates strains; thus, the combined analysis allows for better phylogenetic resolution and implicates virus origins, recombination, and virus-host interaction.

3. Gene repertoires in discriminating FCoV and CCoV genotypes

In general, the coronavirus genome contains six ORFs flanked by 5′ and 3′ untranslated regions. The viral RNA is covered by the nucleocapsid protein (N), which is enveloped by membrane proteins directly encoded by at least three viral genes involved in the synthesis of the structural spike protein (S), envelope protein (E), and membrane protein (M). Some coronaviruses have an additional membrane glycoprotein, hemagglutinin esterase, and the accessory proteins 3a–c and 7a–b (Masters et al., 2006). Of these important components, the trimeric S protein forms characteristic viral peplomers, and is a major driver of viral tropism and pathogenesis (Delmas and Laude, 1990; de Groot et al., 1989). This distinctive S protein is found in serotype I viruses with seropositivity of up to 90% infection of FCoVs, while the S protein of serotype II has been identified as a recombinant protein between feline and canine enteric coronaviruses (Shiba et al., 2007). Type II FCoVs can use feline aminopeptidase N (fAPN), a cell surface metalloprotease on the intestinal, lung, and kidney epithelial cells, as its receptor, but this is impossible for type I FCoVs, suggesting that the two serotypes use different receptors for cell entry (Hohdatsu et al., 1998; Dye et al., 2007). In contrast, Tresnan and colleagues (1996) showed that the type I FCoV strain UCD-1 also can use fAPN

receptors. Evidence regarding the receptor for the attachment and entry of type I FCoVs remains conflicting.

CCoVs were also divided into two genotypes. Based on their genetic relation to FCoV-I, FCoV-like CCoVs were designated type I CCoV (accounting for about 20% of CCoV infections) and the typical reference CCoVs were termed type II CCoVs (Pratelli et al., 2003). In contrast to FCoVs, both genotypes of CCoV are commonly co-infected (nearly 36% of CCoV-infected dogs) in dogs with diarrhea, and thus genetic recombination is allowed to occur (Pratelli et al., 2004; Decaro et al., 2010; Soma et al., 2011). Type I and type II CCoVs could be differentiated based on the single gene encoding for S protein, ORF3 (absent in all other alphacoronaviruses), or M protein (Decaro and Buonavoglia, 2008; Pratelli et al., 2006, Jeoung et al., 2014). In type II CCoVs (accounting for about 44% of CCoV infections), three sub-genotypes (CCoV-IIa, IIb, IIc) were classified based on the sequence of the first 300 amino acids of the N-terminal domain (NTD) in the S protein that is an important determinant of intestinal tropism in closely related porcine coronaviruses (TGEV, transmissible gastroenteritis virus) (Krempl et al., 1997; Schultze et al., 1996; Decaro et al., 2009). Infection by the type IIa CCoV strain, the highly virulent pantropic CCoV (e.g., strain CB/05), has been detected in tissues other than the intestine, including the lungs, spleen, liver, kidney, and brain, and the patient exhibits clinical signs of fever, lethargy, vomiting, hemorrhagic diarrhea, and acute lymphopenia and neurological signs, followed by death (Buonavoglia et al., 2006; Decaro et al., 2007, 2008). Moreover, type IIb CCoV is detected in 20% of type II CCoV infections and is thought to have emerged via double recombination between CCoV-IIa and TGEV (the so-called TGEV-like CCoVs), suggesting that co-

infection has occurred in at least one host species (Decaro et al., 2009, 2010; Pedersen et al., 1984). The recently characterized type IIc CCoV (with strain A76 as a prototype) has been reported in Sweden and the United States. CCoV strain A76 has a recombinant S protein, a product of recombination between type I and II CCoV sequences and a serotype I-like S1 NTD, while the rest of the protein is serotype II-like (Regan et al., 2012; Whittaker et al., 2018).

In the evolutionary study of FCoVs and CCoVs, it has been proposed that type I FCoVs and CCoVs originate from a common ancestor. Presumably, the acquisition of the ORF3 gene after the divergence of type I FCoVs or the loss of this gene in their common ancestor may have resulted in type I CCoVs. Meanwhile, the gain of the new S gene and the loss of the ORF3 gene led to the emergence of type II CCoVs and then gave rise to type II FCoVs through recombination with type I FCoVs (Lorusso et al., 2008). Furthermore, no cross-protection from pantropic CCoV infection was observed in a dog immunized by an enteric CCoV strain (Decaro et al., 2010). These results indicate that the dynamics of the gain/loss of accessory genes may generally cause shift of infection in the host and then result in more virulent strains and increased severity of enteritis disease.

The pan-genome analysis with the gene present or absent clearly demarcated strains not according to which host species the strains infect (Fig. 3), as was observed when performing the analysis with complete genomes (Whittaker et al., 2018). Moreover, the distribution of the clinical isolates across the categories in the hierarchical phylogenetic model enabled the visualization of their original ecological niche rather than their isolation source, as infections are extremely rare events and evolutionary dead-ends. Based on the accessory genes, we distinguished FCoVs and CCoVs into seven different

categories (Fig. 4). Among these, category 1 consisted predominantly of type I/II FCoV strains, and some strains were closely related to TGEV (Fig. S3), indicating that recombination events might occur between these FCoVs. In particular, type II FCoV grouped with the TGEV genetically related CCoV strain HLJ-073 (accession no. KY063618) in category 2, indicating an approach for identifying sub-genotype CCoV-IIb (Decaro et al., 2009). On the other hand, the FCoV strain NTU156 in category 2 was identified as a natural interspecies recombination between type I FCoV and type II CCoV and was originally isolated from the pleural effusion of a FIP cat (Lin et al., 2013). Thus, the presence of CCoV strain KY063618, an enteric strain with ORF3abc deletion, in category 2 may indicate its dynamic infection, for example, its alternative tissue tropism from the intestinal tract to systemic infection and its viral cell tropism from dogs to humans (Chen et al., 2019). Some studies (e.g. Alfano et al., 2019; Xia et al., 2020) have described three CCoV strains, i.e., HLJ-071, HLJ-072, and HLJ-073, as belonging to CCoV-IIa. However, the pan-genome analysis separated strain HLJ-073 (KY063618) from other identified CCoV-IIa strains (e.g., strain HLJ-072 with accession no. KY063617 and HLJ-071 with accession no. KY063616). This is consistent with a previous result that showed a close relationship between strain HLJ-073 with members of the type II FCoV cluster rather than with members of the CCoV-I or CCoV-II cluster. Based on recombination analysis of its S, E, and M genes, it is probably a recombinant of TGEV, FCoV-II, and CCoV-I/II (Chen et al., 2019). Thus, the pan-genome analysis may discriminate clearly between the different pathogenic forms of FCoVs and CCoVs, including their potential threat to distinct host cell tropism.

With the presence of pantropic CCoV (pCCoV)-IIa strain CB/05, the CCoVs in category 3 are considered sub-genotype IIa (pCCoV-IIa), while type I FCoVs were mainly distributed in this category. Compared with the closely related TGEV strains, the FIP virus (FIPV) strain UU17 (accession no. HQ012367) clustered to the TGEV Purdue and Miller strains (Fig. S3), showing the higher genetic relatedness between pCCoV, FIPV, and TGEV.

Our findings also show that CCoV strains K378 and 171 appear to be even closer to FIPVs and FECVs (category 4) than to the other CCoVs, consistent with the previous conclusion for strain K378 based on analysis of the S protein (Wesseling et al., 1994). In addition, the pattern of gene repertory similarities may reflect the geographical origin of these coronaviruses, e.g., two FCoVs originate from the USA and the Netherlands, while three CCoVs originate from the USA, Italy, and Germany. This may indicate their epidemiological transition of geographical distribution between FCoVs and CCoVs.

The remaining FCoVs in the other categories are capable of triggering genetic recombination in both FECVs and FIPVs. FIPV may arise through mutations in the viral genome during FECV (feline enteric coronavirus) infection and lose its enterocyte tropism. Factors such as stress, re-infection, or super-infection that may trigger disease progression have been described for both naturally and experimentally infected cats (Desmarets et al., 2016). In the host, the virus faces high selection pressure to form a genetically heterogeneous virus population. For example, the distribution of FCoV mutations through deletions and/or amino acid changes related to protein M, S1, S2, N, and 7b has been demonstrated when compared to its originating FECV strain (accession no. KU125419) and during infection (Lowiese et al., 2016). However, that study could

not find the hitherto described FIPV-specific mutations in the genes encoding spike and 3c, and did not indicate the ability of gradual adaptation of FECV at the level of whole gene repertory comparison. Here, the pan-genome analysis showed that FCoV with accession no. KU215420 (exposed cat 1, 7 day(s) post-infection) clustered with type I FECV, while FCoV with accession no. KU215422 (exp. cat 2, 21 dpi) is close to type I FIPV (Fig. 4). In late infection, FCoV with accession no. KU215424 (exp. cat 1, 28 dpi) and KU215427 (with S1 deletion, exp. Cat 3, 28 dpi), isolated from hosts with clinical signs such as diminished appetite and moderate weight loss, were clustered to type I FIPVs (with accession no. FJ938061 and KY566209 harvested from ascites samples) and type IIc CCoV strain A76. These data support the rapid adaptation of FECV and its formation of new variants, posing a threat to the host. Our analysis of the gene repertories derived from FECV and FIP might indicate the relation of FECV infections; it also useful for understanding the pathogenesis and further designing accurate biomarkers for differentiating FIPV and FECV, on which many scientists focus continually (e.g. Guan et al., 2020).

The four FCoVs assigned by strain HLJ (accession no. KY566209, KY566210, KY566211, KY292377) were sampled from FIP−suspected cats in China. Previous study (Li et al., 2019) showed a cluster of three strains (KY566209, KY566210, KY566211) as a potentially new type I FCoV when compared with reference strains of type I and type II FCoVs, and the highest phylogenetic relationship was with a reference strain isolated from Denmark (cat 1 Karlslunde, accession no. KX722530), while strain KY292377 formed in another clade distinguished from three other HLJ strains. This result was not entirely in line with our pan-genome analysis, where four strains were differently

distributed to category 6 and 7 (Fig. 4). In contrast to the type I FCoV strain with accession no. KY566211 (from a single-cat household environment) clustered in category 6, three of four FCoV genomes with accession no. KY566209, KY566210, and KY292377 [all isolated from multi−cat environments; Li et al., (2019)] clustered in category 7 and separated into sub-clusters of type I and II FCoVs. In particular, the FCoV strain with accession no. KY292377 clustered to the strain with accession no. KX722530, isolated from cat lung (Denmark) and the strain with accession no. KX722529 isolated from naturally infected cats with FIP (Belgium) and type II FCoV strain 79-1683 with accession no. JN634064 (Herrewegh et al., 1998; Pedersen et al., 2014). These data suggest that the high seroprevalence of type I FCoVs in the cat population may be distinguished well by pan-genome analysis when combined with their isolation sources, clinical characteristics of infection, and cell tropism. This result is in line with the conclusion that strain 79-1683 was not a true FECV, where it was demonstrated to grow readily in Crandell-Rees feline kidney (CRfk) cells and harbor a mutated ORF3c in the sequences, while FECVs are different (Pedersen et al., 2014). Our pan-genome analysis can be used to estimate the viral tropism and pathogenesis of coronavirus.

In addition to the strain HLJ-073 (accession no. KY063618), which was separate from other identified CCoV-IIa strains (e.g., strain HLJ-072 with accession no. KY063617 and HLJ-071 with accession no. KY063616) based the pan-genome analysis, these fecal strains can be re-demonstrated as a potential pathogenic species when clustered together with strains isolated from ascites, pleura, or lung of diseased animals. Indeed, cell isolation and tropism experiments have shown that fecal isolate HLJ-073 could induce cytopathic effects in CRfk cells, and replicate effectively in canine

macrophages/monocytes and human THP-1 cells. Likewise, this pantropic isolate has functional genes form a category 2 with FCoV strain NTU156 (accession no. GQ152141) isolated from pleura (Fig. 4), indicating an alternative approach to finding a new pantropic isolate.

Moreover, our findings are in agreement with a recent study (Xia et al., 2020) that showed CCoV infecting the canine digestive system and that included strain HLJ-071, HLJ-072, and HLJ-073 with reduced genomic I CpG that could spread to the respiratory system in canids and that became a severe canine pathogen. These enteric CCoVs also exhibited a much lower GC% and I CpG combination than CRCoVs infecting the canine respiratory system. The presumably strong selection against CpG in the viral RNA genome in canid intestine resulted in rapid evolution of the virus that may suggest a hypothesis of the origin and initial transmission of pantropic isolates (including other animal and human strains, e.g., BatCoV RaTG13 and SARS-CoV-2). Likewise, the category 6 type II CCoV strain TN_449 (accession no. JQ404410), isolated from the host died of gastroenteritis, and strain 1_71 (accession no. JQ404409), isolated from the feces of a diarrheic host, exhibited CpG deficiency (Xia et al., 2020). This suggests the importance of monitoring coronavirus infections in the canine digestive system, where the transformation of the viral lineage can be induced to gain a low-GC genome and spread to other species. Thus, analysis of a pan-genome, CpG, and viral tropism should be combined to clarify the characteristics of virulent coronaviruses.

4. The identification of biomarkers for diagnosis

Type I CCoV isolates are not culturable in cell culture systems, which has severely hampered the study of these viruses. *In vivo*, type I CCoVs co-circulate extensively with

type II CCoVs, often occurring as co-infections (Decaro et al., 2011; Ntafis et al., 2011; Costa et al., 2013). Classification of CCoV-IIa and CCoV-IIb has been based on differences in pathogenicity and tissue tropism. The common CCoV-IIa is restricted to the small intestine and causes enteritis, while the pantropic CCoV-IIa can spread systemically, and causes leukopenia. Like TGEV, the second variant CCoV-IIb causes enteritis in neonatal animals. CCoV-IIb has been detected by PCR assays in various organs outside the intestinal tract primarily in dogs co-infected with canine parvovirus (Decaro et al., 2008, 2012; Marinaro et al., 2010), but also in dogs with uncertain disease status (Decaro et al., 2013). In culture, type II CCoV strain 1-71 typically grows readily with the A-72 cell line, which are canine tumor fibroblast cells widely used for virus propagation, but also grows well in a variety of feline cell lines (e.g., CRfk). However, it is not grow in many other canine cell lines (Regan et al., 2012).

Similar to CCoVs, type I strains predominate throughout the world, but type II strains appear to be more adaptable to tissue culture. Both type I and type II FCoVs co−circulate in FIP−affected cats (Li et al., 2019). Measuring antibody levels against FCoV is rarely of value in etiological diagnosis of FIP, and histopathological examination of infected tissues is commonly used (O'Brien et al., 2012; Sharif et al., 2010). In spite of the similarities between these viruses and the frequent sub-clinical infections, it is clear that systemic and lethal FIP is a much more common outcome for FCoV infection in cats, compared to CCoV infection in dogs. Thus, there is an urgent need for a universal method for detecting FCoV/CCoV variants in clinical specimens based on genome-wide analysis, and numerous studies based on the RT-PCR method have been developed (e.g. Decaro et al., 2005; Tanaka et al., 2015).

In our analysis, polymorphism of dispensable genes among the FCoV and CCoV strains provides very valuable information on species-specific control measures against coronavirus disease. For example, the presence/absence of a gene (Fig. 4, white box) may be a good marker in molecular techniques, an unending search to accurately identify pantropic CCoV, and type IIb and IIc CCoV isolates, especially when differentiating new species from common and/or pantropic species (Licitra et al., 2014; Tanaka et al., 2015). On further examination, the combination of phenotyping and visualizing differential gene content with downstream analysis such as GeneMarkS, RAST annotation subsystems, as well as *in silico* modeling of surface proteins (UCSF Chimera, University of California) will help discover new biological insights into the evolution of pathogenesis and explore strain-specific drug targets against coronavirus disease in animals. Finally, our pan-genome interpretation, pan-RT-PCR, a highly discriminatory PCR assay based on highly informative identified genetic targets according to their presence or absence (Yang et al., 2013), will be a routine tool in the lab that can distinguish all clinically relevant FCoV and CCoV variants.

5. Conclusions

The amount of coronavirus genetic information available in the NCBI GenBank Genome database allows us to begin addressing the genetic complexity of veterinary coronaviruses, which originate from several molecular mechanisms, including insertion/deletion events, different nucleotide substitution rates, and intragenotype and intergenotype recombination and re-assortment events. Of course, pan-genome analyses are an effective tool that could yield deep insights into the comparison of virulence genes among viral strains, and could also enable further understanding of mutualistic

interactions and/or host–virus interactions. Integrated analyses using tools such as ANI, pan-genome, and phylogenomic analysis are used for the taxonomy of viral strains isolated from different host ecosystems. For example, whole-genome ANI showed that FCoVs and CCoVs had 80.34–88.72% similarity. Serotype II CCoV accession no. KC175339 shared high similarity (ANI > 99%) with serotype II FCoVs (accession no. DQ010921, DQ286389, JQ408980, GQ152141, JN634064), suggesting that ANI analysis can be conducted quickly for assessing the very close antigenic relationship between FCoV and CCoV and its potential for cross-species infection. Downstream analysis may therefore be indicative of whether an isolate is an emerging potential zoonotic pathogen. For example, strain HLJ-073 in China has biological characteristic differences from other reported CCoVs, and hierarchical clustering can be useful for screening the CCoV special strains. In addition, we distinguished FCoVs and CCoVs into seven different categories based on the distribution of their accessory genes. The distribution of the clinical isolates across the categories in the hierarchical phylogenetic model enables the visualization of their original ecological niche rather than their isolation source, as infections are extremely rare events and evolutionary dead-ends. This interpretation enables the development of novel control methods against disease, e.g., a rapid detection assay such as pan-RT-PCR, as well as a promising candidate for vaccine development.

6. Conflict of interest statement

The authors declare no conflict of interest.

7. Data Availability Statement

All data of this article are fully available without restriction on request from the corresponding author

8. References

Alfano, F., Dowgier, G., Valentino, M. P., Galiero, G., Tinelli, A., Nicola, D., Fusco, G. (2019). Identification of Pantropic Canine Coronavirus in a Wolf (*Canis lupus italicus*) in Italy. J Wildl Dis. 55(2):504-508. doi:10.7589/2018-07-182.

Benetka, V., Kolodziejek, J., Walk, K., Rennhofer, M., Möstl, K. (2006). M gene analysis of atypical strains of feline and canine coronavirus circulating in an Austrian animal shelter. Vet Rec.159:170-175.

Blom, J., Kreis, J., Spänig, S., Juhre, T., Bertelli, C., Ernst, C., Goesmann, A. (2016). EDGAR 2.0: An enhanced software platform for comparative gene content analyses. Nucleic Acids Res. 44:W22-W28.

Buonavoglia, C., Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Castagnaro, M., & Tempesta, M. (2006). Canine coronavirus highly pathogenic for dogs. Emerging infectious diseases, *12*(3), 492–494. https://doi.org/10.3201/eid1203.050839.

Chen, S., Liu, D., Tian, J., Hongtao, K., Guo, D., Qian, J., Jiasen, L., Zhijie, Li, Xiaoliang H., Liandong Qu. (2019). Molecular characterization of HLJ-073, a recombinant canine coronavirus strain from China with an ORF3abc deletion. Arch Virol. 164(8):2159-2164. https://doi.org/10.1007/s00705-019-04296-9

Costa, E. M., de Castro, T. X., Bottino Fde, O., Garcia Rde, C. (2013). Molecular characterization of canine coronavirus strains circulating in Brazil. Vet. Microbiol. 168:8-15.

De Groot, R. J. van Leen, R. W. Dalderup, M. J. M. Vennema, H. Horzinek, M. C., Spaan, W. J. M. (1989). Stably expressed FIPV peplomer protein induces cell fusion and elicits neutralizing antibodies in mice. Virology. 171, 2:493-502.

Decaro, N., Buonavoglia, C. (2008). An update on canine coronaviruses: viral evolution and pathobiology. Vet Microbiol. 132:221-234.

Decaro, N., Buonavoglia, C. (2011). Canine coronavirus: Not only an enteric pathogen. Vet. Clin. N. Am. Small Anim. Pract. 41:1121-1132.

Decaro, N., Campolo, M., Lorusso, A., Desario, C., Mari, V., Colaianni, M.L., Elia, G., Martella, V., Buonavoglia, C. (2008). Experimental infection of dogs with a novel

strain of canine coronavirus causing systemic disease and lymphopenia. Vet. Microbiol. 128:253-260.

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, P. J., Rusvai, M., Shields, S., Xylouri, E., Xu, Z., & Buonavoglia, C. (2013). European surveillance for pantropic canine coronavirus. Journal of clinical microbiology, 51(1), 83–88. https://doi.org/10.1128/JCM.02466-12.

Decaro, N., Elia, G., Martella, V., Campolo, M., Mari, V., Desario, C., Lucente, M. S., Lorusso, E., Kanellos, T., Gibbons, R. H., & Buonavoglia, C. (2010). Immunity after natural exposure to enteric canine coronavirus does not provide complete protection against infection with the new pantropic CB/05 strain. Vaccine, *28*(3), 724–729. https://doi.org/10.1016/j.vaccine.2009.10.077

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. Journal of virology, *83*(3), 1532–1537. https://doi.org/10.1128/JVI.01937-08.

Decaro, N., Mari, V., Elia, G., Addie, D. D., Camero, M., Lucente, M. S., Martella, V., & Buonavoglia, C. (2010). Recombinant canine coronaviruses in dogs, Europe. Emerging infectious diseases, *16*(1), 41–47. https://doi.org/10.3201/eid1601.090726.

Decaro, N., Martella, V., Ricci, D., Elia, G., Desario, C., Campolo, M., Cavaliere, N., Di Trani, L., Tempesta, M., & Buonavoglia, C. (2005). Genotype-specific fluorogenic RT-PCR assays for the detection and quantitation of canine coronavirus type I and type II RNA in faecal samples of dogs. Journal of virological methods, *130*(1-2), 72–78. https://doi.org/10.1016/j.jviromet.2005.06.005

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 research, *125*(1), 54–60. https://doi.org/10.1016/j.virusres.2006.12.006.

Delmas, B., Laude, H. (1990). Assembly of coronavirus spike protein into trimers and its role in epitope expression. Journal of Virology. 64, 11:5367-5375.

Desmarets, L. M., Vermeulen, B. L., Theuns, S., Conceição-Neto, N., Zeller, M., Roukaerts, I. D., Acar, D. D., Olyslaegers, D. A., Van Ranst, M., Matthijnssens, J., & Nauwynck, H. J. (2016). Experimental feline enteric coronavirus infection reveals an aberrant infection pattern and shedding of mutants with impaired infectivity in enterocyte cultures. Scientific reports, *6*, 20022. https://doi.org/10.1038/srep20022

Dye, C., Temperton, N., Siddell, S.G. (2007). Type I feline coronavirus spike glycoprotein fails to recognize aminopeptidase N as a functional receptor on feline cell lines. The Journal of general virology. 88(Pt 6):1753-1760. https://doi.org/10.1099/vir.0.82666-0

Escutenaire, S., Isaksson, M., Renstrom, L.H., Klingeborn, B., Buonavoglia, C., Berg, M., Belak, S., Thoren, P. (2007). Characterization of divergent and atypical canine coronaviruses from Sweden. Arch. Virol. 152:1507-1514.

Faria, N. R., Quick, J., Claro, I.M., Theze, J. de Jesus, J.G. Giovanetti, M., et al. (2017). Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 546:406-410.

Faria, N.R., Kraemer, M.U., Hill, S. de Jesus, J.G. de Aguiar, R.S. Iani, F.C., et al. (2018). Genomic and epidemiological monitoring of yellow fever virus transmission potential. Science. 361, 6405:894-899. https://doi.org/10.1126/science.aat7115

Guan, X., Li, H., Han, M., Jia, S., Feng, B., Gao, X., Wang, Z., Jiang, Y., Cui, W., Wang, L., & Xu, Y. (2020). Epidemiological investigation of feline infectious peritonitis in cats living in Harbin, Northeast China from 2017 to 2019 using a combination of an EvaGreen-based real-time RT-PCR and serum chemistry assays. Molecular and cellular probes, *49*, 101495. https://doi.org/10.1016/j.mcp.2019.101495.

Herrewegh, A. A. P. M., Smeenk, I., Horzinek, M. C., Rottier, P. J. M., de Groot, R. J. (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(5):4508-4514.

Hohdatsu, T., Izumiya, Y., Yokoyama, Y., Kida, K., Koyama, H. (1998). Differences in virus receptor for type I and type II feline infectious peritonitis virus. Arch Virol 143: 839-850.

Hora, A.S., Tonietti, P.O., Taniwaki, S.A., Asano, K.M., Maiorka, P., Richtzenhain, L.J., Brandao, P.E. (2016). Feline coronavirus 3c protein: A candidate for a virulence marker? Biomed. Res. Int. 8560691.

Horzinek, M.C., Lutz, H., Pedersen, N.C. (1982). Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect Immun. 37:1148-1155.

Jeoung, S.Y., Ann, S. Y., Kim, H.T., Kim, D. (2014). M gene analysis of canine coronavirus strains detected in Korea. Journal of veterinary science. 15(4):495-502. https://doi.org/10.4142/jvs.2014.15.4.495

Krempl, C., Schultze, B., Laude, H., Herrler, G. (1997). Point mutations in the S protein connect the sialic acid binding activity with the enteropathogenicity of transmissible gastroenteritis coronavirus. J. Virol. 71:3285-3287.

Li, C., Liu, Q., Kong, F., Guo, D., Zhai, J., Su, M., & Sun, D. (2019). Circulation and genetic diversity of Feline coronavirus type I and II from clinically healthy and FIP-suspected cats in China. Transboundary and emerging diseases, *66*(2), 763–775. https://doi.org/10.1111/tbed.13081

Li, X., Song, Y., Wong, G., Cui, J. (2020). Bat origin of a new human coronavirus: there and back again. Sci China Life Sci. 63:461-462.

Licitra, B.N., Duhamel, G.E., Whittaker, G. R. (2014). Canine enteric coronaviruses: emerging viral pathogens with distinct recombinant spike proteins. Viruses. 6(8):3363-3376. https://doi.org/10.3390/v6083363

Lin, C.N., Chang, R.Y., Su, B.L., Chueh, L.L., (2013). Full genome analysis of a novel type II feline coronavirus NTU156. Virus Genes. 46(2):316-322. doi: 10.1007/s11262-012-0864-0.

Lorusso, A., Decaro, N., Schellen, P., Rottier, P.J., Buonavoglia, C., et al. (2008). Gain, preservation, and loss of a group 1a coronavirus accessory glycoprotein. J Virol. 82: no. 20:10312-10317.

Desmarets, L. M., Vermeulen, B. L., Theuns, S., Conceição-Neto, N., Zeller, M., Roukaerts, I. D., Acar, D. D., Olyslaegers, D. A., Van Ranst, M., Matthijnssens, J., &

Nauwynck, H. J. (2016). Experimental feline enteric coronavirus infection reveals an aberrant infection pattern and shedding of mutants with impaired infectivity in enterocyte cultures. Scientific reports, *6*, 20022. https://doi.org/10.1038/srep20022.

Marinaro, M., Mari, V., Bellacicco, A. L., Tarsitano, E., Elia, G., Losurdo, M., Rezza, G., Buonavoglia, C., Decaro, N. (2010). Prolonged depletion of circulating CD4+ T lymphocytes and acute monocytosis after pantropic canine coronavirus infection in dogs. Virus Res. 152:73-78.

Masters, P.S. (2006). The molecular biology of coronaviruses. Advances in Virus Research. 65:193-292.

McArdle, F., Bennett, M., Gaskell, R. M., Tennant, B., Kelly, D. F., Gaskell, C. J. (1992). Induction and enhancement of feline infectious peritonitis by canine coronavirus. Am J Vet Res. 53:1500-1506.

Naylor, M. J., Walia, C. S., McOrist, S., Lehrbach, P. R., Deane, E. M., Harrison, G. A., (2002). Molecular characterization confirms the presence of a divergent strain of canine coronavirus (UWSMN-1) in Australia. J Clin Microbiol. 40:3518-3522.

Ntafis, V., Mari, V., Decaro, N., Papanastassopoulou, M., Papaioannou, N., Mpatziou, R., Buonavoglia, C., Xylouri, E. (2011). Isolation, tissue distribution and molecular characterization of two recombinant canine coronavirus strains. Vet. Microbiol. 151: 238-244.

O'Brien, S. J., Troyer, J. L., Brown, M. A., Johnson, W. E., Antunes, A., Roelke, M. E., et al. (2012). Emerging viruses in the Felidae: shifting paradigms. Viruses. 4:236-57.

OIE report 2020, https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33892

Pedersen, N. C. (2014). An update on feline infectious peritonitis: virology and immunopathogenesis. Vet J. 201(2):123-32. doi: 10.1016/j.tvjl.2014.04.017.

Pedersen, N. C., J. W., Black, J. F. Boyle, J. F. Evermann, A. J., McKeirnan, R. L., Ott. (1984). Pathogenic differences between various feline coronavirus isolates. Advances in Experimental Medicine and Biology. 173:365-380.

Pratelli, A., (2006). Genetic evolution of canine coronavirus and recent advances in prophylaxis. Vet. Res. 37:191-200.

Pratelli, A., Decaro, N., Tinelli, A., Martella, V., Elia, G., Tempesta, M., Cirone, F., Buonavoglia, C. (2004). Two genotypes of canine coronavirus simultaneously detected in the fecal samples of dogs with diarrhea. J Clin Microbiol. 42:1797-1799.

Pratelli, A., Martella, V., Pistello, M., Elia, G., Decaro, N., Buonavoglia, D., Camero, M., Tempesta, M., Buonavoglia, C. (2003). Identification of coronaviruses in dogs that segregate separately from the canine coronavirus genotype. Journal of Virological Methods. 107(2):213-222.

Regan, A. D., Millet, J. K., Tse, L.P., Chillag, Z., Rinaldi, V. D., Licitra, B. N., Dubovi, E. J., Town, C. D., Whittaker, G. R. (2012). Characterization of a recombinant canine coronavirus with a distinct receptor-binding (S1) domain. Virology. 430:90-99. https://doi.org/10.1016/j.virol.2012.04.013.

Schultze, B., Krempl, C., Ballesteros, M. L., Shaw, L., Schauer, R., Enjuanes, L., Herrler, G. (1996). Transmissible gastroenteritis coronavirus, but not the related porcine respiratory coronavirus, has a Sialic acid (N-Glycolylneuraminic acid) binding activity? J. Virol. 70:5634-5637.

Sharif, S., Arshad, S. S., Hair-Bejo, M., Omar, A.R., Zeenathul, N. A., Alazawy, A. (2010). Diagnostic methods for feline coronavirus: a review. Vet Med Int. 7.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., Zhao, Y., Liu, P., Liang, L., Cui, P., Wang, J., Zhang, X., Guan, Y., Tan, W., Wu, G., Chen, H., … Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science (New York, N.Y.)*, *368*(6494), 1016–1020. https://doi.org/10.1126/science.abb7015

Shiba, N., Maeda, K., Kato, H., Mochizuki, M., Iwata, H. (2007). Differentiation of feline coronavirus type I and II infections by virus neutralization test. Vet Microbiol. 124:348-52.

Soma, T., Ohinata, T., Ishii, H., Takahashi, T., Taharaguchi, S., Taharaguchi, S., Hara, M. (2011). Detection and genotyping of canine coronavirus RNA in diarrheic dogs in. Japan. Res Vet Sci. 90: 205-207.

Sturn, A., Quackenbush, J., Trajanoski, Z., 2002. Genesis: cluster analysis of microarray data. Bioinformatics. 18:207-208,

Tanaka, Y., Sasaki, T., Matsuda, R., Uematsu, Y., Yamaguchi, T. (2015). Molecular epidemiological study of feline coronavirus strains in Japan using RT-PCR targeting nsp14 gene. BMC veterinary research. 11, 57. https://doi.org/10.1186/s12917-015-0372-2

Terada, Y., Matsui, N., Noguchi, K., Kuwata. R., Shimoda, H., Soma, T., Mochizuki, M., Maeda, K. (2014). Emergence of pathogenic coronaviruses in cats by homologous recombination between Feline and Canine Coronaviruses. PLoS ONE 9(9): e106534.

Tettelin, H., Riley, D., Cattuto, C., Medini, D. (2008). Comparative genomics: The bacterial pan-genome. Curr. Opin. Microbiol. 11:472-477.

Tresnan, D. B., Levis, R., Holmes, K. V. (1996). Feline aminopeptidase N serves as a receptor for feline, canine, porcine, and human coronaviruses in serogroup I. J Virol 70:s 8669-8674.

Wesseling, J. G., Vennema, H., Godeke, G. J., Horzinek, M. C., Rottier, P. J. (1994). Nucleotide sequence and expression of the spike (S) gene of canine coronavirus and comparison with the S proteins of feline and porcine coronaviruses. J Gen Virol. 75 (Pt 7):1789-1794. doi:10.1099/0022-1317-75-7-1789

Whittaker, G. R., André, N. M., & Millet, J. K. (2018). Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the *Alphacoronavirus 1* Species. *mSphere*, *3*(1), e00463-17. https://doi.org/10.1128/mSphereDirect.00463-17

Woo, P. C., Lau, S. K., Lam, C. S., Lau, C. C., Tsang, A. K., Lau, J. H., Bai, R., Teng, J. L., Tsang, C. C., Wang, M., Zheng, B. J., Chan, K. H., & Yuen, K. Y. (2012). Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *Journal of virology*, *86*(7), 3995–4008. https://doi.org/10.1128/JVI.06540-11.

Xia, X. (2020). Extreme genomic CpG deficiency in SARS-CoV-2 and evasion of host antiviral defense. Mol Biol Evol. msaa094. doi:10.1093/molbev/msaa094.

Yang, J. Y., Brooks, S., Meyer, J. A., Blakesley, R. R., Zelazny, A. M., Segre, J. A., Snitkin, E. S. (2013). Pan-PCR, a computational method for designing bacterium-

typing assays based on whole-genome sequence data. Journal of clinical microbiology 51(3):752-758. https://doi.org/10.1128/JCM.02671-12

Zicola, A., Jolly, S., Mathijs, E., Ziant, D., Decaro, N., Mari, V., Thiry, E. (2012). Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. J. Small Anim. Pract. 53:297-300.

Figure legends

Figure 1: The Heat-map of Average Nucleotide identity (ANI) matrix and phylogenetic relationship of 99 CoV genomes. The color bar represents the host species. Strains names with isolation source and accession numbers are listed in Table S1.

Figure 2: Pan genome analysis of CCoVs and FCoVs. (A and B) Calculated pan genome of CCoV and FCoV strains, respectively. These were extracted by using EDGAR (Blom et al., 2016). (C and D) Sizes of pan-genome and core genome of CCoV and FCoV strains, respectively, indicated an open pan-genome according to Heap's Law model.

Figure 3: Comparison of hierarchical clustering and phylogenetic tree of studied CoVs genomes. Both hierarchical clustering (right panel) based on their relative shared gene content and phylogenetic tree (left panel) based on their concatenated orthologous genes were performed on all 99 strains. Color strings connecting the same strains of both trees aims at highlighting the degree of similarities between both tree methods (Sturn et al., 2002; Blom et al., 2016).

Figure 4: Comparison of hierarchical clustering of FCoV and CCoV genomes indicating a potential in discriminating FCoV and CCoV genotypes. Function of gene

presence and absence of gene in each genome are indicated as red and black, respectively. Strain labeled with colour-dot is closely related to TGEV in Fig. S2.

Supplementary Figure S1: The Heat-map of Average Nucleotide identity (ANI) matrix and phylogenetic relationship of miscellaneous CoV genomes. The color bar represents the host species. Strains names with isolation source and accession numbers are listed in Table S1.

Supplementary Figure S2: Comparison of hierarchical clustering of CoV genomes derived from many host species indicating a potential relationship of FCoV and HCoV (NL63, 229E) genotypes. Function of gene presence and absence of gene in each genome are indicated as red and black, respectively.

Supplementary Figure S3. Comparison of hierarchical clustering of FCoV and CCoV genomes to TGEV, transmissible gastroenteritis virus. (A) Hierarchical clustering of CCoV and TGEV genomes indicating a potential in discriminating type IIb/c CCoV. (B) Hierarchical clustering of FCoV and TGEV genomes indicating a natural interspecies recombination. Function of gene presence and absence of gene in each genome are indicated as red and black, respectively. Strains names with isolation source and accession numbers are listed in Table S1.

Supplementary Table 1. List of CoV genomes in this study.

Highlights

Whole-genome ANI between FCoV and CCoV strains showed 80.3–88.72% similarity, with some exceptions.

ANI analysis can be quickly conducted to assess the very close antigenic relationship between FCoVs and CCoVs and its potential for cross-species infection.

Pan-genomic analysis and the hierarchical phylogenetic model clearly demarcate strains according to the distribution of clinical isolates.

Combined analysis of CCoVs; FCoVs; and the HCoV strains HKU1, OC43, NL63, and 229E also indicate that cats are highly susceptible to HCoVs, while dogs have low susceptibility to the virus.

The distribution of the pantropic isolates across the categories in the hierarchical phylogenetic model allows the prediction of their potential for infection, and the selection of good markers for differentiating new species from common and/or pantropic isolates.