Molecular epidemiology of type I and II feline coronavirus from cats with suspected feline infectious peritonitis in China between 2019 and 2021

Lishan Lin · Dawei Yao · Lei Wu · Rubin Fan · Yang Liu · Zhenlei Zhou

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Abstract
Feline infectious peritonitis (FIP) is one of the deadliest diseases of cats in China. In this study, 120 ascitic fluid samples from FIP-suspected cats were collected from veterinary hospitals in 21 provinces in China between 2019 and 2021. One hundred nine samples were positive for feline coronavirus (FCoV), with no feline immunodeficiency virus infections and one feline leukemia virus infection (1/109, 0.92%). The prevalence of FCoV was significantly associated with age ($p < 0.01$) and was not highly associated with gender, breed, geographical location, or viral coinfection ($p > 0.01$). One unique strain, SD/202012/003, contained a six-nucleotide deletion in the spike gene. Sequence analysis showed that 94.68% (89/94) of the isolates had a mutation of methionine to leucine at position 1058 in the spike protein. The epidemiological data obtained of FCoV in this study may be beneficial for clinical monitoring of FCoV in China.

Introduction
Feline infectious peritonitis (FIP) is a fatal disease with a high mortality rate, especially in kittens, that is typically characterized by granulomatous inflammatory lesions in several organs and/or protein-rich serous effusion in body cavities [1]. The etiologic pathogen of FIP has been identified as feline infectious peritonitis virus (FIPV), which originated from feline enteric coronavirus (FECV) [2]. FIPV and FECV are biotypes of feline coronavirus (FCoV), a single-stranded positive-sense RNA virus belonging to the family Coronaviridae, order Nidovirales [3]. FCoV has two genotypes, FCoV-I and FCoV-II [4]. The latter genotype resulted from recombination between a type I FCoV and canine coronavirus (CCoV) [5].

FIP is a consequence of a viral infection. However, it is usually associated with several risk factors, such as age, environment, and viral coinfections. FIP occurs mostly in 6-month- to 2-year-old cats [6, 7]. This is possibly attributable to less-effective control of replication of FCoV in these animals [8]. Thus, critical mutations are more likely to appear. Furthermore, a multiple-cat environment is considered a significant risk factor for FIP because it increases the risk of FECV infection [9]. Recovery from FIP is generally dependent on the elimination of FECV infection. Moreover, it has been reported that cats with viral coinfections are more likely to develop FIP due to the immunosuppressive effect of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) [10–12].

Taking into consideration the sparse information related to FIPV in domestic cats in China, the aims of this study were (a) to carry out an epidemiological survey in China between 2019 and 2021 regarding age, breed, gender, and geographical location, (b) to determine the genotype of FCoV and the presence of viral coinfection with FeLV and FIV, (c) to identify amino acid substitutions at position 1058 in the spike protein, and (d) to investigate the genetic divergence of the identified FCoV isolates for the currently circulating coronaviruses.
Materials and methods

A total of 120 samples were collected from veterinary hospitals between 2019 and 2021, and basic information was recorded. The samples were tested for the presence of feline coronavirus and other viruses, after which the FCoV genotype was determined. Sanger sequencing was performed to identify the M1058L mutation in the S gene, and a phylogenetic tree was constructed for phylogenetic analysis. Further details are provided as a supplementary file.

Results

One hundred nine out of 120 samples tested positive for FCoV by PCR. Detailed information about the 120 samples is given in Supplementary Table S1. The percentage of sampling sites located in northern and southern China was 52.29% (57/109) and 47.71% (52/109), respectively. The percentage of male and female cats in this study was 53.21% (58/109) and 46.79% (51/109), respectively. The purebred and cross-breed cats accounted for 41.28% (45/109) and 58.72% (64/109), respectively. The age of the FIP-suspected cats ranged from 1 week to 67 months. The median age was 3 months, with a proportion of 16.51%. The percentage of cats less than 12 months and 24 months old was 91.74% and 97.25%, respectively. FIV was not detected in any of the samples, while one of the 109 FCoV-positive samples and one of the 11 FCoV-negative samples were positive for FeLV. The prevalence of FCoV was significantly associated with age ($p = 0.003$) and was not highly associated with gender ($p = 0.731$), breed ($p = 0.268$), geographical location ($p = 0.908$), or viral coinfection ($p = 0.434$) (Table 1).

Partial S gene sequences were determined for 78 (71.56%, 78/109) of the samples (Supplementary Table S1). Sequence analysis indicated that the positive rate for FCoV I and FCoV II was 98.72% (77/78) and 1.28% (1/78), respectively. The nucleotide and amino acid sequence identity among the 77 FCoV I strains was 81.00%-100.00% and 82.1%-100%, respectively. The 77 type I strains exhibited 80.4%-89.3% nucleotide sequence identity and 80.4%-94.6% amino acid sequence identity to the type I FCoV reference strain C1Je. The nucleotide and amino acid sequence identity of the FCoV type II strain to the 77 identified type I strains was 52.9%-58.9% and 55.2%-67.2%, respectively. The FCoV type II strain exhibited 94.0% nucleotide and 98.2% amino acid sequence identity to the type II FCoV reference strain 79-1146. It is interesting to note that one strain, SD/202012/003, contained a six-nucleotide deletion compared with the other 76 FCoV I strains (Fig. 1).

The partial S genes of 94 (94/109, 86.24%) samples were amplified to identify the mutation M1058L in the S gene. Sequence analysis showed that 89 (89/94, 94.68%) had a T (78/94, 82.98%) or C (11/94, 11.70%) at position 23,531, while five had an A (5/94, 5.32%) at the same position, indicating that 94.68% of the strains had a mutation from methionine to leucine at position 1058 in the spike gene.

Phylogenetic analysis of partial S genes of 78 FCoV strains showed that the phylogenetic tree was divided into 11 clusters of type I FCoV and one cluster of type II FCoV (Fig. 2). The type II FCoV strain AH/202007/002 exhibited the closest relationship to a FCoV strain from Taiwan and a raccoon dog coronavirus strain. Strain SD/202012/003

| Table 1 Correlation of FCoV positivity with age, gender, breed, geographical location, and viral coinfections |
|---------------------------------|-----------------|-----------------|------|-------|
| Variable                        | Number of positive samples | Number of negative samples | OR   | 95% CI | $p$-value |
| Age                             |                  |                  |      |       |          |
| <12 months                      | 96               | 6                | 1.615| 0.937-2.782 | 0.003    |
| ≥12 months                      | 13               | 5                | 0.262| 0.115-0.598 |          |
| Gender                          |                  |                  |      |       |          |
| Male                            | 58               | 7                | 0.836| 0.517-1.352 | 0.731    |
| Female                          | 51               | 4                | 1.287| 0.574-2.884 |          |
| Breed                           |                  |                  |      |       |          |
| Pure                            | 45               | 7                | 0.649| 0.394-1.069 | 0.268    |
| Cross                           | 64               | 4                | 1.615| 0.727-3.584 |          |
| Geographical location           |                  |                  |      |       |          |
| North China                     | 57               | 5                | 1.150| 0.588-2.252 | 0.908    |
| South China                     | 52               | 6                | 0.875| 0.493-1.553 |          |
| Viral coinfections              |                  |                  |      |       |          |
| FeLV positive                   | 1                | 1                | 0.101| 0.007-1.504 | 0.434    |
| FeLV negative                   | 108              | 10               | 1.090| 0.903-1.315 |          |

$OR$ odds ratio, $CI$ confidence interval
contained a six-nucleotide deletion in the spike gene, and
two other strains, BJ/201811/MG892420 and BJ/201811/
MG892451, had a different six-nucleotide deletion.

Discussion

In this study, the percentage of FIP-suspected cats less than
12 months old and less than 24 months old was 91.74% and
97.25%, respectively, which is in agreement with a previous
report. In this study, no significant correlation was found
between FIP and breed or gender ($p > 0.05$). A previous
study showed that breed and gender were not associated
with FIP [13], but other studies have indicated that male
sex was significantly correlated with FIP [6, 14]. There is
also evidence of a predilection for FIP in some breeds [8].
Therefore, the data regarding risk factors for FIP are still
controversial.

In this study, viral coinfection with FCoV was inves-
tigated as a potential risk factor. FIV was not detected in
any of the samples, while only one FCoV-positive and one
FCoV-negative sample were positive for FeLV. The preva-
ence rate of FIV has been reported to be approximately 10%
in Hungary, Malaysia, and Ireland [15–17]. However, the
positive rate of FIV in China is only 1.3–1.5% [18, 19]. The
reason for this difference is unclear. In Hungary, Malaysia,
and Ireland, the positive rate of FeLV was reported to be
17.3%, 12.0%, and 10.4%, respectively, while the prevalence
rate of FeLV in China was found to be 11.33% [20]. In this
study, no significant association was found between FCoV
infection and FIV or FeLV infection ($p = 0.434$). The pos-
sible reason may be the low morbidity rate, and more clinical
samples were required.

The positive rate of FCoV I and FCoV II was 98.72%
(77/78) and 1.28% (1/78), respectively, while the genotype
of the remaining samples could not be determined due to
a low viral load in the samples or possibly to mismatching
between primers and templates. The results indicated that
FCoV I is the dominant genotype in China, which is in
agreement with what has been reported in Japan [14] and
Malaysia [21]. Type II FCoVs, which are recombinants of
a type I FCoV and CCoV, are still far less prevalent than
type I FCoVs. There is little evidence that differences in
infectivity, disease severity, or lethality are associated with
the differences in prevalence of the two types of FCoV.

Studies focusing on differentiating between FECV and
FIPV have been conducted mainly at the molecular level.
Several studies have revealed that mutations in the 3C, 7a,
and 7b genes may play an important role in the genotype
switch [22, 23]. However, accurate detection methods need
to be established to reliably distinguish these two biotypes
of FCoV. One study indicated that $95.83\% (23/24)$ of the
??viruses from animals with FIP?? had the amino acid
substitution M1058L in the spike protein, while none of
??those from animals without FIP?? had this substitu-
tion, indicating that the substitution M1058L is correlated
with FIP [24]. In this study, we found that $94.68\%$ of the
94 samples had the M1058L mutation in the spike gene,
which is consistent with previous studies [24, 25]. Thus,
the M1058L substitution in the S gene could be an indica-
tor of FIP.

A phylogenetic tree based on partial S sequences showed
that 77 type I FCoV strains maintained genetic diversity,
while only one type II FCoV strain exhibited a close rela-
tionship to a FCoV strain from Taiwan and a raccoon dog
coronavirus strain. One study indicated that the above two
strains, BJ/201811/MG892420 and BJ/201811/MG892451,
with a six-nucleotide deletion in the spike gene were not
reported in the current GenBank database [26]. The lim-
ited information currently available suggests that the six-
nucleotide deletion may be one of the characteristics
of Chinese regional type I FCoV strains. In this study, strain
SD/202012/003 contained a different six-nucleotide dele-
tion, which was also not recorded in the GenBank database.
Therefore, this strain might also be Chinese regional type I
FCoV strain.
This study was carried out to investigate the epidemiology of FCoV in China during 2019 to 2021 regarding age, breed, gender, geographical location, and viral coinfections. The prevalence of FCoV was significantly correlated with age ($p < 0.01$) but was not highly associated with gender, breed, geographical location, or viral coinfection ($p > 0.01$). Type I FCoV was more predominately detected than type II FCoV. More than 90% of the strains had a mutation from methionine to leucine at position 1058 in the spike protein, indicating that this could serve as an indicator of FIP.

Fig. 2 Phylogenetic analysis of type I and II FCoV strains based on partial S gene sequences (168 bp)
data provide useful information about the epidemiology of FCoV in China.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05291-9.

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Data availability The data from this study are freely available in the GenBank database (https://www.ncbi.nlm.nih.gov/) under the accession numbers MW858252-MW858329.

Declarations Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The study was approved by the Laboratory Animal Welfare and Ethics Committee of Nanjing Agricultural University. The study did not involve endangered or protected species. No specific per-

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