Comparison of the Amino Acid Sequence and Phylogenetic Analysis of the Peplomer, Integral Membrane and Nucleocapsid Proteins of Feline, Canine and Porcine Coronaviruses

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Received October 13, 1995; in revised form, February 19, 1996. Accepted March 1, 1996

Abstract: Complete nucleotide sequences were determined by cDNA cloning of peplomer (S), integral membrane (M) and nucleocapsid (N) genes of feline infectious peritonitis virus (FIPV) type I strain KU-2, UCD1 and Black, and feline enteric coronavirus (FECV) type II strain 79-1683. Only M and N genes were analyzed in strain KU-2 and strain 79-1683, which still had unknown nucleotide sequences. Deduced amino acid sequences of S, M and N proteins were compared in a total of 7 strains of coronaviruses, which included FIPV type II strain 79-1146, canine coronavirus (CCV) strain Insavc-1 and transmissible gastroenteritis virus of swine (TGEV) strain Purdue. Comparison of deduced amino acid sequences of M and N proteins revealed that both M and N proteins had an identity of at least 90% between FIPV type I and type II. The phylogenetic tree of the M and N protein-deduced amino acid sequences showed that FIPV type I and type II form a group with FECV type II, and that these viruses were evolutionarily distant from CCV and TGEV. On the other hand, when the S protein-deduced amino acid sequences was compared, identity of only about 45% was found between FIPV type I and type II. The phylogenetic tree of the S protein-deduced amino acid sequences indicated that three strains of FIPV type I form a group, and that it is a very long distance from the FIPV type II, FECV type II, CCV and TGEV groups.

Key words: Feline infectious peritonitis virus, Feline enteric coronavirus, Amino acid sequence

Feline infectious peritonitis virus (FIPV), family Coronaviridae, genus Coronavirus, causes a chronic, progressive, immunologically mediated disease in domestic and exotic cats. The family Coronaviridae is divided into 3 distinct antigenic groups on the basis of serologic tests (18). One group contains mouse hepatitis virus, neonatal calf diarrhea coronavirus, human coronavirus OC43, hemagglutinating encephalomyelitis virus of swine and rat coronavirus. The second group contains avian infectious bronchitis virus. The third group consists of human respiratory coronavirus 229E, transmissible gastroenteritis virus (TGEV) of swine, porcine respiratory coronavirus, canine coronavirus (CCV), FIPV and feline enteric coronavirus (FECV). By using monoclonal antibodies we have shown the existence of at least 2 serotypes of FIPV, and the antigenicity of type II strain of FIPV and FECV were closer to TGEV and CCV than to type I FIPV (7, 8). Both types I and II FIPV cause infectious peritonitis in cats, and the pathogenicity of type II FIPV is greater than that of type I FIPV (25). However, in the field, the prevalence of FIPV type I is high, and about 70% of feline cases of FIP are due to infection with type I (9).

FIPV is an enveloped RNA virus with a single-stranded positive-sense RNA genome and the virions consist of three main structural proteins, peplomer (S) glycoprotein, integral membrane (M) glycoprotein and nucleocapsid (N) protein. The virus genome is at least 20 kilobases long.
The complete nucleotide sequences of these genes have been established with FIPV type II strain 79-1146 (3, 5, 31) and several other serologically related coronaviruses, such as TGEV and CCV (10, 12-14, 26). With regard to FECV strain 79-1683, the sequences of the peplomer (S) gene and 3' end 1-kb gene have been reported (32, 33). Wesseling et al (33) have determined, from the phylogenetic tree constructed on the basis of the S protein-deduced amino acid sequences, that 2 strains of CCV (strain Insavc-1 and strain K378) are evolutionarily more closely related to FIPV strain 79-1146 and FECV strain 79-1683 than TGEV. We have recently determined the nucleotide sequence of the S gene of strain KU-2 classified as type I, and compared it with that of FIPV type II strain 79-1146, CCV strain Insavc-1 and TGEV strain Purdue (17). Deduced amino acid sequences of S protein showed identities of 90.9% and 80.9% between FIPV type II and CCV and between FIPV type II and TGEV, respectively. However, deduced amino acid sequences of S protein showed identity of only 45.9% between FIPV type II and FIPV type I. These findings supported the results of our study on antigenic relationships among feline, porcine and canine coronaviruses with monoclonal antibodies (7, 8). However, it is not clear whether a similar pattern would be obtained by phylogenetic analysis by comparison of integral membrane (M) and nucleocapsid (N) genes or whether other strains of FIPV type I would be differentiated from the groups in a way similar to that for KU-2.

In this study, complete nucleotide sequences of the S, M and N genes of FIPV type I strain UCD1 and strain Black, and of the M and N genes of FIPV type I strain KU-2 and FECV strain 79-1683 were determined, in order to clarify the phylogenetic relationships among these viruses. Genetic relationships among feline coronaviruses, FIPV type I, FIPV type II and FECV type II, were also estimated from the phylogenetic tree of S, M and N proteins.

Materials and Methods

Viruses and their genomic RNA. FIPV strains KU-2, Black and UCD1, and FECV strain 79-1683 were used for cDNA cloning and sequencing. FIPV strain KU-2 was isolated by Hohdatsu et al (8). FIPV strain UCD1 was supplied by Dr. Niels C. Pedersen of the University of California, Davis (19, 21). FIPV strain Black was supplied by Dr. Janet K. Yamamoto of the University of Florida (1, 22). FECV strain 79-1683 was supplied by Dr. Alison J. McKeirnan of Washington State University, Pullman (16, 24). Genomic RNA was extracted from virus-infected Felis catus whole fetus cells (fcwf 4) by the method used by Motokawa et al (17).

DNA primers. DNA primers were custom-synthesized by Bex Corp. (Tokyo) by using di-amidite chemistry and an automatic DNA synthesizer. Table 1 shows the nucleotide sequences of the primers used in cloning. Recognition sites of restriction enzymes for cloning were included in the primers. The binding locations of primers are shown in Fig. 1. A minus-sense primer

Table 1. Nucleotide sequences of DNA primers used for cloning

| Primer     | Nucleotide sequence | Region amplified | Sense |
|------------|--------------------|-----------------|-------|
| ISPr-8F    | 5'-CGCTGGATCAATGTTAAGTACTAACT-3' | region S1 | + (position 445) |
| ISPr-9R    | 5'-GGGGAAATTCTGCACTTAAGCTGCC-3' | region S1 | - (position 1537) |
| BUSPr-12F  | 5'-GGGGGAATCTCAACCAATTATTACAGATGGAATG-3' | region S2 | + (position 1524) |
| BUSPr-13R  | 5'-GGGGAAATCGGTCAGCTGCAACATG-3' | region S3 | - (position 2674) |
| ISPr-10F   | 5'-GGGGGATCAAGCTAACATATTACGATACC-3' | region S3 | + (position 2663) |
| ISPr-11R   | 5'-GGGGGAATTCAAGCGTAAATACCTTAAGTG-3' | region S4 | - (position 3310) |
| BUSPr-14F  | 5'-GGGGGATTCGAGGCTAGCCTGAAATATG-3' | region S4 | + (position 3268) |
| ISPr-2     | 5'-GGGGGAATTCTGAGGCTAAATATG-3' | region S4 | - (position 3268) |
| IM5T       | 5'-CCCCGGCGTTTCTTCTGACTAATGCAA-3' | region MN2 | - |
| IMPr-1     | 5'-GGGGGATGCCATGGCTTCTGAAATGCAA-3' | region MN3 | + |
| IMPr-3     | 5'-GGGGGATGCACTGCTGAAATGCAA-3' | region MN4 | - |
| IN3R       | 5'-GGGGGATCAAGCTAACATATTACGATACC-3' | region S3 | + (position 2663) |
| BEP(DT)    | 5'-CGTGTTACGCTGAAATGCAA-3' | region S3 | - (position 2663) |

a) Primer prepared with reference to nucleotide sequence of FIPV strain KU-2. b) Primer prepared with reference to nucleotide sequences of FIPV strains Black and UCD1. c) Primer prepared with reference to nucleotide sequences of FIPV strain 79-1146. d) Region amplified by RT-PCR. e) Binding location in FIPV strain KU-2 S gene (Accession No. D32044).
was used for reverse transcription (RT) for synthesis of
cDNA from genomic RNA.

cDNA cloning. The M and N genes of FIPV strain
KU-2 were divided into the three regions (region MN1,
MN2 and MN4) shown in Fig. 1. Region MN1 was
sequenced by using the previously reported cDNA clone
pFPSI-1 (17). Genomic template RNA was amplified by
RT-polymerase chain reaction (PCR). The PCR products
were cloned into pUC18, then were subcloned into
M13mp18/19 for determination of the nucleotide
sequence.

The S genes of FIPV strains Black and UCD1 were
divided into four regions (region S1 through S4), and the
M and N genes of FIPV strains Black and UCD1 and
FECV strain 79-1683 were divided into two regions
(region MN2 and MN3), as shown in Fig. 1. The RT-
PCR products were directly cloned into M13mp18/19 for
determination of the nucleotide sequence.

DNA sequencing and analysis. The single-stranded
DNA was sequenced by means of dideoxynucleotide
chain termination using the Dye Primer cycle sequencing
kit (Applied Biosystems Inc., Foster City, Calif., U.S.A.).
The sequence was resolved with an automated DNA
sequencer (Applied Biosystems model 373S). At least
eight clones for one type of cDNA were sequenced to
avoid artifact mutations due to misreading by reverse
transcriptase and Taq polymerase for PCR.

The sequences determined were then analyzed with
the GENETYX computer program (Software Develop-
ment Co., Ltd., Tokyo). Homology including the deleted
sequence was calculated (30). Multiple sequence align-
ment and evolutionary distances between amino acid
sequences were estimated with the PAM250 matrix (2),
which compares amino acid changes according to empi-
rically determined probabilities of change. The phylo-
genetic tree was prepared by the UPGMA method (29).

Results

Nucleotide Sequence of 3' End 4.5 Kilobases of FIPV
Type I Strain KU-2

Two new cDNA clones, pFPMI (region MN2) and
pFPNI (region MN4), of FIPV strain KU-2 were
obtained in the present experiment (Fig. 1). The distance
between the 3' end of the S gene and the poly A tail of
FIPV type I strain KU-2 was 4,496 bases. The M gene,
of 789 bases, coded 263 amino acids, and it was esti-
mated to express proteins of 29.9 kilodaltons (kDa)
(Fig. 2). The N gene, of 1,131 bases, coded 377 amino
acids, and it was estimated to express proteins of 42.5
kDa (Fig. 3).

S, M and N Genes of FIPV Strains Black and UCD1, and
FECV Strain 79-1683

The M genes of FIPV strain Black and FECV strain
79-1683 were 789 bases, which is the same as that of
strain KU-2. The M gene of strain UCD1 was 786
bases, which is less than that of the above viruses by 3
bases. The M gene of FIPV strain Black and FECV
strain 79-1683 coded 263 amino acids and the M gene of
FIPV strain UCD1 coded 262 amino acids. They were
estimated to express proteins of about 29.9 kDa (Fig. 2).

The N genes of FIPV strain Black and strain UCD1
were 1,131 bases, which is the same as that of strain
KU-2. The N gene of FECV strain 79-1683 was 1,128
bases, which is less by 3 bases than that of these viruses.
FIPV strain Black and strain UCD1 N genes coded 377
amino acids, and FECV strain 79-1683 N gene coded
376 amino acids, and they were estimated to express pro-
teins of 42.3, 42.7 and 42.4 kDa, respectively (Fig. 3).

FIPV strain Black S gene was 4,386 bases, and strain
UCD1 S gene was 4,371 bases. They were less than the

![Fig. 1. Map of the binding location of cloning regions and DNA primers. Hatched boxes show the regions of RT-PCR and cDNA cloning. Arrowheads indicate the binding location of DNA primers which are shown in Table 1. *In FIPV strain KU-2, cDNA clones pFPMI and pFPNI were obtained by recombination of regions MN1 and MN4 with pUC18.](image-url)
4,392 bases of strain KU-2 by 6 and 21 bases, respectively. FIPV strain Black S gene coded 1,462 amino acids, and strain UCD1 S gene coded 1,457 amino acids, and these two were estimated to express proteins of about 164 kDa (Fig. 4). There were 37 N-glycosylation sites in strain Black as well as in strain UCD1. The number of the sites was smaller by 4 sites than the 41 sites in strain KU-2.

**Homology Analysis**

Deduced amino acid sequences of M protein showed identity of at least 82% among FIPV type I strains KU-2, Black and UCD1, FIPV type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue. ...: indicates the same amino acid as that in KU-2. -: indicates a gap.
Fig. 4. Deduced amino acid sequence alignment of S protein of FIVI mouse type I strains KU-2, Black and UCD1, FIVI type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insac-1 and TGEV strain Purdue. ... indicates the same amino acid as that in KU-2. - indicates a gap.
acids, and the sequence of residues 23–45 showed particularly low homology (Fig. 2). The phylogenetic tree prepared from the deduced amino acid sequence of M protein showed the shortest evolutionary distance among FIPV strains (Fig. 5a).

The deduced amino acid sequence of N protein showed identity ranging from 90.2 to 93.9% among three strains of FIPV type I, FIPV type II and FECV strain 79-1683 (Table 2). Deduced amino acid sequences of CCV and TGEV N proteins showed identity of about 75% with those of the feline coronaviruses. The phylogenetic tree prepared from the deduced amino acid sequence of N protein showed that individual feline coronaviruses have approximately equivalent evolu-
tionary distances, that FECV strain 79-1683 is not different from FIPV and that the feline coronavirus group is distinctly different from CCV and TGEV (Fig. 5b).

The deduced amino acid sequence of S protein showed identity ranging from 79.3 to 95.4% among FIPV type II, FECV strain 79-1683, CCV and TGEV (Table 3). However, FIPV type I showed identity of only 45% with these viruses in the S protein amino acid sequence of any of the strains KU-2, Black and UCD1. The homology among strains of FIPV type I showed 89.2% identity between strains KU-2 and UCD1, and 89.6% identity between KU-2 and Black and between UCD1 and Black. The phylogenetic tree prepared from the deduced amino acid sequence of S protein showed that three strains of FIPV type I were a very long evolutionary distance from FIPV type II, FECV strain 79-1683, CCV and TGEV groups (Fig. 5c).

Discussion

FIPV forms one antigenic cluster with FECV, CCV and TGEV, and the antigenic structures of these viruses are serologically closely related to each other (6, 11, 20, 28). Some studies have shown that S protein amino acid sequences of FIPV type II, FECV strain 79-1683, CCV and TGEV have identity of about 75% or more (10, 33). However, we have recently reported that the S protein amino acid sequence of FIPV type I strain KU-2 has only about 45% identity with FIPV type II, CCV and TGEV (17). In the present study, S proteins of FIPV type I strain UCD1 and strain Black showed results similar to those of strain KU-2. The phylogenetic tree prepared from the deduced amino acid sequence of S protein showed that three strains of FIPV type I were a very long evolutionary distance from FIPV type II, FECV strain 79-1683, CCV and TGEV groups. However, the phylogenetic tree newly prepared from the M and N proteins showed a pattern which differed from that of the S protein; the phylogenetic tree of the M and N proteins suggested that feline coronaviruses, i.e., FIPV type I, FIPV type II and FECV strain 79-1683, constitute a single group.

FIPV type I and type II can be said to belong to the same group of viruses in that both induce FIP. In addition, the length of the open reading frame (ORF) 6b of FIPV type I was the same as that of FIPV type II ORF 6b (data not shown), and there was not as much deletion as in FECV strain 79-1683 (32). However, the homology of the deduced amino acid sequence of FIPV type I and type II S proteins was very low, and it was impossible to regard the two virus types as members of the same group from the aspect of nucleotide sequence. S protein plays an important role in neutralization and adsorption to cellular receptors, suggesting that the function of type I is very different from that of type II. The results of our analysis of neutralizing epitopes using monoclonal antibodies support this suggestion (7). On the other hand, FECV type II strain 79-1683 causes only mild enteritis, and the homology of its S, M and N genes is the highest with FIPV type II. The homology of the S protein amino acid sequence between FIPV type II and FECV strain 79-1683 was higher than that among three strains of FIPV type I. That is, on the basis of pathogenicity, FIPV causing FIP is divided into type I and type II, and another virus, FECV, that does not cause FIP is thought to exist. If pathogenicity is neglected, and nucleotide sequence is considered as the basis, FIPV type II and FECV type II will be included in the same group of viruses. One study has shown that a type I virus strain is present in FECV as well as in FIPV (23). However, since it is impossible to culture FECV type I in vitro, its study has been delayed. The viruses including this type should be analyzed in the future.

The homology of the S protein amino acid sequence among FIPV type I strains was determined. There was 89.2% identity between strains KU-2 and UCD1, and 89.6% identity between KU-2 and Black and between UCD1 and Black. However, it has been reported that in

Table 3. Homology (%) of deduced amino acid sequence of S protein

| Strain          | FIPV type I | FIPV type II | FECV 79-1683 | CCV | TGEV |
|-----------------|-------------|-------------|-------------|-----|-----|
| KU-2            | 100         | 46.0        | 46.2        | 45.7| 45.2|
| Black           | 89.6        | 45.4        | 45.4        | 45.1| 44.0|
| UCD1            | 89.2        | 46.4        | 46.4        | 45.7| 45.3|
| FIPV type II    | 100%        | 100%        | 100%        | 100 | 100 |
| FECV            | 100%        | 90.9        | 81.2        | 79.3| 79.3|
| CCV             | 100%        | 91.6        | 81.2        | 79.3| 79.3|
| TGEV            | 100%        | 80.9        | 81.2        | 79.3| 79.3|

*Percentage of identical amino acid residues in amino acid sequences including deleted sequences. a) FIPV strain 79-1146 (Accession No. D00150). b) FECV strain 79-1683 (Accession No. Q25539). c) CCV strain Insavc-1 (Accession No. D13096). d) TGEV strain Purdue (Accession No. M21950).
strains FIPV type II S protein amino acid sequences showed identity of 99.6% between strain 79-1146 and strain DF2 (27). The homology of the S gene amino acid sequence among FIPV type II strains was almost completely conserved, as compared to that of the S gene amino acid sequence among FIPV type I strains. Does FIPV type II that is currently prevalent throughout the world have such homology? If so, FIPV type II may be a virus with very high conservation of the nucleotide sequence of genomic RNA or it may be a virus that has recently appeared and become prevalent. It has to be shown in the future to what degree FIPV type II strains are different from each other. In the field, FIPV type I is significantly more prevalent than FIPV type II (9). FIPV type II may be the prototype of FIPV. Some reports have shown that CCV infects cats (15). FIPV type II was considered to be a new FIPV type, which may have arisen from recombinants between FIPV type I and CCVs.

This work was supported by private contributions of Ajinomoto General Foods, Inc., Japan.

References

1) Black, J.W. 1982. Recovery and in vitro cultivation of a coronavirus from laboratory-induced cases of feline infectious peritonitis (FIP). Vet. Med. [small anim clin] 75: 811-814.
2) Dayhoff, M.O., Schwaryz, R.M., and Orcutt, B.C. 1987. A model of evolutionary change in proteins, p. 345-352. In Dayhoff, M.O. (ed), Atlas of protein sequence and structure, Vol. 5, suppl. 3, National Biomedical Research Foundation, Washington, D.C.
3) de Groot, R.J., Maduro, J., Lenstra, J.A., Horzinek, M.C., van der Zeijst, B.A., and Spaan, W.J. 1987. cDNA cloning and sequence analysis of the gene encoding the peplomer protein of feline infectious peritonitis virus. J. Gen. Virol. 68: 2639-2646.
4) de Groot, R.J., ter Haar, R.J., Horzinek, M.C., and van der Zeijst, B.A. 1987. Intracellular RNAs of the feline infectious peritonitis coronavirus strain 79-1146. J. Gen. Virol. 68: 995-1002.
5) de Groot, R.J., Andeweg, A.C., Horzinek, M.C., and Spaan, W.J. 1988. Sequence analysis of the 3'-end of the feline coronavirus FIPV 79-1146 genome: comparison with the genome of porcine coronavirus TGEV reveals large insertions. Virology 167: 370-376.
6) Fiscus, S.A., and Teramoto, Y.A. 1987. Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. J. Virol. 61: 2607-2613.
7) Hohdatsu, T., Okada, S., and Koyama, H. 1991. Characterization of monoclonal antibodies against feline infectious peritonitis virus type II and antigenic relationship between feline, porcine, and canine coronaviruses. Arch. Virol. 117: 85-95.
8) Hohdatsu, T., Sasamoto, T., Okada, S., and Koyama, H. 1991. Antigenic analysis of feline coronaviruses with monoclonal antibodies (MAbs): preparation of MAbs which discriminate between FIPV strain 79-1146 and FECV strain 79-1683. Vet. Microbiol. 28: 13-24.
9) Hohdatsu, T., Okada, S., Ishizuka, Y., Yamada, H., and Koyama, H. 1992. The prevalence of types I and II feline coronavirus infections in cats. J. Vet. Med. Sci. 54: 557-562.
10) Horsburgh, B.C., Brierley, I., and Brown, T.D. 1992. Analysis of a 9.6 kb sequence from the 3' end of canine coronavirus genomic RNA. J. Gen. Virol. 73: 2849-2862.
11) Horzinek, M.C., Lutz, H., and Pedersen, N.C. 1982. Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect. Immun. 37: 1148-1155.
12) Jacobs, L., de Groot, R.J., van der Zeijst, B.A., Horzinek, M.C., and Spaan, W. 1987. The nucleotide sequence of the peplomer gene of porcine transmissible gastroenteritis virus (TGEV): comparison with the sequence of the peplomer protein of feline infectious peritonitis virus (FIPV). Virus Res. 8: 363-371.
13) Kapke, P.A., and Brian, D.A. 1986. Sequence analysis of the porcine transmissible gastroenteritis coronavirus nucleocapsid protein gene. Virology 151: 41-49.
14) Kapke, P.A., Tung, F.Y., Brian, D.A., Woods, R.D., and Wesley, R. 1987. Nucleotide sequence of the porcine transmissible gastroenteritis coronavirus matrix protein gene. Adv. Exp. Med. Biol. 218: 117-122.
15) McKeirnan, A.J., and Evermann, J.F., Hargis, A., Miller, L.M., and Ott, R.L. 1981. Isolation of feline coronavirus from two cats with diverse disease manifestations. Feline Pract. 11: 16-20.
16) Motokawa, K., Hohdatsu, T., Aizawa, C., Koyama, H., and Hashimoto, H. 1995. Molecular cloning and sequence determination of the peplomer protein gene of the feline infectious peritonitis virus type I. Arch. Virol. 140: 469-480.
17) Olsen, C.W. 1993. A review of feline infectious peritonitis virus: molecular biology, immunopathogenesis, clinical aspects, and vaccination. Vet. Microbiol. 36: 1-37.
18) Pedersen, N.C. 1976. Morphologic and physical characteristics of feline infectious peritonitis virus and its growth in autochthonous peritoneal cell cultures. Am. J. Vet. Res. 37: 567-572.
19) Pedersen, N.C., Ward, J., and Mengeling, W.L. 1978. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. Arch. Virol. 58: 45-53.
20) Pedersen, N.C., Boyle, J.F., and Floyd, K. 1981. Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. Am. J. Vet. Res. 42: 363-367.
21) Pedersen, N.C., and Black, J.W. 1983. Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus. Am. J. Vet. Res. 44: 229-234.
ences between various feline coronavirus isolates. Adv. Exp. Med. Biol. 173: 365–380.

24) Pedersen, N.C., Evermann, J.F., McKeirnan, A.J., and Ott, R.L. 1984. Pathogenicity studies of feline coronavirus isolates 79-1146 and 79-1683. Am. J. Vet. Res. 45: 2580–2585.

25) Pedersen, N.C. 1987. Virologic and immunologic aspects of feline infectious peritonitis virus infection. Adv. Exp. Med. Biol. 218: 529–550.

26) Rasschaert, D., and Laude, H. 1987. The predicted primary structure of the peplomer protein E2 of the porcine coronavirus transmissible gastroenteritis virus. J. Gen. Virol. 68: 1883–1890.

27) Reed, A.P., Klepfer, S., Miller, T., and Jones, E. 1993. Cloning and sequence analysis of the spike gene from several feline coronaviruses. Adv. Exp. Med. Biol. 342: 17–21.

28) Sanchez, C.M., Jimenez, G., Laviada, M.D., Correa, I., Sune, C., Bullido, M., Gebauer, F., Smerdou, C., Callebaut, P., and Escribano, J.M. 1990. Antigenic homology among coronaviruses related to transmissible gastroenteritis virus. Virology 174: 410–417.

29) Sokal, R.R., and Michener, C.D. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 28: 1409–1438.

30) Takashi, K., and Gotoh, P. 1984. Sequence relationship among various 4.5 S RNA species. J. Biochem. 92: 1173–1177.

31) Vennema, H., de Groot, R.J., Harbour, D.A., Horzinek, M.C., and Spaan, W.J. 1991. Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. Virology 181: 327–335.

32) Vennema, H., Rossen, J.W., Wesseling, J., Horzinek, M.C., and Rottier, P.J. 1992. Genomic organization and expression of the 3' end of the canine and feline enteric coronaviruses. Virology 191: 134–140.

33) Wesseling, J.G., Vennema, H., Godeke, G.J., Horzinek, M.C., and 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: 1789–1794.