Genetic Analysis of the RNA Polymerase Gene of Caliciviruses from Dogs and Cats

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ABSTRACT. Caliciviruses that infect animals including humans cause a specific disease syndrome in their respective hosts. Feline calicivirus (FCV) is a major pathogen of respiratory disease of cats, and human caliciviru is a causative agent of diarrhea. It has been suggested, furthermore, that FCV and newly recognized canine calicivirus (CaCV) may also be possible causes of diarrhea in these animal species. In this study nucleotide sequence of the RNA polymerase gene of two caliciviruses of canine origin, namely CaCV strain No. 48 and FCV-like strain Sapporo/283, and a number of FCV strains of respiratory and enteric origins was examined. The length of sequenced region, from the 5'LKDEL motif through the 3'YGDD motif of the gene, was 555 bp for CaCV No. 48 strain and 552 bp for the other FCV strains including Sapporo/283 strain. In phylogenetic analysis, CaCV No. 48 strain grouped as a distinct branch sharing ancestral roots with San Miguel sea lion virus, and FCVs formed one compact group in which Sapporo/283 strain was included.—KEY WORDS: amino acid sequence, calicivirus, canine, feline, RNA polymerase gene.

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Caliciviruses are important veterinary and human pathogens and have been isolated from a wide range of vertebrate species. Caliciviruses that infect animals, like vesicular exanthema of swine virus, feline calicivirus (FCV), San Miguel sea lion virus (SMSV) and rabbit hemorrhagic disease virus (RHDV) cause a specific disease syndrome in their respective hosts [2, 3].

In humans, calicivirus has been known as a major cause of diarrhea since Norwalk virus (NV) was detected by an immune electron microscopy in the stool of a patient associated with an outbreak of gastroenteritis in Norwalk, Ohio, in 1972 [13]. Research in the nature of human calicivirus (HuCV) has been difficult due to the lack of cell culturing system and low numbers of virus particles shed during infection. Initially, HuCVs have been classified based on the virion morphology and immunological characteristics. In 1990, viruses from volunteers were used for the molecular cloning of the NV genome [12]. Development of PCR protocols in that study and subsequent nucleotide and amino acid (aa) sequence analyses of a part of the RNA polymerase gene from many HuCV isolates had led to classify of HuCVs into three genetic groups (NV genogroup [10, 11], Snow Mountain virus genogroup [10, 11], and Sapporo/82 genogroup [17]).

FCV is a clinically important pathogen for cats. Main clinical manifestation of infection is respiratory illness, tongue ulcers, chronic stomatitis and lameness [8]. It has also been etiologically associated with diarrhea in cats and the virus has been isolated from both normal and diarrheic feces [15]. However, apart from capsid protein sequences of some strains, molecular characterization of FCVs from cats with the diseases other than acute respiratory infections has not been reported, and therefore it has been unclear what genetic properties may be related to the different clinical manifestations until recently [8, 14].

Calicivirus is not normally considered to be a pathogen of dogs. However, caliciviruses antigenically related to FCV were isolated from feces of dogs with glossitis [6] and diarrhea [7, 19]. On two occasions in the USA, canine caliciviruses (CaCV) that were antigenically distinct from FCV were isolated from dogs with diarrhea [20] and with vesicular genital infection [4]. Based on the results of seroepidemiological surveys, CaCV is thought to be widely distributed in dog populations [16, 20]. However the molecular characteristics of CaCV have remained unknown because the virus is seldom detected in clinical specimens and there are only a few CaCV isolates at present. In Japan, two calicivirus strains (CaCV No. 48 and Sapporo/283) were isolated from feces of dogs with diarrhea by Mochizuki *et al.* [16, 19], on two separate occasions. CaCV No. 48 strain has no antigenic relationship to FCV as determined by serum neutralization and complement-fixation tests [16] and Sapporo/283 is closely related to FCV antigenically [19].

In this paper, the genetic analysis of the RNA polymerase gene of calicivirus isolates recovered from feces of cats and dogs was performed. Because no RNA polymerase gene sequences of calicivirus isolates from dogs have been described so far, we focused on the characterization of CaCV No. 48 strain and Sapporo/283 strain. Furthermore, we investigated possible correlation between disease and genetic properties of the RNA polymerase gene of caliciviruses isolated from both respiratory and enteric diseases of dogs and cats.

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Table 1. Calicivirus strains analysed in the present study

Strain	Origin	Typing[a)]	GenBank access.	Reference
CaCV No. 48	Alimentary tract	Enteric	AF053720	[16]
Sapporo/283	Alimentary tract	Respiratory	AF098932	[19]
FCV F2	Respiratory tract	Respiratory	AF098929	[22]
FCV F14	Respiraotry tract	Respiratory	AF098928	[22]
FCV FC35	Alimentary tract	Enteric	AF098930	[15]
FCV FC61	Alimentary tract	Enteric	AF098931	[15]
FCV F4	Respiratory tract	Unknown	D31836	[22]
FCV F9	Respiratory tract	Respiratory	Z11536	[1]
FCV CFI/68	Respiratory tract	Unknown	U13992	[5]
FCV Urbana	Respiratory tract	Unknown	U07130	[21]

a) The virus was typed as either enteric or respiratory by the method described previously [15].

MATERIALS AND METHODS

Viruses: Virus strains analysed are presented in Table 1. CaCV No.48 strain was isolated in 1990 from a two-month-old Japanese domestic 'shiba' dog with fatal diarrhea in Aichi, Japan [16]. Sapporo/283 strain was isolated in 1992 from a 3-year-old mongrel male dog with watery diarrhea in Sapporo, Japan [19].

FCV F2 and F14 strains were isolated from cats with respiratory symptoms by Takahashi *et al.* [22]. These strains are the respiratory type FCVs (R-FCV) according to the results of the bile and protease tests described previously [15].

FCV FC35 and FC61 strains were isolated from normal feces during a survey of cats with enteric disorders carried out in the periods 1989–90 in Kagoshima, and they are enteric type FCVs (E-FCV) [15].

CaCV No. 48 and Sapporo/283 strains are known as enteric and respiratory types, respectively. Of other FCVs listed in Table 1, FCV F9 strain is a respiratory type, however types of F4, CFI/68 and Urbana strains are unknown though they were respiratory origin.

Viral RNA extraction: Viral RNA was extracted from infected cells using GLASSMAX RNA Microisolation Spin Cartridge System (Life Technologies Inc., Maryland, U.S.A.). In the case of Sapporo/283 strain, viral RNA was extracted from 250 μl of cell supernatant using ISOGEN-LS (Nippongene Co., Toyama, Japan).

PCR: For CaCV No. 48 strain, RT-PCR was carried out using an RNA PCR kit (AMV) ver. 2.1 (TaKaRa, Kyoto, Japan) according to the manufacturer's instructions. The RNA polymerase region was amplified and cloned as two fragments. Reverse transcription was carried out using random primers (TaKaRa). For the first amplification, cDNA was produced using a primer set based on the amino acid motifs (primer GLPSG: 5'-GGVCTVCCATCTG GWTTSCC-3', primer YGDD: 5'-AATCTCATCATCAT CACCATA-3') [10]. Thermal cycling conditions for the PCR consisted of 35 cycles of denaturation at 94°C for 15 sec, annealing at 45°C for 20 sec and extension at 72°C for 60 sec. For the second amplification, PCR was carried out using an upstream sense primer based on published sequences (primer CVs-F1: 5'-CTMCCWCAYGART ACRCMMTTGG-3') and an antisense primer based on the obtained sequence from the GLPSG-YGDD region (primer CaCV2: 5'-GCCGATCCCACAAGAAGACAGTG-3').

For the other strains, the polymerase region was amplified as one fragment using the primers CVs-F1 and YGDD. Complementary DNA was synthesized by random primers using AMV Reverse Transcriptase XL (TaKaRa). The PCR was carried out with 2.5U of Taq polymerase (TaKaRa) in 50 μl reactions containing 0.5 μl of cDNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture and 0.5 pM of each primer. Thermal cycling conditions for the PCR consisted of DNA denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 45°C for 1 min and primer extension at 72°C for 1 min. The PCR products were visualized under UV illumination by agarose gel electrophoresis.

Sequencing: The PCR products were purified using Wizard PCR Preps DNA Purification System (Promega, Wisconsin, U.S.A.). TA cloning was done using Regular pT7Blue(R) T-vector Kit (Novagen, Wisconsin, U.S.A.), and ligation mixtures were transformed into Nova Blue (Novagen) or Epicurian Coli XL2-Blue MRF' (Stratagene, California, U.S.A.) competent cells.

For each sample, 10 to 20 plasmids containing the PCR insert were purified using Wizard Minipreps DNA purification System (Promega) and sequenced with Cy5-labelled "forward" and "reverse" primers of AutoRead Sequencing Kit (Amersham Pharmacia Biotech, Tokyo, Japan).

Sequence analysis: Nucleic acid sequences were analysed and aligned using GENETYX-WIN Software (ver. 2.1, Software Development Co., Japan). Alignments were input into Clustal W (Ver. 1.7, Thompson 1997) and PHYLIP modules (Ver. 3.57c, Felsenstein 1995) for bootstrapping and construction of phylogenetic trees. Tree construction was done without outgroup specification, while bootstrap analysis was done with Clustal W over 1000 trials with seed 111, including gap positions, without multiple

substitution correction.

The following published sequences were used in alignments and comparative analyses: FCV strains F4 (GenBank/D31836), F9 (Z11536), CFI/68 (U13992) and Urbana (L40021) and SMSV-1 strain (M87481), SMSV-4 strain (M87481), NV (M87661), Southampton virus (L07418), foot-and-mouth disease virus (FMDV) (X00871) and RHDV (M67473).

RESULTS

Amplification of the RNA polymerase gene: RT-PCR of CaCV No. 48 strain was carried out in two steps. First, RT-PCR was carried out by using a primer set of GLPSG and YGDD which designed from the previously described sequences of GLPSG and YGDD motifs for other animal and human caliciviruses. As shown in Fig. 1, a single band of approximately 150 bp by electrophoresis was obtained. Then, the rest portion to the LKDEL motif [12] was amplified by using a primer set of CVs-F1 and CaCV2. This step also resulted in a single band of the expected size (approximately 400 bp).

For the other strains, RT-PCR was carried out using the CVs-F1 and YGDD primers. A single band of approximately 600 bp was generated from these strains.

Sequencing and analysis of the RNA polymerase gene: The length of the amplified region, from the 5' LKDEL motif through the 3' YGDD motif was 555 bp for CaCV No. 48 strain, and 552 bp for all the other FCV strains including Sapporo/283 strain. Alignment of the predicted aa sequence for the RNA polymerase gene with relevant published sequences is shown in Fig. 2. Analysis of aa sequence of the canine isolates CaCV No. 48 and Sapporo/283 strains showed that these viruses contained the LKDEL and GLPSG motifs commonly found in caliciviruses of other animal and human origins.

As shown in Table 2, sequence homology of CaCV No.48 strain was highest to SMSVs (71–72%), followed by FCVs (58–61%). In comparison, homology among strains of the same calicivirus species was much higher: among FCVs: 91–94%, SMSVs: 99% and HuCVs: 94%. Homology of Sapporo/283 strain sequence was highest to FCV F9 strain (99.5%).

Phylogenetic comparison between the canine and feline strains used in this study and previously published sequences from various animal calicivirus and HuCV strains resulted in the feline strains into one compact group, separable from all other caliciviruses with the exception of the canine isolate Sapporo/283 strain which co-localized with the feline strains (Fig. 3). The other canine isolate, CaCV No. 48 strain, grouped as a distinct branch sharing the ancestral root with SMSV strains.

When only phylogenetic relationships among the canine and feline isolates were analysed, CaCV No. 48 strain again separated from the other isolates (Fig. 4). Sapporo/283 strain localized in close proximity of FCV strain F9. Branching along the clinical characteristics or the sampling sites of animals from which the strains were isolated, respiratory or enteric, was not seen.

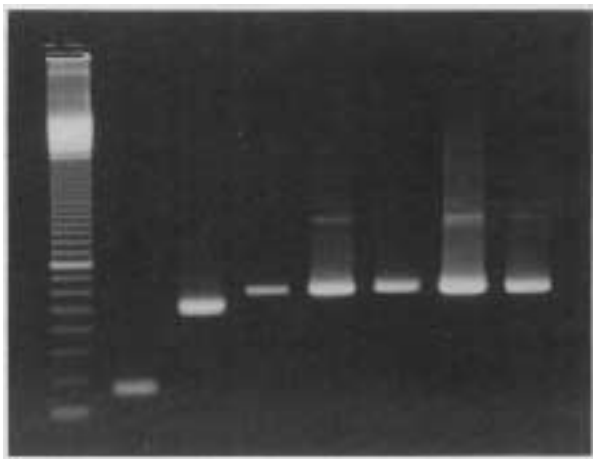


Fig. 1. Electorophoresis of RT-PCR products from CaCV and FCVs. From left to right lanes 1 to 8; lane 1: 100 bp ladder size marker; lane 2: CaCV No. 48 strain amplified using a primer set of GLPSG and YGDD; lane 3: CaCV No. 48 strain amplified using a primer set of CVs-F1 and CaCV2; lanes 4 to 8: Sapporo/283, FCV F2, FCV F14, FCV FC35 and FCV FC61 strains, respectively amplified using a primer set of CVs-F1 and YGDD.

DISCUSSION

The molecular characterization of CaCV No. 48 strain and Sapporo/283 strain isolated from the feces of diarrheic dogs was done in order to elucidate molecular biology of calicivirus infection in domestic dogs. As for analysis of the deduced aa sequence, CaCV No.48 strain as well as Sapporo/283 strain both contain the aa motifs LKDEL and GLPSG which are well conserved in RNA polymerase genes of *Caliciviridae*. This result demonstrated that dog isolates are indeed caliciviruses on a genetic level. The region spanning LKDEL and YGDD motifs in CaCV No. 48 strain contains 555 nucleotides, coding for 185 aa. Sapporo/283 strain contains 552 nucleotides, coding for 184 aa, which is the same as in all FCVs using in this study.

As shown in Table 2, the level of aa identity of CaCV No.48 strain was highest when calculated against SMSV strains, followed by FCV strains. In contrast, the aa identity between strains of the same calicivirus species was higher than 90%. Figure 3 gives the result of phylogenetic analysis of the sequence alignment shown in Fig. 2. CaCV No. 48 strain positioned distinctly separable from the SMSV and FCV clades, and even further apart from HuCVs, RHDV and FMDV. On the bases of the previous findings on serological evidence (no antigenic relationship to FCV) and biophysical evidence (growth only in canine cell cultures) [16], as well as the present sequence analysis of the RNA polymerase CaCV No. 48 strain can indeed be identified as a canine calicivirus.

In the case of Sapporo/283 strain, which was also isolated

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CaCV No.48        LKDELRPKDKI AVGKRRLIWGCDVGVAVVAASAFKEVSSAI MAMSEFDFIQVGINMDGTAVETLYKRL :  68
Sapporo/283       -------VE-V SE----M---------T-C-A---G--D-- T-NHQYGP--------SPS--A-FQ-I :  68
FCV F2            -------IE-V QE----M---------T-C-A---G--D-- T-NHQYGPV-------SPS--V--Q-I :  68
FCV F14           -------VE-V -E----M---------T-C-A---A--D-- T-NHQYGPV-------SPS--S-FQ-I :  68
FCV FC35          -------VE-V -E----M---------T-C-A---A--D-- T-NHQYGPV-------SPS--A--Q-I :  68
FCV FC61          -------IE-V QE----M---------T-C-A---G--D-- T-NHQYGPV-------SPS--A--Q-I :  68
FCV F4            -------IE-M QE----M---------T-C-A---G--D-- T-NHQYGP--------SPS--V--Q-I :  68
FCV F9            -------VE-V SE----M---------T-C-A---G--D-- T-NHQYGP--------SPS--A-FQ-I :  68
FCV CFI/68        -------IEKV QE----M---------T-C-A---G--D-- T-NHQYGP--------SPS--A-FQ-I :  68
FCV Urbana        -------VEKV -E----M---------T-C-A---G--D-- T-NHQYGPV-------SPS--A-HQ-I :  68
SMSV 1            ----------V --------------ST-CRA---R--ES- --NHALG----------P---DPF---  :  68
SMSV 4            ----------V --------------ST-CRA---R--ES- --NHALG----------P---D-F---  :  68
RHDV              -------L--V KE--K--L---------C--AV-HNICYKL KMVAR-GP-A--VD-TSRD-DVIINN-  :  68
Norwalk virus     -----VKPE--YQKV-K--L--A-L-TV-R--R--GPFCD-- KSHVIKLP-K--M-TIEDGPLIYAEHA  :  69
Southampton virus -----VKPE-VYQKV-K--L--A-L-TV-R--R--GPFCD-- KSHTIKLP-K--M-SIEDGPLIYAEHS  :  69
FMDV              ----I--LE-V RA--T-IVDVLPVEHILYTRMMIGRFCAQMHSNNGPQIGSA--C-P-VDWQRFGTHFA  :  69

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CaCV No.48        YTPGTHRYCVDYSKWDSTQPPNVTRMSLELLRHFTDKSPVVD SAVATLSSPSIAVFGGVSFKTNGGLPS : 137
sapporo/283       K SAAKVFA----------SAR-SAA-IDI--Y-S-R--I-- --SN--K--PV-I-N--AV-VSS---- : 136
FCV F2            K SAAKVFA----------S-R-SAA-IDI--Y-S-R--I-- --AN--K--P--I-N--AV-VAS---- : 136
FCV F14           K SAAKVFA----------S-R-SAA-IDI--Y-S-R--I-- ---N--K--PV-I-N--AV-VSS---- : 136
FCV FC35          K SAAKVFA----------S-R-SAA-IDI--Y-S-R--L-- --AN--K--PV-I-N--AV-VTS---- : 136
FCV FC61          K SAAKVFA----------S-R-SAA-IDI--Y-S-R--I-- --AN--K--P--I-N--AV-VSS---- : 136
FCV F4            K SAAKVFA--F-N-----S-R-SAA-IDI--Y-S-R--I-- --AN--K--P--I-N--AV-VAS---- : 136
FCV F9            K SAAKVFA----------S-R-SAA-IDI--Y-S-R--I-- --SN--K--PV-I-N--AV-VSS---- : 136
FCV CFI/68        K SARKVFA----------S-R-SAA-IDI--Y-S-RT-I-- --TN--K--P----N--AV-VSS---- : 136
FCV Urbana        K SAAKV-A----------S-R-SAA-IDI--Y-S-R--I-- --AN--K--P--I-N--AV-VSS---- : 136
SMSV 1            ER-KHD---------------K--SQ-IDI---------I-- --C---K-NP-GI-N--A--VA----- : 137
SMSV 4            ER-KHD---------------K--SQ-IDI---------I-- --C---K-NP-GI-N--A--VA----- : 137
RHDV              TSKASDFL-L--------MS-C-V-LAIDI-ADCCEQTELTK -V-L--K-HPMTILDAMIVQ-KR---- : 137
Norwalk virus     K  YKNHFDA--TA-----NRQIMTE-FSIMSRL-ASPELAE VVAQD-LA--EMDV-DYVIRVKE---- : 136
Southampton virus K  YKYHFDA--TA-----NRQIMTE-FSIMCRL-ASPELAS VVAQD-LA--EMDV-DYVIRVKE---- : 136
FMDV              Q  YRNVWD----AF-ANHCSDAMNIMF EEVFR-EFGFHPNAEWILKTLVNTEHAYENKRITVG--M-- : 136

                  *                                          ****
CaCV No.48        GMPLTSILNSLNHCLLVGSAIIQVLESKGVDVNWNIYDTIDLFTYGDD                          : 185
Sapporo/283       ------VI-------Y--C--L-S--A-AIP-T--LFS-F-IM-----                          : 184
FCV F2            ------VI------MY--C--L-S--ARQIP-T--LFSSF-MM-----                          : 184
FCV F14           ------VI-------Y--C--L-S--ARN-P-T--LFS-F-IM-----                          : 184
FCV FC35          ------VI-------Y--C--M-S--A-NIP-T--LFSSF-MM-----                          : 184
FCV FC61          ------VI------MY--C--L-S--ARNIP-T--LFSSF-MM-----                          : 184
FCV F4            ------VI------MY--C--L-S--ARQIP-T--LFSSF-MM-----                          : 184
FCV F9            ------VI-------Y--C--L-S--A-AIP-T--LFS-F-IM-----                          : 184
FCV CFI/68        ------VI-------Y--C--L-S--ARN-P-T--LFS-F-MM-----                          : 184
FCV Urbana        ------VI-------Y--C--L-S--AR--P-T--LFS-F-MM-----                          : 184
SMSV 1            -------I-------M----VVKA--DS--Q-T---F-SM--------                          : 185
SMSV 4            -------I-------M----VVKA--DS--Q-S---F-SM--------                          : 185
RHDV              ---F--VI--IC-W--WSA-VYKSC AEIGLHCS-L-EDAPFY-----                          : 184
Norwalk virus     -F-C--QV--I--WIITLC-LSEA    TGLSPDVVQSMSYFSF----                          : 181
Southampton virus -F-C--QV--I--W-ITLC-LSE-    TGLSPDVIQSMSYFSF----                          : 181
FMDV              -CSA---I-TILNNIY-LY-L       RRHYEGVEL--YTMIS----                          : 178
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Fig. 2. Alignment of amino acid sequence of the RNA polymerase gene of caliciviruses. For CaCV No. 48 strain, sequencing was carried out for two RT-PCR products separately amplified by using two sets of primers GLPSG and YGDD, and CVs-F1 and CaCV2. For Sapporo/283, FCV F2, F14, FC35 and FC61 strains, a set of primer CVs-F1 and YGDD was applied. Asterisks indicate conserved motifs of *Caliciviridae.*

Table 2. Amino acid similarity of the RNA polymerase gene

Strain	CaCV No. 48	Sapporo/ 283	FCV F2	FCV F14	FCV FC35	FCV FC61	FCV FC4	FCV F9	FCV CFI/68	FCV Urbana	SMSV 1	SMSV 4	RHDV	Norwalk	South- ampton	FMDV
CaCV No. 48	■	59	58	60	60	58	58	60	60	61	71	72	40	30	31	20
Sapporo/283		■	92	95	94	94	91	a)	94	94	65	65	43	31	31	22
FCV F2			■	93	94	98	98	93	94	95	66	66	43	31	32	22
FCV F14				■	95	94	91	96	94	96	67	67	44	31	31	22
FCV FC35					■	95	92	94	91	94	65	66	44	31	32	22
FCV FC61						■	96	94	95	96	65	65	43	32	32	22
FCV F4							■	92	92	92	65	65	42	31	31	21
FCV F9								■	94	95	66	66	44	31	32	23
FCV CFI/68									■	95	66	66	44	31	32	23
FCV Urbana										■	68	68	43	30	31	23
SMSV 1											■	99	41	27	28	24
SMSV 4												■	41	27	28	24
RHDV													■	31	32	20
Norwalk virus														■	94	25
Southampton virus															■	24
FMDV																■

a) This value is 99.5.

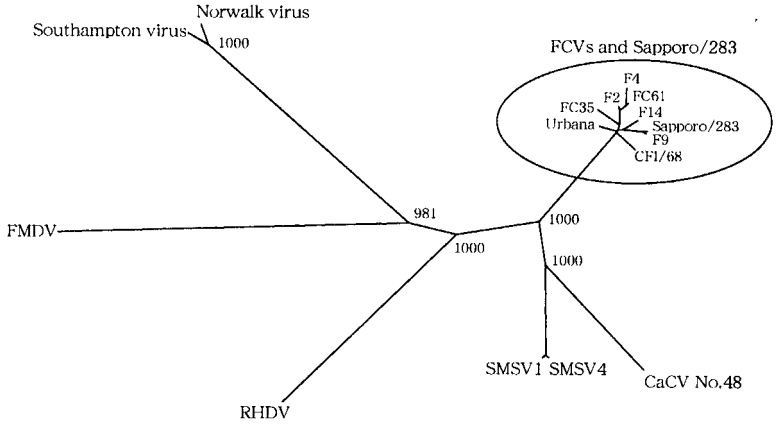


Fig. 3. Phylogenetic analysis of the RNA polymerase gene of caliciviruses. The phylogeny was calculated with the program PHYLIP version 3.75c. Numbers indicate bootstrap values of the respective branch nodes.

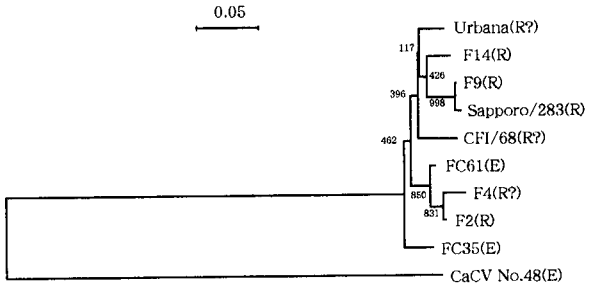


Fig. 4. Phylogenetic analysis of the RNA polymerase gene of canine and feline caliciviruses. The phylogeny was visualized with the program njplot. Numbers indicated bootstrap values of the particular branch node after 1000 bootstrap assays.(E): enteric type calicivirus, (R): respiratory type calicivirus.

from the diarrheic feces of a dog, genetic analysis of the polymerase gene suggested that it is genetically more closely related to FCVs rather than to CaCV. Amino acid identity of Sapporo/283 strain was highest against FCV F9 strain (99.5%) and at least 90% against other FCV strains. In the result of phylogenetic analysis, this novel dog isolate was positioned within the FCV cluster. This result supports the observation that Sapporo/283 strain was neutralized by the antisera against FCV strains but not by the antiserum against CaCV No. 48 strain [19]. Sapporo/283 strain was also isolated from diarrheic feces, but it is a respiratory type calicivirus. The present case, of an FCV-like calicivirus isolated from a dog, hints at the possibility of interspecies transmission from cats to dogs. However, there is as yet no direct evidence for the transmission of FCV to dogs in nature.

During preparation of this manuscript, there published some reports that investigated possible correlations between genetic properties and pathogenicity of FCV isolates based on analysis of the capsid protein gene [8, 18]. One study

used both capsid protein sequence data and the reactivity of monoclonal antibodies raised against a stomatitis and a respiratory strains, but failed to find clustering of strains in either case [8]. These reports described strains associated with respiratory symptoms but not diarrhea. To the best of our knowledge there are no reports describing molecular characteristics of FCV isolates from diarrheic cases.

The genetic relation charted in Fig. 4, indicated that respiratory and enteric type isolates show very high levels of sequence identity in RNA polymerase genes, but do not result in significant subclustering. The two E-FCV strains FC35 and FC61 did not specially group together, but rather showed closer relation to respiratory isolates than to each other. As is clear from Fig. 3, which compares these strains to caliciviruses from a variety of animal species, the feline cluster is a very homogeneous group of isolates. Also, bootstrap analysis showed that bootstrap values were low at a number of branch nodes within the feline cluster. In order to obtain more information on the genetic determinants, if any, of pathogenicity of the calicivirus of dogs and cats, it will be necessary to examine other regions of the calicivirus genome and add to data from more isolates, representing the various disease manifestations.

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