Isolation of a Calicivirus Antigenically Related to Feline Caliciviruses from Feces of a Dog with Diarrhea

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ABSTRACT. A CPE-producing agent was recovered in feline cell cultures from feces of a male dog suffering from intermittent watery diarrhea. Antigenic analysis of this isolate, Sapporo/283, was performed using the plaque reduction neutralization and complement fixation assays and it was neutralized by antisera against feline calicivirus (FCV) but not against canine calicivirus (CaCV). Likewise, it showed common CF antigenicity with the other FCV strains included in the experiments. These findings revealed that the isolate was more closely related antigenically to FCV than CaCV, indicating the possibility of interspecies transmission. It was also suggested that the isolate was a respiratory type calicivirus. Epizootiological results suggested, however, that FCV seldom infects dogs under natural condition. — KEY WORDS: calicivirus, canine, feline.

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Caliciviruses are small, non-enveloped, positive-stranded RNA viruses possessing a distinct virion morphology characterized by cup-shaped depression on the spherical capsid surface [14]. The single-stranded RNA genome is about 8 Kb in length and only a single major species of capsid protein has been identified [3]. Although the virus had been associated with infections affecting a wide host range, the well-characterized members of this genus have been isolated from porcine (vesicular exanthema of swine virus), pinniped (San Miguel sea lion virus), and feline (feline calicivirus:FCV) species. The primary diseases associated with calicivirus infections are vesicular in nature and those affecting the respiratory system [14]. In canine, reports had also emerged regarding the isolation of caliciviruses from cases of glossitis [5], diarrhea [6, 10, 13] and vesicular genital infections [4]. Identification of the earlier isolates were based on electron microscopic studies as well as serologic tests using antibody against FCV. A close antigenic relationship between the early isolates and FCV was established [5, 6]. The origin of these FCV-like isolates reported in the U.S.A. may be the feline combined vaccines used to protect dogs from canine parvovirus infections [1]. However, recent canine calicivirus (CaCV) isolates [4, 10, 13] appeared to be different serologically from FCV, and with one isolate, only a close relation with the stunting syndrome agent of chicken was found [13]. This short communication describes the isolation and identification of a calicivirus isolated from a dog with diarrhea. Serological tests revealed that this canine isolate is antigenically related to FCV, but any serological evidence showing a possibility for FCV infections of dogs was not obtained.

We isolated two calicivirus strains from dogs by examining 533 diarrheal feces for seven years from 1989. One is CaCV No. 48 strain reported previously [10], and the other is described here. Suffering from intermittent watery diarrhea, a 3-year-old mongrel male dog was brought to the Takahashi Animal Hospital in Sapporo. Other than this problem, the dog was apparently active and had good appetite. Vaccination record was unknown. The fecal sample was prepared for virological examination as described previously [10] and inoculated into three kinds of cell cultures: CRFK (Crandell feline kidney), fcwf-4 (*Felis catus* whole fetus) and MDCK (Madin-Darby canine kidney). A CPE, characterized by cell rounding and detaching from the surface, occurred in the feline cells but not in MDCK cells. The sample was checked for the presence of coronavirus by a cell culturing method using fcwf-4 cells, rotavirus by a reverse passive hemagglutination test [9] and parvovirus by a polymerase chain reaction [8], but none of these viruses were found. Upon further analysis, neither inclusion bodies nor syncytia were produced in the cells infected. Also, the virus did not lose infectivity after the treatment with ether. Serum samples of the patient were not available.

Determination of the antigenic properties of this virus, which we designated as Sapporo/283 strain, was done by using the plaque reduction neutralization (PRN) and the complement fixation (CF) assays. The PRN assay was carried out as described before [12] using rabbit hyperimmune sera against FCV strains F4 and F14 as well as CaCV strain No. 48 [10, 16]. Sapporo/283 strain was tested together with other FCV and CaCV strains: FCV F-9 [2], FCV F14 [15], FCV FC168 and FCV FC193 (our recent isolates from diarrheal feces of cats) and CaCV No. 48. The PRN titer was expressed as the highest dilution of antiserum neutralizing 75% or more of the input virus. The CF assay was performed using the CF-Kit (Denka Seiken, Tokyo) with the hyperimmune serum against FCV F14. The CF titers were expressed as the highest dilution of antiserum giving 75% or more fixation. Results of the PRN and the

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Table 1. Antigenic relations among canine isolate Sapporo/283 strain, feline calicivirus (FCV) and canine calicivirus (CaCV) by plaque reduction neutralization (PRN)[a] and complement fixation (CF)[b] assays

Virus strains	PRN antibody titers of the immuneserum against:			CF antibody titers of the immuneserum against:
	FCV F4	FCV F14	CaCV No. 48	FCV F14
Sapporo/283	5,120	320	<2	128
FCV F-9	1,280	80	<2	128
FCV F41	640	20,480	<2	128
FCV FC168	NT[c]	NT	NT	128
FCV FC193	NT	NT	NT	64
CaCV No. 48	<10	<10	128	<2

a) PRN titers were expressed as the highest dilution of antiserum neutralizing 75% or more of the input virus.
b) CF titers were expressed as the highest dilution of antiserum giving 75% or more fixation.
c) NT: not examined.

Table 2. Effect of bile and protease treatments on infectivity of canine isolate Sapporo/283 strain, feline calicivirus (FCV), and canine calicivirus (CaCV)

Virus strains	Titer reduction (logs) resulted from the treatment indicated:	
	Bile (deoxycholic acid)[a]	Protease (trypsin)[b]
Sapporo/283	3.9	≥4.26
FCV FC168	0.35	2.4
FCV FC193	0.6	0.4
CaCV No. 48	0.37	1.19

a) Virus fluid was mixed with an equal amount of 0.2% DOC in Eagle's medium, and incubated at 37°C for 1 hr.
b) Virus fluid was mixed with an equal amount of 1.0% trypsin (1:250) in the medium, and incubated at 37°C for 1 hr.

CF assays are presented in Table 1. Sapporo/283 strain was neutralized by the antisera against FCV strains F4 and F14 but not against CaCV strain No. 48. Results of the CF assay also revealed the common antigenic properties of Sapporo/283 strain with FCVs since it reacted with the antiserum against FCV F14 strain as the other FCV strains did. These findings, together with the observation that Sapporo/283 strain replicated efficiently only in the feline cells but not in MDCK cells (data not shown), are strongly indicative that it was probably of feline origin, hence, antigenically similar to FCV. This suggests the possibility of an interspecies transmission of the caliciviruses as reported on group A rotaviruses [11] and feline parvoviruses [8] in these animal species.

One hundred fifty-two dog serum samples collected during 1981 and 1985 were examined by the micro-neutralization assay described previously [16] to see whether they possess neutralizing activity against Sapporo/283 and FCV F4 strains. In addition, 100 dog serum samples collected in 1985 and 1991 [10] were examined to see whether they possess the CF antibody against an antigen resided on the capsid protein which is commonly detected among FCVs [15]. As a result, neither neutralizing activity (antibody titer is less than 1:20) nor CF antibody (less than 1:4) was detected in them, indicating that FCV seldom infects dogs.

In a recent study conducted in our laboratory, it was established that sensitivity to bile (deoxycholic acid) and protease (trypsin) of the different FCV strains may be used to differentiate between their enteric and respiratory nature *in vivo* [7]. We tested the canine isolate Sapporo/283 together with other FCV and CaCV strains using the methods described before [7]. The results are presented in Table 2. Sapporo/283 strain was markedly inhibited by the treatments as compared to the FCV strains FC168 and FC193 as well as to the CaCV strain No. 48, all of which were isolated from feces of diarrheic animals. Although Sapporo/283 strain was also isolated from the diarrheic feces, it is quite different from the other strains defined as enteric type FCVs, that is, it is rather a respiratory type calicivirus. The presence of the virus in the feces could just have been an incidental finding which may not have caused the diarrhea. Additional studies are needed for defining the clinical importance of these FCV-related caliciviruses found in dogs described here and previously [5, 6] as a disease-causing agent.

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