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Short Communication

Different stabilities to bile among feline calicivirus strains of respiratory and enteric origin

M. Mochizuki
Laboratory of Veterinary Microbiology, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, Korimoto 1-21-24, Kagoshima 890, Japan

ABSTRACT

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Feline calicivirus (FCV) strains isolated from feces (E-FCV) were compared with FCV strains of respiratory origin (R-FCV). All strains were shown to be labile at pH 3.0. All strains except one strain of E-FCV were found to be sensitive to the action of trypsin. When exposed to bile salt (deoxycholic acid sodium salt), all R-FCV strains were markedly inactivated, but none of the E-FCV strains was inactivated. It was possible to select bile-resistant substrains from a bile-sensitive strain.

INTRODUCTION

Feline calicivirus (FCV) is a common cause of clinical and subclinical respiratory infections of domestic cats and other felids (Povey and Hale, 1974; Studdert, 1978). During the acute phase and also for prolonged periods after convalescence, the virus is shed in ocular, nasal and oropharyngeal secretions (Bartholomew and Gillespie, 1968; Povey and Johnson, 1970; Povey et al., 1973; Wardley, 1976; Wardley and Povey, 1977a). Persistence of virus in the tonsil has been demonstrated (Kahn and Gillespie, 1971; Povey et al., 1973; Povey and Hale, 1974; Wardley and Povey, 1977b; Dick et al., 1989). Although the etiological role of FCV in feline enteric diseases has not been well documented, the virus has been occasionally associated with diarrheic cases (Spradbrow et al., 1970; Love and Baker, 1972; Povey and Hale, 1974; Wardley and Povey, 1977b). During our survey of feline enteric viruses, five strains of FCV (E-FCV) were recovered from either normal or diarrheic stools. In the present communication, some in vitro characteristics of these E-FCV strains are compared with those of FCV strains of respiratory origin (R-FCV).

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MATERIALS AND METHODS

Viruses

Nine strains of FCV were examined in the present study. Strains F-9 (Bittle et al., 1960), and F2, F14 and F17 (Takahashi et al., 1971) were respiratory origin. Five E-FCV strains, 9-2, Y12, FC30, FC35 and FC61, were isolated from diarrheal feces (FC30) or normal feces (9-2, Y12, FC35 and FC61) of cats during a survey carried out in the periods 1985-86 and 1989-90 from 215 stool samples collected at Kagoshima in Japan. Feline herpesvirus (FHV) C7301 strain (Mochizuki et al., 1977) was used as a reference virus. All strains were cultivated in the CRFK cell culture (Mochizuki et al., 1987).

Virus titration and serology

Infective titer was determined by a plaque assay in the CRFK cell culture by a method described previously (Mochizuki et al., 1987).

Antigenic properties of FCV strains were examined by plaque-reduction neutralization test (PRNT) and complement fixation (CF) test with hyperimmune sera against F4 and F14 strains of R-FCV (Tohya et al., 1990). The PRNT method was the same as used previously (Mochizuki et al., 1987). The CF test was performed by a conventional microtiter method by using the CF-KIT (Denka Seiken, Tokyo). The CF antigen was prepared by the method described recently (Tohya et al., 1990). The CF titer was expressed as the reciprocal of the highest serum dilution giving at least 75% fixation.

Physicochemical tests

In the acid lability test, the virus was diluted 1:10 in Eagle's minimum essential medium (EMEM) adjusted to pH 3.0 and to pH 7.0 (untreated virus control) with tris buffer, and infectivity titrations were carried out after 3 h incubation at room temperature.

Bile sensitivity test was performed by using deoxycholic acid sodium salt (DOC, Nacalai Tesque Inc., Kyoto). The virus fluid was mixed with an equal amount of 0.2% DOC in EMEM, the mixture was incubated at 37°C for 1 h, and then its infective titer was determined. Untreated virus control was mixed with an equal amount of EMEM and treated in the same manner.

Protease sensitivity test was performed by using trypsin (1:250, Nakarai Tesque Inc., Kyoto). The virus fluid was mixed with an equal amount of 1% trypsin in EMEM, the mixture was incubated at 37°C for 1 h, and then titrated. Untreated virus control was also titrated.

RESULTS

Antigenicity

Table 1 shows antigenic relations among FCV strains determined by PRNT and CF. All strains possessed the same CF antigenicity, but they showed var-

TABLE 1

Antigenic relations among feline calicivirus (FCV) strains by plaque-reduction neutralization and complement-fixation (CF) tests

Virus strains (Origin)[1]	Neutralization antibody titers of the immuneserum against:		CF antibody titers of the immuneserum against FCV F14 strain
	FCV F4 strain	*FCV F14 strain*	
F-9(R)	1 280	80	128
F2(R)	10 240	160	128
F14(R)	640	20 480	256
F17(R)	1 280	320	128
9-2(E)	320	40	128
Y12(E)	640	40	128
FC30(E)	640	320	128
FC35(E)	<10	10	128
FC61(E)	80	160	64

[1]R, respiratory tract; E, feces.

TABLE 2

The effect of acid, bile and protease treatment on infectivity of feline calicivirus (FCV) strains

Virus strains (Origin)[1]	Titer reduction (logs) resulted from the treatment indicated:		
	Acid(pH 3.0)	*Bile(deoxycholic acid)*	*Protease(trypsin)*
FHV[2] C7301	7.7	5.6	4.3
FCV F-9(R)	1.7	2.3	1.9
F2(R)	2.9	4.5	3.2
F14(R)	2.3	3.3	2.7
F17(R)	2.2	4.3	2.8
9-2(E)	2.6	0.3	2.3
Y12(E)	3.6	0.8	2.9
FC30(E)	3.3	0.1	2.7
FC35(E)	2.9	0	1.3
FC61(E)	2.8	0	0.4

[1]See Table 1 legend.
[2]Feline herpesvirus

ious degrees of neutralization titers when reacted with the hyperimmune sera against two R-FCV strains. However, the neutralization titers of R-FCV strains were relatively higher than those of E-FCV strains when the immune serum against FCV F4 strain was used in PRNT.

Physicochemical tests

Results of the physicochemical tests are presented in Table 2. The F-9 strain was more acid stable than the other FCV strains which were regarded as acid labile as indicated by a reduction in titer of a hundred fold or greater.

TABLE 3

The effect of consecutive treatments with deoxycholic acid (DOC) on infectivity of feline calicivirus F14 strain

DOC treatment[1]	Infective titers (PFU/0.2 ml)	
	treated	Control
Before		
Once	5.0×10^{4}	1.0×10^{8}
Twice	4.0×10^{6}	9.8×10^{7}
Three times	2.0×10^{7}	3.9×10^{8}
Four times	1.5×10^{7}	4.5×10^{7}

[1]The virus was once grown before each DOC treatment.

With the exception of FC61 strain, the FCV strains were sensitive to the action of trypsin, which resulted in a reduction of titer ranging from 1.3 (FC35 strain) to 3.2 (F2 strain) logs. The FC61 strain was found to be a trypsin-resistant FCV, exhibiting a loss in titer of 0.4 $\log_{10}$ units.

All R-FCV strains were sensitive to the action of DOC. The reduction of infective titer ranged from 2.3 (F-9 strain) to 4.5 (F2 strain) $\log_{10}$. By contrast, no significant loss in titer was observed with E-FCV strains. The strains FC30, FC35 and FC61 were resistant, and the 9-2 and Y12 strains exhibited a loss in titer of 0.3 and 0.8 $\log_{10}$, respectively. The F14 strain of R-FCV was repeatedly treated with DOC to know whether bile-sensitive, R-FCV strain contains a bile-resistant virus fraction in its population. As shown in Table 3, DOC-resistance was found after four treatments and bile-resistant substrains were selected from the bile-sensitive strain.

DISCUSSION

Calicivirus has been recognized mainly as a respiratory pathogen in cats, and diarrhea is not a sign that is commonly associated with FCV infection. Because the virus is continuously shed into oropharyngeal secretions for prolonged periods, it is expected that the virus can be recovered from feces independently of the clinical condition (Povey and Hale, 1974). Although FCV strains of respiratory origin were judged to be sensitive to the actions of acid, protease and bile in the present study, part of the virus strain was still infective after the treatments. Thus virus is carried out through the alimentary tract avoiding inactivation, and are transmitted via the fecal–oronasal route among cats. After several repetitions of this transmission mode, the strain may constitute virions which are stable under adverse conditions as shown experimentally using DOC in vitro (Table 3). All FCV strains isolated from the feces were resistant to the action of bile salt and one of them was also

found to be resistant to the action of trypsin. Such strains, e.g. FC61, may infect epithelial cells of not only respiratory but also alimentary tracts, which results in respiratory as well as intestinal illness. Further probable examples found in the literature are as follows: the CFI strain which was isolated from nasopharyngeal area, but was resistant to the action of bile salt, and was recovered from feces of experimentally infected kittens (Bartholomew and Gillespie, 1968); and the M8 (Wardley and Povey, 1977a) and BF-71 (Povey and Hale, 1974) strains which caused clinical signs of both the upper respiratory illness and diarrhea in the experimentally infected cats, though it is unknown whether they are sensitive to the actions of bile salt or trypsin.

Another possibility is that FCV strains exist possessing affinities and pathogenicity to the alimentary tract of cats in nature (Bürki, 1966). In recent years, reports concerning the linkage of calicivirus and enteric diseases have been appeared for viruses from humans (McSwiggan et al., 1978; Chiba et al., 1979; Cubitt et al., 1979, 1981), dogs (Evermann et al., 1985; Schaffer et al., 1985), swine (Saif et al., 1980), and cattle (Woode and Bridger, 1978; Bridger et al., 1984). Caliciviruses are intermediate between the rhinovirus and the enterovirus of the family Picornaviridae in their stability at low pH, as were FCV strains examined in the present study as described generally (Studdert, 1978). However, it was shown that the FCVs of fecal origin were stable to bile salt. FCVs might be divided into two groups based in the difference in their susceptibility against the bile salt: that is, respiratory-type and enteric-type. Further investigations to ascertain the genetic background of this different DOC susceptibility among FCV strains may provide deeper understanding of the grouping and pathogenicities of FCVs as well as their relation to enteric caliciviruses of other animal species.

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