

Short communication

Differentiation of feline coronavirus type I and II infections by virus neutralization test

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Abstract

Feline coronavirus (FCoV) is divided into two types I and II, based on their growth *in vitro* and antigenicity. In this study, virus neutralization (VN) test was applied for type differentiation of FCoV infections. Sera of cats which were clinically and serologically diagnosed as feline infectious peritonitis (FIP) possessed significantly higher VN titers to type I FCoV, and sera from cats experimentally infected with FIPV type II had high VN titers to type II but not type I viruses. A total of 79 cat sera collected in the years between 2004 and 2005 were examined to evaluate seroprevalence by the VN test, showing the following results: (1) 50 cats (63.3%) were sero-positive to FCoV; (2) of the 50 FCoV positive cat serum samples, 49 (98%) showed significantly higher titers to type I virus and only one (2%) for type II virus. These results indicate that the VN test described here can be used for serological differentiation of FCoV infections of cats, and that FCoV type I is a dominant type in recent years of Japan.

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1. Introduction

Feline infectious peritonitis (FIP) is a highly fatal and immune-mediated pyogranulomatous disease that occurs in both domestic and wild felidae. The disease is caused by feline coronavirus (FCoV). FCoV is a member of the family *Coronaviridae*, genus *Coronavirus* which is a group of enveloped and positive-

stranded RNA viruses. Antigenically and genetically, coronaviruses are currently grouped into at least four groups. FCoV belongs to group I coronavirus which includes canine coronavirus (CCoV), transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus and human coronavirus 229E (Wege et al., 1982).

Two pathotypes of FCoVs are also known: one is feline enteric coronavirus (FECV) which causes from asymptomatic infection to severe enteritis and another is feline infectious peritonitis virus (FIPV) that causes

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fatal immune-mediated disease FIP (Pedersen, 1987; Pedersen et al., 1981). It was demonstrated that FIPV arises inside of cats by mutation from infected FECV (Vennema et al., 1998). However, it is difficult to distinguish between FECV and FIPV by *in vitro* serological and genetical methods (Boyle et al., 1984; Fiscus and Teramoto, 1987; Pedersen et al., 1984).

Both FECV and FIPV are divided into two types I and II, based on their neutralization reactivity with spike (S) protein-specific mAbs (Fiscus and Teramoto, 1987; Hohdatsu et al., 1991b, 1992) and sequence analysis of the S protein gene (Motokawa et al., 1995, 1996). In addition to this serological property, some other biological characteristics to each type have been described. While FCoV type I grows poorly in cell culture, type II can grow well in many different cell lines (Pedersen et al., 1984). Furthermore, type II FCoV shows close antigenic and genetic relationship to CCoV and TGEV, suggesting that FCoV type II arose from double recombination between FCoV type I and CCoV (Herrewegh et al., 1998)

FCoV type II strains are more frequently utilized than type I strains for *in vitro* experiments because of higher efficient growth. For serological survey of FCoV infection, antibody has been detected by indirect fluorescent antibody assay (IFA) or enzyme-linked immunosorbent assay (ELISA) using FCoV (Pedersen, 1976; Scott, 1979; Horzinek and Osterhaus, 1979; Ishida et al., 1987). Alternatively CCoV or TGEV are also used as a target antigen because of serological close relations to FCoV. However, discrimination ability (specificity) of these methods using heterologous antigens seems to be low since they detect also cross-reactive antibodies between types I and II, such as antibody to nucleocapsid (N) protein.

In this study, we developed a plaque-reduction neutralization test (PRNT) to serologically distinguish FCoV type I and II infections in cats, and we applied it for field cases.

2. Materials and methods

2.1. Cell cultures

Feline whole fetus cells (fcwf-4 cells), which are fetal feline lung cells having characteristics of macrophages (Jacobse-Geels and Horzinek, 1983)

were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were maintained in a humidified 5% CO₂ incubator at 37 °C.

2.2. Viruses

FIPV type I strains C3663 and Yayoi and FIPV type II strains KUK-H/L and M91-267 were used in this study. KUK-H strain was isolated from the spleen sample of effusive form FIP case in 1987 by using CRFK cells, and KUK-H/L was plaque-purified from the KUK-H strain (Mochizuki et al., 1997). M91-267 strain was isolated from the spleen sample taken at the postmortem examination of effusive form FIP case in 1991 (Mochizuki et al., 1997). These two strains were classified into type II by using FCoV type-specific mAbs, kindly provided by Dr. T. Hohdatsu (Hohdatsu et al., 1991a, b). C3663 strain was isolated from a cat with an effusive form of FIP in 1994 and used as a reference FCoV type I (Mochizuki et al., 1997). In addition, it was genetically confirmed that C3663 belongs to type I FCoV and M91-267 and KUK-H/L do to type II (manuscript in preparation). Yayoi strain was isolated from liver homogenate from non-effusive form of FIP by serial passage in suckling mouse brain and then in fcwf-4 cells and have been used as Japanese prototype strain of FCoV type I (Hayashi et al., 1981). C3663, KUK-H/L and M91-267 strains were used in subsequent experiments within 10 passages in fcwf-4 cells. Although the passage history of Yayoi strain is unknown, it was propagated less than three times in our laboratory.

2.3. Cat sera

Four sera (Nos.1–4) were collected from cats clinically diagnosed as FIP and serologically identified as type I infection by indirect immunofluorescent assay using unfixed fcwf-4 cells infected with FCoV type I or II. On the other hand, we could not obtain any sera from naturally FCoV type II infected cats. Therefore, two sera collected from cats experimentally infected with M91-267 or KUK-H/L were used in this study (Mochizuki et al., 1997).

To evaluate the seroprevalence of FCoV types I and II in Japan, sera were collected from 79 cats, which

Table 1
VN titers to FCoV types I and II in FIPV-infected cats

FCoV type	Strain	Sera to FCoV type I ^a				Sera to FCoV type II	
		No. 1	No. 2	No. 3	No. 4	No. 5 ^b	No. 6 ^c
Type I	C3663	4000	4000	4000	4000	160	1280
	Yayoi	1000	16000	2000	2000	80	640
Type II	M91-267	40	<10	40	<10	20000	128000
	KUK-H/L	10	<10	40	<10	4000	128000

VN titers are expressed as the reciprocal value of the highest dilution.

^a Sera were collected from cats clinically diagnosed as FIP and serologically diagnosed as FIPV type I infection.

^b Serum was collected from a cat experimentally inoculated with M91-267.

^c Serum was collected from a cat experimentally inoculated with KUK-H/L.

visited to one veterinary hospital in 2004 and 2005. Two of them were clinically and serologically diagnosed as FIP in the hospital.

2.4. Plaque-reduction neutralization test (PRNT)

Serological test was performed by PRNT using serum samples inactivated by heating at 56 °C for 30 min. The sera diluted with DMEM containing 2% FCS and each virus diluted to approximately 2×10^3 PFU/ml with DMEM containing 2% FCS were equally mixed and incubated at 37 °C for 1 h. Then 50 μ l of the mixture was inoculated onto fcwf-4 cells monolayer in 24-well plate (SUMITOMO BAKELITE, Japan). After absorption at 37 °C 1 h, the inoculum was removed and 0.8% agarose (SeaPlaque[®] Agarose, TaKaRa, Japan) in growth medium was overlaid. The infected cells were incubated at 37 °C until cytopathic effect (CPE) appeared, fixed with phosphate-buffered formalin and stained with crystal violet. When the number of plaques was less than 75% of those in control wells, the diluted sera were judged to be positive. For screening of cat sera, all sera finally diluted to 1:10 were examined for PRNT. The positive sera were further diluted to 1:33, 1:100, 1:333, 1:1000, 1:3333, and 1:10000 to determine virus-neutralizing titers. The titers were expressed as the highest serum dilution showing 75% plaque reduction or more.

3. Results

3.1. Development of virus neutralization (VN) test

To determine the antigenic relationship between FCoV types I and II, serum samples from cats

experimentally infected with FIPV type II M91-267 or KUK-H/L and naturally infected with FIPV type I were examined by PRNT. Four sera from FIPV type I infected cats showed VN titers to C3663 and Yayoi ranging from 1:1000 to 1:16000, and those to M91-267 and KUK-H/L ranging from <1:10 to 1:40 (Table 1). Sera collected from the experimentally

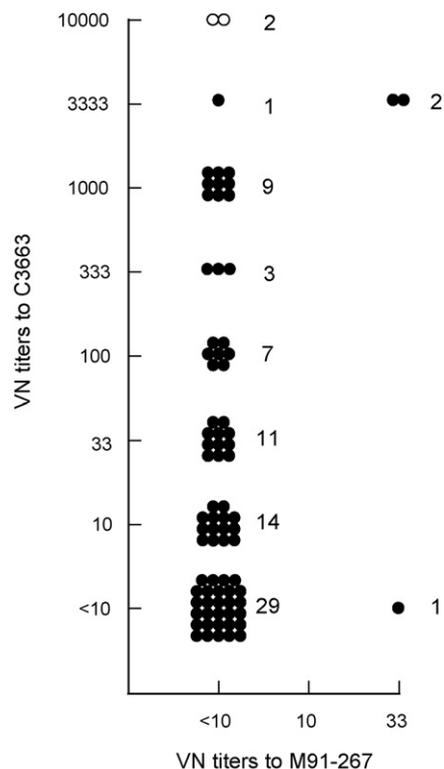


Fig. 1. Distribution of virus-neutralizing titers to FIPV types I and II among cats. VN titers are expressed as the reciprocal value of the highest dilution. Open circles show cats diagnosed as FIP.

FIPV type II infected cats had higher VN titers to type II ranging from 1:4000 to 1:128000 and lower VN titers to type I ranging from 1:80 to 1:1280 (Table 1). These results indicated that FCoV-infected cat sera had significantly higher VN titers to homologous type, but not to heterologous type. Therefore, this PRNT using both types of FCoV must be able to distinguish serologically between FCoV type I and II infections.

3.2. Prevalence of FCoV types in Japan

A total of 79 cat sera collected in Japan were examined for the prevalence of antibodies to FCoV types I and II by PRNT using C3663 and M91-267 strains. The result showed that 50 (63.3%) cats were seropositive to FCoV (Fig. 1). Among these seropositive samples, 49 (98%) possessed significantly higher titers against type I, and only one (2%) did against type II. Although three samples had VN titers of 1:33 to type II, two of them had higher VN titers to type I (1:3333). Since one sample did not have VN titer to type I, it was regarded as positive for type II. All two samples from FIP cases had the highest VN titers to type I (1:10000), meanwhile, these were negative (<1:10) to type II.

4. Discussion

In this study, we distinguished types of FCoV which had infected cats by VN test. At first, we determined cross-reactivities of the known sera from FIP cats (Table 1). VN titers difference between homologous type was five-fold or less, and to heterologous type was 25-fold or more, indicating that the present PRNT can clearly differentiate FCoV types. It is known that a major difference between types I and II is located in S protein responsible for eliciting VN antibodies (Fiscus and Teramoto, 1987; De Groot et al., 1989; Corapi et al., 1992).

Using the PRNT, we examined the recent status of FCoV seroprevalence in field cats. More than 60% of the cats were found to be FCoV seropositive and this was similar to the positive rate described in other countries (Pedersen et al., 1981; Loeffler et al., 1978; Kummrow et al., 2005; Bell et al., 2006). In Japan, Hohdatsu et al. (1992) first described that 21.3% of chronically diseased cats and 13.4% of non-diseased

cats had antibodies to FCoV by IFA to detect FCoV infection. The seroprevalence rate obtained in the present study is about three times higher than that in the previous report. Since different methods and samples were used for epidemiology, it is impossible to directly compare the prevalence of FCoV in Japan. However, most probable explanation for this discrepancy is that FCoV has been widespread among Japanese cat population during the last decade.

Previous studies conducted in several countries including Japan have revealed that 69–91% of seropositive cats were suspected to be type I infection (Hohdatsu et al., 1992; Vennema, 1999; Kummrow et al., 2005). In the present study, it was found that 49 cats (98%) possessed type I specific antibodies and only one cat (2%) to type II. The prevalence of type I infection revealed here was significantly higher than the previous report (80.4% in chronically diseased cats) by competitive ELISA (Hohdatsu et al., 1992). Although this and the previous report (Hohdatsu et al., 1992) were different in methods and samples, it might suggest that type II FCoV infection has been decreasing in Japanese domestic cats in recent years.

In conclusion, our newly established VN test can specifically detect and distinguish between types of FCoV infection. In addition, FCoV type I, but not type II, has widely spread among cats population in Japan.

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