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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.

Keywords: Feline coronavirus; Feline infectious peritonitis virus; Virus neutralization test

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% CO_2 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