

## The Prevalence of Types I and II Feline Coronavirus Infections in Cats

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**ABSTRACT.** The types of feline coronaviruses that are prevalent throughout Japan were determined by competitive enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody (MAb) to feline infectious peritonitis virus (FIPV) Type II and neutralizing test using Type II FIPV as challenge virus. A total of 1,079 cat serum samples were tested by indirect fluorescent antibody (IFA) assay for FIPV Type II antigen, all 42 sample from natural cases of FIP, 138 of 647 (21.3%) from cases with some chronic diseases and 57 of 390 (14.6%) from apparently non-diseased cases were positive. Of the 42 cases with FIP, 29 (69%) and 13 (31%) were found to have infection with FIPV Types I and II, respectively. Of the cases with chronic diseases, 111 (80.4%) were shown to have infection with FIPV or FECV Type I, while 14 (10.1%) with FIPV or FECV Type II. All of the 57 apparently non-diseased cases seemed to have been infected with FIPV or FECV Type I. These results indicated that feline coronavirus Type I is more high prevalent in Japan—**KEY WORDS:** coronavirus, feline, FECV, FIPV, competitive ELISA.

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For serological diagnosis of feline infectious peritonitis virus (FIPV) infection, indirect fluorescent antibody (IFA) assay has been widely used [7, 10, 18, 19]. However, the presence of feline enteric coronavirus (FECV) antigenically cross-reacting with FIPV and causing mild enteritis makes serological diagnosis indefinite [12, 15–17]. Both FIPV and FECV are divided into Types I and II, on the basis of the disease types, that is, the ability of the viruses to cause peritonitis or, to proliferate in cell cultures and the antigenic relationship to transmissible gastroenteritis virus (TGEV) and canine coronavirus (CCV) [14]. Using monoclonal antibodies (MAb) we have shown the presence of at least 2 serotypes of FIPV and the antigenicity of Type II strains of FIPV and FECV which were closer to TGEV and CCV than to Type I FIPV [8, 9]. In this study the types of FIPV-FECV prevalent in Japan were determined by competitive enzyme-linked immunosorbent assay (ELISA) using a Type II FIPV-specific MAb and by neutralizing (NT) test using Type II FIPV as challenge virus.

### MATERIALS AND METHODS

**Viruses:** Type II FIPV strain 79–1146 and Type I strain UCD-1 were kindly supplied from Dr. H. C. Horzinek, The State University, Utrecht, and from Dr. N. C. Pedersen, The University of California, Davis, respectively. These viruses were grown in feline whole fetus cells (fcwf-4). The fcwf-4 cells

were grown in Eagle's minimum essential medium containing L-15 medium (20%), fetal calf serum (10%), penicillin (100  $\mu$ /ml) and streptomycin (100  $\mu$ g/ml). The cells were maintained in a humidified 5% CO<sub>2</sub> incubator at 37°C. For preparation of virus antigen, strain 79–1146 infected culture fluid was subjected to ammonium sulfate precipitation and an about tenfold concentrated was layered onto a discontinuous sucrose density gradient (20 and 60%) in a RPS 28 rotor (Hitachi Koki, Japan) and centrifuged at 27,000 r.p.m. for 2 hr. The virus bands formed were collected, diluted in NTE buffer (0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA) and centrifuged at 80,000  $\times$  g for 1 hr. The resulting pellet was suspended in a 1/500 volume of NTE buffer and used as virus antigen.

**Antisera:** Either strain 79–1146 or strain UCD-1 was oronasally inoculated into about 6 month old cats which had been tested for feline coronavirus antibody, and serum was sampled at regular intervals.

**MAb:** The MAb (No. 6–4–2) to strain 79–1146 was produced as previously described [8]. The MAb recognized protein S of the virus and neutralized strain 79–1146 and FECV Type II strain 79–1683, but it did not react with Type I FIPV strains, UCD-1, UCD-2, UCD-3, UCD-4, NW-1, Black. Peroxidase-labeled MAb was prepared by the method of Nakane and Kawaoi [13].

**Test serum samples:** A total of 1,079 feline serum samples were divided into 3 groups: 1) 42 samples

from natural cases of FIP with febrile effusive peritonitis, jaundice, nephropathy and, feline coronavirus antibody, 2) 647 samples from cases with a variety of chronic diseases, 3) 390 samples from non-diseased cases bred in Aomori Prefecture, Japan. The samples from natural cases of FIP and cases with a variety of chronic diseases were submitted by private veterinarians in Fukuoka, Osaka, Gifu, Shizuoka, Niigata, Kanagawa, Tokyo, Saitama, Fukushima, Iwate, Akita, Aomori Prefecture, Japan for diagnosis of FIP and serological tests for viral infection.

**ELISA:** The virus antigen (see above) was appropriately diluted with carbonate buffer (0.05 M, pH 9.6) and 100  $\mu$ l of dilutions was given into each well of 96-well flat-bottomed Microelisa plates. The plates were allowed to stand overnight at 4°C, washed with PBS containing 0.02% Tween-20, and 100  $\mu$ l of the test serum sample was added to each well. Horseradish peroxidase-conjugated goat anti-cat IgG (Cooper Biomedical, Malvern, U.S.A.) were diluted to the optimal concentration with PBS containing 10% calf serum and 0.05% Tween-20 and 100  $\mu$ l of the dilution was added to each well of the plates. After incubation at 37°C for 30 min, each well received 100  $\mu$ l of substrate solution and incubated at 25°C for 20 min in a dark room. The substrate solution was prepared by dissolving O-phenylenediamine dihydrochloride at a concentration of 0.4 mg/ml in 0.1 M citric acid- 0.2 M Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 4.8) and adding 0.2  $\mu$ l/ml of 30% H<sub>2</sub>O<sub>2</sub>. The reaction was stopped with 3 N H<sub>2</sub>SO<sub>4</sub> solution and the optical density (OD) at 492 nm was determined.

**Competitive ELISA:** Each well of 96-well Microelisa plates coated with virus antigen received 100  $\mu$ l of a 1:100 dilution of cat serum in PBS containing 10% calf serum and 0.05% Tween-20, and the plates were incubated at 37°C for 1 hr. After washing with PBS containing 0.02% Tween-20 3 times, an optimal dilution of peroxidase-conjugated MAb in PBS containing 10% calf serum and 0.05% Tween-20 was added and the plates were incubated at 37°C for another 30 min. The subsequent treatment was the same as for conventional ELISA (described above). The percent inhibition was calculated by the formula 100 (A-B/A), where A and B were the OD data in the dilution buffer and test serum, respectively.

**Indirect fluorescent antibody (IFA) assay:** FIPV strain 79-1146 infected cells were fixed for 15 min in cold acetone and air dried. Serial dilutions of the

test sera were added to acetone-fixed cells and allowed to stand at 37°C for 30 min. After washing with PBS three times, the specimens were stained with anti- cat IgG goat serum conjugated with fluorescein isothiocyanate (Southern Biotechnology Assoc., Birmingham, U.S.A.). After incubation at 37°C for 30 min, they were washed with PBS, mounted in 50% glycerol buffer and observed a fluorescence microscope.

**Neutralization (NT) test:** Serial twofold dilutions of the test sera were mixed with an equal volume of an FIPV strain 79-1146 suspension containing approximately 200 TCID<sub>50</sub> and the mixtures were incubated at 37°C for 60 min. Each mixture was then inoculated into the fcwf-4 cell cultures in flat-bottomed microplates (Corning Glass Works, Corning, New York, U.S.A.), and incubation was made in an atmosphere of 5% CO<sub>2</sub> in air at 37°C for 3 days. For each serum dilution tests were duplicated. The antibody titer was expressed as a reciprocal of the highest dilution of MAb that completely inhibited viral cytopathic effect.

## RESULTS

Of the 1,079 test serum samples 273 (22.0%) had IFA titers of 1:64 or higher against FIPV Type II strain 79-1146 (Table 1). All 42 of the samples from cases with FIP had higher titers of 1:256 to 1:4,096, while 138 of 647 (21.3%) samples from cases with some chronic diseases and 57 of 390 (14.6%) samples from non-diseased cats were also positive for IFA.

The 237 positive samples were discriminated for Types I and II of FIPVs/FECVs infections by competitive ELISA with MAb specific for FIPV Type II and by NT test with Type II strain 79-1146 as challenge virus. Specificity of the competitive ELISA was checked using the antiserum from cats experimentally infected with Type II FIPV strain 79-1146 and Type I strain UCD-1, revealing that antiserum to strain 79-1146 showed increasing

Table 1. Serodiagnosis of feline coronavirus infection by IFA assay

Case	Positive (1: 64 $\leq$ )/ Tested (%)
FIP	42/ 42(100 )
Chronic ally diseased	138/647( 21.3)
Non-diseased	57/390( 14.6)
Total	237/1079( 22.0)

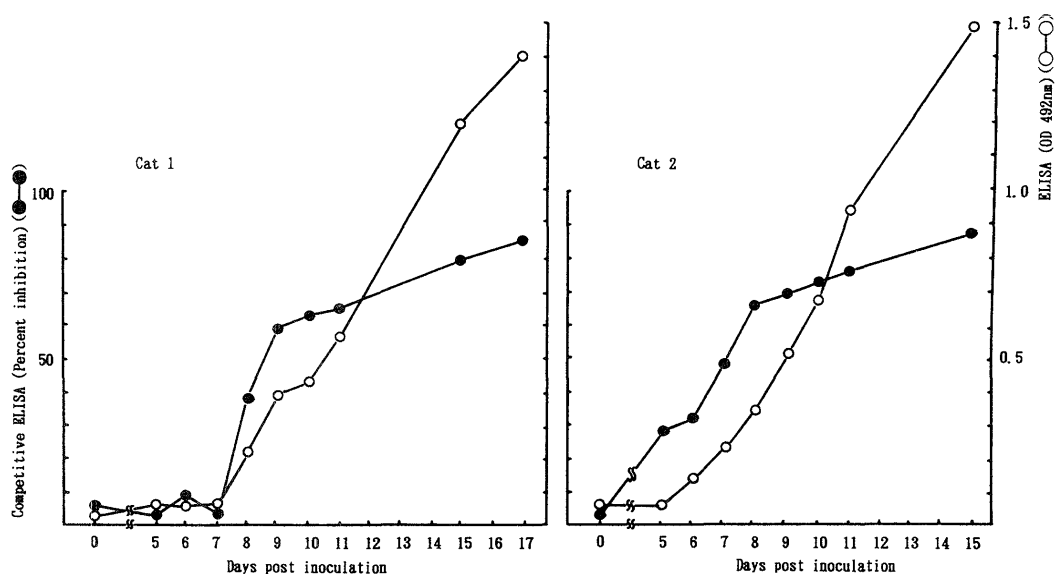


Fig. 1. Competitive ELISA and ELISA of antisera from cats inoculated with Type II FIPV strain 79-1146.

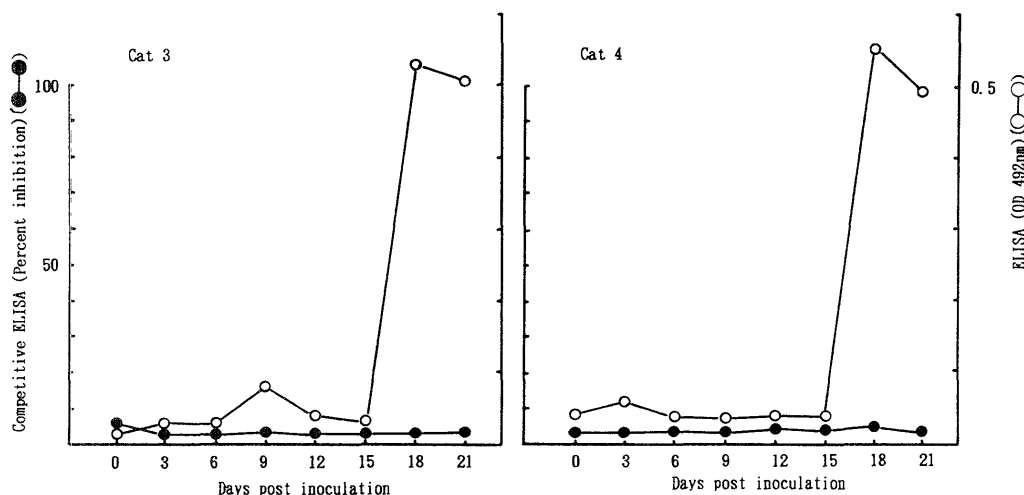


Fig. 2. Competitive ELISA and ELISA of antisera from cats inoculated with Type I FIPV strain UCD-1.

percent inhibitions in competitive ELISA along with increasing OD in ELISA (Fig. 1) while antiserum to strain UCD-1 did not (Fig. 2).

The results were compared between competitive ELISA and IFA or NT test, and between NT test and IFA, in cases with FIP, as shown in Fig. 3. Some of the FIP cases that had higher IFA titers showed lower percents of inhibition in competitive ELISA and NT titers. From the results of competitive ELISA and NT test, the test sera were divided into two groups: 1) 29 (69.0%) cases giving lower values in both tests, and 2) 13 (31.0%) cases showing higher values in both tests. Similar results were obtained with the serum samples from cases with

chronic diseases, that is, 111 of 138 (80.4%) showed lower values in both tests while 14 (10.1%) higher values. Another group of 13 (9.4%) cases showed 30 to 80% inhibition in competitive ELISA (Fig. 4). All the test samples from non-diseased cases showed low values in both tests (Fig. 5).

Table 2 shows the discrimination of feline coronavirus infection based on the results of competitive ELISA.

DISCUSSION

About 10 to 30% of household cats examined were described to carry antibody to feline coronavir-

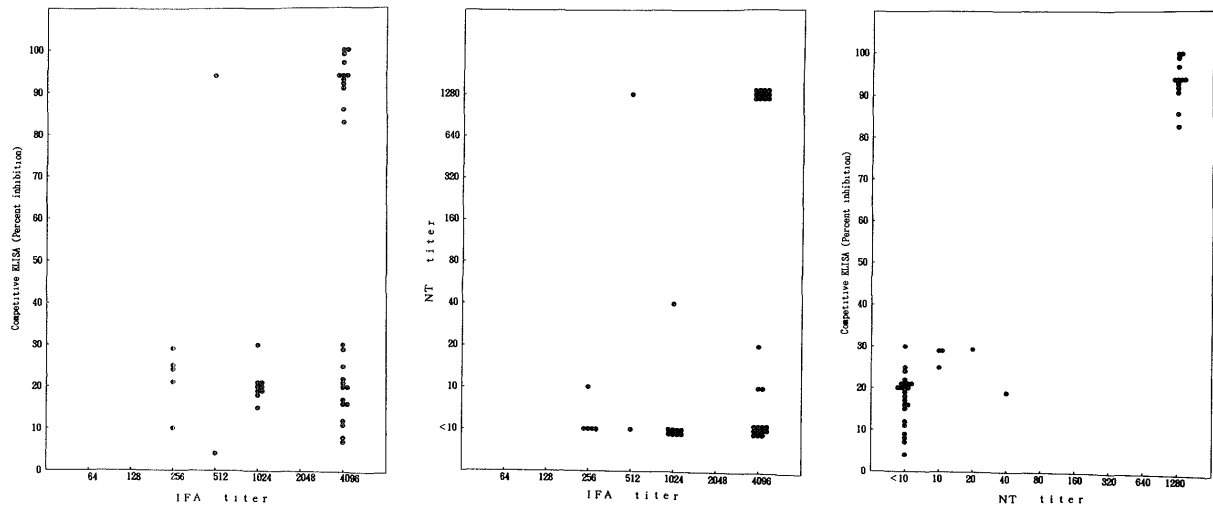


Fig. 3. Competitive ELISA data, and NT titers and IFA titers of sera from cats with FIP.

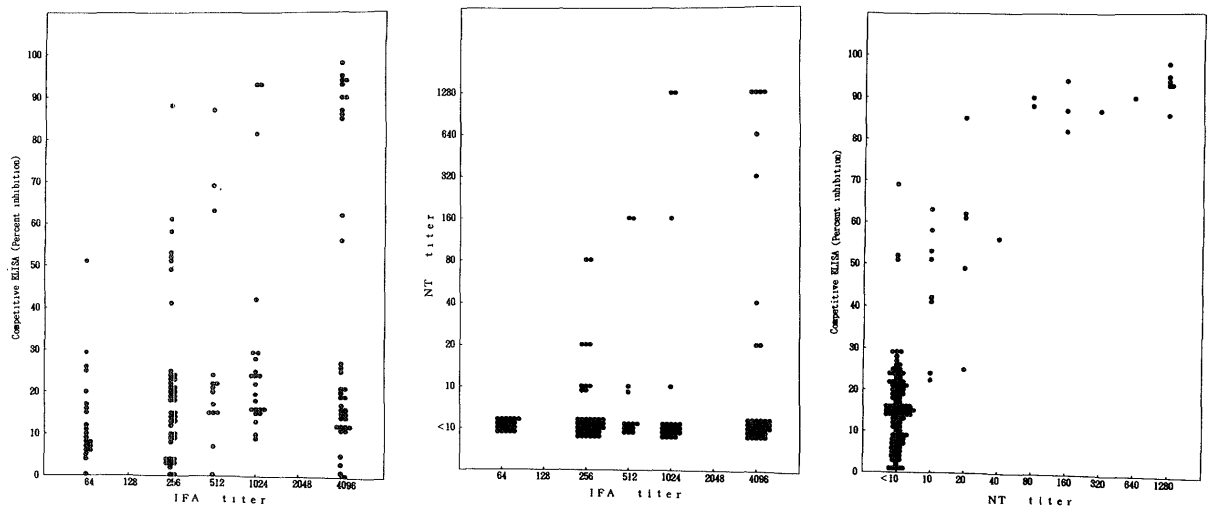


Fig. 4. Competitive ELISA data, and NT titers and IFA titers of sera from cats with some chronic diseases.

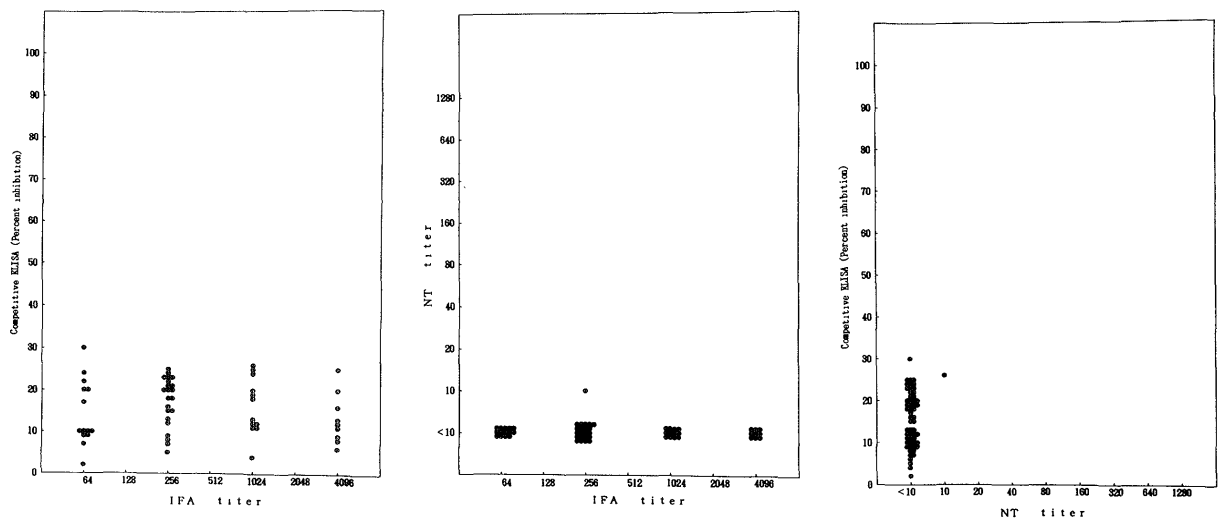


Fig. 5. Competitive ELISA data, and NT titers and IFA titers of sera from non-diseased cats.

Table 2. Typing of feline coronaviruses by competitive ELISA and its prevalence

Cases	Number of cases tested	<30% <sup>1)</sup>		30~80%	80%<	
		Type I FIPV	Type I FIPV or Type I FECV	?	Type II FIPV	Type II FIPV or Type II FECV
FIP	42	29(69.0%) <sup>2)</sup>			13(31.0%)	
Chronic ally diseased	138		111(80.4%)	13(9.4%)		14(10.1%)
Non-diseased	57		57(100%)			

1) % inhibition in competitive ELISA.

2) Number of cases showing % inhibition indicated; percentage in parenthesis.

uses FIPVs/FECVs [7, 10, 18, 19]. In catteries which had experienced the occurrence of FIP in the past 80 to 90% of cats were reported to be positive for the antibody [10, 18, 19], and in the present study, 237 of 1,079 (22.0%) cases were positive for IFA. The incidence of FIP was lower than the rate of antibody positivity, suggesting the existence of inapparent infections. Pedersen *et al.* [15, 17] and McKeirnan *et al.* [12] isolated FECV antigenically cross reactive with FIPV. Although both FIPV and FECV can be into Types I and II [14], the prevalent virus in this country have remained unknown.

As shown in Figs. 1 and 2, FIPV Type I infection was discriminated from that of FIPV Type II by competitive ELISA using a MAb specific for FIPV Type II virus [8]. Fiscus *et al.* [2, 3] reported that competitive ELISA using MAb was useful for discrimination between FIPV infections with virulent and avirulent viruses [2, 3]. Pedersen *et al.* [14] reported that antiserum to FIPV Type I strongly reacted with Type I virus in NT test, but very weakly reacted with FIPV Type II and FECV Type II viruses. In the present study of competitive ELISA and NT test using FIPV strain 79-1146, the typing of FIPVs/FECVs was attempted with 237 serum samples from cats showing IFA titers of 1:64 or higher. The FIP cases were assigned to one group showing a lower percent inhibition on competitive ELISA and NT titers and another group showing a higher percent inhibition and NT titers. When positivity for IFA and negativity in competitive ELISA and NT test indicates FIPV Type I infection, and positivity in competitive ELISA and NT test did Type II infection, about 70 and 30% of FIP cases were considered to have infection with Types I and II, respectively. Pedersen *et al.* [14] found that many cases of FIP were due to Type I virus. Similar

grouping of cases with chronic diseases was possible. The percentage of Type II infection in this group, however, was 10.1%, unlike the cats with FIP. It was not clear whether or not the chronic disease group included FIP cases, whether all the cases showing low values in competitive ELISA and NT test had infection with Type I FIPV and whether all the cats showing high competitive ELISA and NT titers were infected with Type II FIPV. In these case without FIP, infection with FECV Types I and II cannot be excluded. Furthermore, there were 13 (9.4%) cases of cats with chronic diseases showing a percent inhibition of 30 to 80% on competitive ELISA, unlike FIP cases. Ten of these 13 cats showed NT titers of 1:10 to 1:40, and it is unclear whether these cats had infection with FIPV or FECV Type I or II. We have previously detected a mixed infection with Types I and II in catteries. These 13 cases may be also considered to be at an early stage of FIPV Type II infection.

All the non-diseased cats showed low competitive ELISA and NT titers, indicating no infection with Type II of FIPV or FECV. FECV Type II such as strain 79-1683 isolated by MaKeirnan *et al.* [12] seems not to appear in Japan. The rate of FIPV Type II detection was low in cases with chronic diseases and in non-diseased cats, while the rate was 31% in cases with FIP. The incidence of FIP due to Type II FIPV might be higher than that due to Type I FIPV.

The present study revealed that the combination of competitive ELISA and NT test is useful for seeing the prevalence of feline coronaviruses in cats. Although the Type II strain 79-1146 has been widely used for the genetic studies of FIPV because of the ease of cell culture [5, 6, 11, 20], the use of Type I FIPV should be recommended because of the high

prevalence of this type in nature. For producing preventive vaccines [1, 4], type difference between virus strains should be taken into account.

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