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AN ENZYME-LINKED IMMUNOSORBENT ASSAY USING CANINE CORONAVIRUS-INFECTED CRFK CELLS AS ANTIGEN FOR DETECTION OF ANTI-CORONAVIRUS ANTIBODY IN CAT

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Abstract—From the reasons that canine coronavirus (CCV) grows more efficiently than feline coronavirus in a cell culture and they are mutually related in their antigenicities, an enzyme-linked immunosorbent assay (ELISA) using CCV-infected feline kidney (CRFK) cells as substrate antigens was developed for detection of anti-coronavirus antibodies in cats. It was indispensable for generating coronavirus-specific ELISA antibody activities that the sample was applied to the mock-infected, normal CRFK cells in parallel with the CCV-infected cells and then the optical density values given by the mock-infected cell antigen were subtracted from those given by the virus-infected cell antigen. On the basis of ELISA antibody titers obtained in sera from the cats experimentally infected with CCV and from the spontaneous feline infectious peritonitis (FIP) cases, the ELISA described in the present study was found to be applicable as a simple and easy serologic test which was able to detect anti-coronavirus antibodies as efficiently as the indirect immunofluorescence assay with homologous FIP virus.

Key words: Coronavirus, feline infectious peritonitis virus, canine coronavirus, enzyme-linked immunosorbent assay, ELISA.

Résumé—Etant donné que coronavirus canin (CVC) prolifère en culture cellulaire avec une meilleure efficacité que coronavirus félin et qu'ils ont une antigénéciteoroisée, un test ELISA utilisant des cellules rénales félines (CRFK) infectées par le CVC comme antigène substrat a été dévélopée pour la détéction des anticorps anti-coronaviraux chez le chat. Pour obtenir une activité anticorps ELISA coronavirus-spécifique, il a été considéré comme indispensable que l'échantillon soit appliqué aux cellules (CRFK) normales, non infectées en parallèle avec les cellules mentionnées ci-dessus et que les valeurs de densité optique données par l'antigéne des cellules noninfectées soient soustraites à celles donnéees par l'antigène des cellules infectées. En se basant sur les titres d'anticorps ELISA obtenus avec les sérums de chats infectés expérimentalement avec le CVC ou atteiuts de péritonite infectieuse féline (PIF) spontanée, l'ELISA décrit dans cette étude s'est révélé applicable comme un test sérologique à la fois simple et maniable qui nous permet de détécter anticorps anti-coronavirus aussi effectivement que l'essai d'immunofluorescence indirecte avec le virus PIF homoloque.

Mots-clefs: Coronavirus, virus de la péritonite infectieuse féline, coronavirus canin, test immuno-penzymatique, ELISA.

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Abbreviations: CCV—canine coronavirus; CRFK—Crandell feline kidney; DK/P—primary dog kidney; Eagle's MEM—Eagle's minimal essential medium; ELISA—enzyme-linked immunosorbent assay; FECV—feline enteric coronavirus; FIP—feline infectious peritonitis; FIPV—FIP virus; IFA—indirect immunofluorescence assay; mAb—monoclonal antibody; MNT—micro-neutralization test; NUS—NU-serum; OD—optical density; PFU—plaque forming unit; PBS—phosphate buffered saline solution; TGEV—transmissible gastroenteritis virus; VNT—virus neutralization test.

INTRODUCTION

The coronaviruses are responsible for a wide variety of diseases, in particular respiratory and enteric disorders in mammalian and avian species [1]. Feline infectious peritonitis (FIP) which is a chronic, progressive, immunologically-mediated coronaviral disease [2–6], is one of the most important viral infections in cats with worldwide occurrence and increasing frequency [7, 8]. A feline enteric coronavirus (FECV), which causes a mild intestinal disease but not FIP in kittens, has also been isolated [9] and considered to be the result of a recombination event between virulent FIP virus (FIPV) and avirulent FIPV [10]. Feline coronavirus (FIPV and FECV) isolates thus far are divided into at least two types according to the serologic cross-reaction with canine coronavirus (CCV) by virus neutralization test (VNT) [4], and there may be at least 2 antigenic groups of FIPV by the monoclonal antibody (mAb) analysis [10].

Human coronavirus 229E, transmissible gastroenteritis virus (TGEV) of swine, CCV, FIPV and FECV compose one antigenically related group in the family of coronaviruses [1, 9, 11, 12]. Cats inoculated with CCV [13] or TGEV [14, 15] elicit immune responses against not only homologous but also heterologous virus species in the group. However, the incidence of infection in general cat population in nature with these heterologous viruses is unknown. Because FIPV has proved difficult to isolate and propagate *in vitro*, heterologous serologic assays (VNT, indirect immunofluorescence assay; IFA, enzyme-linked immunosorbent assay; ELISA, and passive hemagglutination test) by use of antigenically related TGEV and CCV have been widely applied for detection of anti-coronavirus antibody in cats [14, 16–23]. In recent years several feline coronavirus isolates cultivable in cell cultures have led to the development of homologous serologic assays [4, 24–27].

The purpose in the present report is to evaluate ELISA that uses CCV-infected cells as antigens for coronavirus serology in cats. Canine coronavirus grows very efficiently in feline cells and the use of such virus-infected cultured cells saves laborious and expensive virus purification.

MATERIALS AND METHODS

Virus strain

A canine coronavirus, designated 5821 strain, was obtained from Dr Masayuki Ajiki, Kyoto-Biken Lab., Kyoto. This strain was originally isolated from feces of a puppy died of enteritides manifesting diarrhea and vomiting in 1984, and had been previously passaged 8 times in primary dog kidney (DK/P) cells. The virus was further cultured twice in DK/P cells and once in Crandell feline kidney (CRFK : ATCC CCL-94) cells in this laboratory, and stored at $-80^{\circ}C$ as a stock virus. Infective titer of the stock virus was determined by the plaque assay described previously [23].

Cells and media

The CRFK cells were used for the plaque assay, micro-neutralization test (MNT) and ELISA. They were cultured in Eagle's minimal essential medium (Eagle's MEM : Nissui Pharm. Co., Ltd, Japan) containing 10% NU-SERUM (NUS: Collaborative Res., Inc., U.S.A.), 10% tryptose phosphate broth (Difco Lab., U.S.A.) and antibiotics

Table 1. Comparison of antibody titers by micro-neutralization test (MNT) and enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescence assay (IFA) using canine coronavirus (CCV) and feline infectious peritonitis virus (FIPV) as substrate antigens, respectively

Specimen*	MNT titer against CCV	ELISA titer against CCV-infected cells	IFA titer against FIPV
1. Serum	<4	400	1600
2. Thoracic fluid	<4	12,800	25,600≦
3. —	<4	3200	25,600≦
4. —	<4	3200	25,600≦
5. Ascites	32	6400	25,600≦
6. —	128	51,200	25,600≦
7. —	<4	1280	1000≦
8. —	<4	320	1000≦

*Specimen nos 1–6 and nos 7 & 8, with known IFA titers determined by indirect IFA using FIPV Yayoi strain infected suckling mouse brain sections [28], were obtained from Department of Veterinary Pathology, University of Tokyo and Department of Veterinary Microbiology, Iwate University, Iwate, respectively.

(100 U of penicillin G, 100 μg of streptomycin and 5 μg of amphotericin B/ml) (Eagle's MEM/10NUS).

Cat sera

Twenty-three sera and 5 ascites were collected from cats clinicopathologically suspected as FIP. One serum, 3 ascites and 2 thoracic fluids, and 2 ascites which were from the pathologically confirmed spontaneous FIP cases and possessed specific IFA antibodies against the Yayoi strain of FIPV [28] as listed in Table 1, were obtained from The Department of Veterinary Pathology, University of Tokyo, Tokyo, and The Department of Veterinary Microbiology, Iwate University, Iwate, respectively.

Experimental inoculation of cats with CCV

Two litters of conventional kittens, which were about 6- to 8-week old, clinically normal and seronegative, were used in the experiments. Each of 5 littermate kittens was orally administered with 4.5×10^6 plaque forming units (PFU) of the virus. Four kittens of another litter received 7.5×10^5 PFU orally and one intramuscular dose of the triple viral vaccine (Panagen FVR*C-P; Pitman-Moore, Inc., U.S.A.) simultaneously. Each of them had another vaccine booster injection 3 weeks later. The vaccine contained a mixture of inactivated feline panleukopenia virus, and attenuated feline calicivirus and feline herpesvirus which were prepared in feline cell cultures. Blood samples were taken from all kittens at appropriate intervals for a 7-week period.

Micro-neutralization test

Neutralizing antibody titer was determined by the method of MNT described elsewhere [23]. More than 1:4 of the titer was regarded as positive.

ELISA

The CRFK cells (8×10^5/ml of cell density in the Eagle's MEM/10NUS) were mixed with an equal volume of the stock virus to be at 0.05 of multiplicity of infection and 100 μl of the mixture was dispensed into each well of a 96-well, flat bottomed, polystyrene plates (Corning Glass Works, U.S.A) as described previously [23]. After 48-h incubation in a humidified chamber containing 5% CO_2, the cells were fixed with methanol containing 0.3% H_2O_2 for 30 min. The wells were then saturated with 250 μl of blocking buffer which

consisted of 3% gelatin in Dulbecco's phosphate buffered saline solution (PBS). After 1 h at room temperature, the blocking buffer was removed from the wells, and to which 50 μl amounts of the test sample serially diluted in PBS containing 1% gelatin and 0.05% Tween 20 (GT/PBS) was added. The plate was incubated at 37°C for 1 h and was washed by emptying, filling with 0.05% Tween 20 in PBS from a wash-bottle and leaving for 3 min. This process was repeated 3 times. Then 50 μl of freshly diluted 1:400 horseradish peroxidase-conjugated anti-cat IgG goat serum (Cooper Biomedical Inc., U.S.A.) in GT/PBS was added to each well and kept for 1 h at room temperature after which plates were washed. The result was read spectrophotometrically (405 nm) by an ELISA reader (MTP-02 type; CORONA Electric Co., Ltd, Japan) 30 min after the addition of ABTS substrate (2,2,′-azino-di(3-ethyl benzylthiazoline sulfonic acid-6)-disodium salt; Polysciences, Inc., U.S.A.) to wells. The test sample was applied to the mock-infected, normal CRFK cells in parallel with the virus-infected cells by the same manner. Optical density (OD) values given by the mock-infected cell antigen were subtracted from those given by the virus-infected cell antigen (the corrected OD value). The ELISA titer was defined as the reciprocal of the highest serum dilution at which the corrected OD value exceeded 0.05 as shown in Fig. 1 in which typical titration curves for positive and negative sera were drawn. The ELISA titer of more than 1:10 was regarded as positive.

RESULTS

Antibody response of cats administered with CCV

All cats manifested no clinical signs throughout the experiments. Rapid rise in both MNT and ELISA antibody titers was detected in all cats administered with CCV on the 8th day as shown in Fig. 2. Both antibody titers gradually rose until the 30th day and declined thereafter in parallel with each other. The geometric means of ELISA titers were about 4–10 times higher than those of MNT titers.

Although ELISA titers of pre-administration sera were regarded to be less than 1:10 by the corrected OD value, all had low reactivities (1:20–1:50) to the mock-infected, normal CRFK cell antigen as shown in Fig. 3. The reactivities against the normal CRFK cell antigens of the cats with no vaccination did not change so drastically as those of the cats administered with the parenteral vaccine. After the vaccination the sera became to be reactive with not only the CCV-infected cell antigen but also the mock-infected cell antigen to comparable degrees. However, the OD values of each dilution of serum against the infected cell antigen were so higher than those against the control cell antigen as to generate the specific values.

Comparison of anti-coronavirus antibody titers in cat sera by MNT, ELISA and IFA

Relationships between MNT and ELISA titers of 28 samples from the cats suspected as FIP were shown in Fig. 4. Twenty-three of them (82.1%) were anti-CCV MNT antibody-positive with titers of 1:4 to 1:512,000 and anti-CCV ELISA antibody with titers of 1:10 to 1:256,000 were detected in all of them. The samples with high MNT titers (1:256 or more; $n = 9$) had proportionally high titers of ELISA antibody as well. The calculated correlation coefficient between both MNT and ELISA titers of them was found to be $r = 0.907$. But significantly high ELISA antibody titers (1:2,560 or more) were detected in 12 out of the other 19 samples possessing low or negative MNT titers (1:64 or less). Table 1 shows MNT and ELISA antibody titers against CCV of the serum and

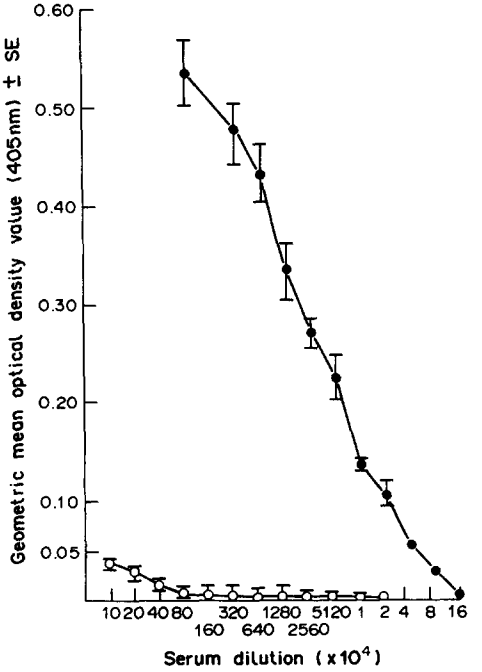

Fig. 1. Typical titration curves for positive (●; $n = 3$; titer 1:41,960) and negative (○; $n = 7$; titer $<1:10$) sera tested by the ELISA.

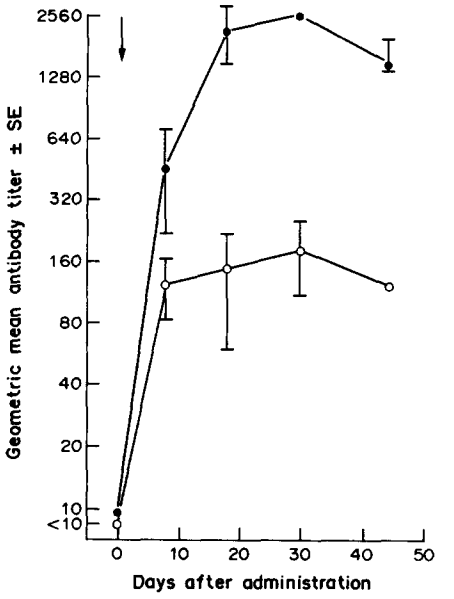

Fig. 2. Comparison of micro-neutralization (○) and ELISA (●) antibody responses in a litter of 5 kittens orally administered with canine coronavirus (CCV). Arrow indicates oral administration of CCV (4.5×10^6 PFU/cat).

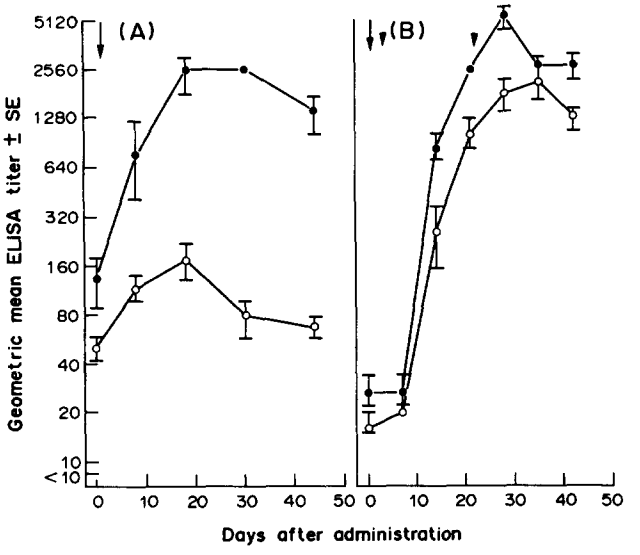

Fig. 3. Serologic responses of canine coronavirus (CCV) administered kittens against CCV-infected cell (●) and mock-infected, control cell (○) antigens without (A) and with (B) vaccination. The ELISA titer was defined as the reciprocal of the highest serum dilution at which the optical density value (405 nm) exceeded 0.05. Arrows indicate oral administration of CCV (A: 4.5×10^6 PFU/cat, B: 7.5×10^5 PFU/cat). Arrow heads indicate intramuscular injection of the vaccine (days 0 and 21; see the text for the vaccine).

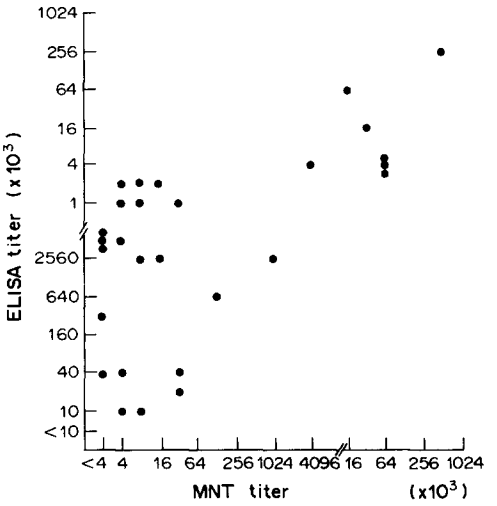

Fig. 4. Comparison of anti-canine coronavirus micro-neutralization test (MNT) and ELISA titers of 28 cats clinicopathologically suspected as feline infectious peritonitis.

the body fluids of 8 FIP cases possessing specific IFA antibodies against FIPV. In these cases, the ELISA titers but not the MNT titers against CCV were correlative with the IFA titers against homologous FIPV. However, the ELISA titers were generally lower than the IFA titers.

DISCUSSION

Clinical diagnosis of FIP is generally performed by evaluation of results of physical and hematological examinations, serum electropholetic analysis, and anti-coronavirus antibody testing. Moreover, biopsy in the living animal or necropsy after death are considered to be the most definitive diagnosis of FIP. The serologic tests are, however, only an aid to diagnosis because anti-coronavirus antibody can be detected not only in FIP cats but also in cats with other diseases and even in healthy animals [8, 18, 19, 22, 28, 29]. The tests used routinely for detection of anti-coronavirus antibodies in felines are IFA or ELISA in which TGEV or CCV have been popularly used as the target antigen [17–19, 21]. The results in the present study indicate that the ELISA using CCV-infected cultured cells as antigens saves laborious purified viral antigen preparation, and it detects anti-coronavirus antibodies in cat sera more extensively than the MNT using CCV, and as efficiently as IFA using homologous FIPV. However, the ELISA titers were generally lower than those obtained by the IFA as shown in Table 1. This may result from qualitical differences of the substrate antigens (i.e. heterologous or homologous coronaviruses for cat) applied in the tests as reported by others [27].

In the histochemical serological assays such as ELISA and IFA, non-specific binding (both γ globulin associated and non-γ globulin associated) of serum samples to the antigen preparations is often an additional problem. It has been described that most but not all of false-positive reactions are attributable to recent vaccination [17, 27, 30, 31]. Some kinds of cell culture vaccines prepared for use in cats have been commonly utilized in Japan as well. Antibodies against vaccine ingredients other than desired viral antigens, especially

serum components in a tissue culture-derived vaccine, may be elicited in cats after parenteral injection and they react with antigenically similar components presented in the antigen preparations of serologic tests. Therefore serum samples should be tested in parallel against negative antigen controls and results are adjusted accordingly as in kinetics-based ELISA described by Barlough *et al.* [17]. In the present study, presumably because whole cultured-cell antigens were used, all cat sera showed a variety of reactivities to the mock-infected, normal CRFK cell antigens as well, and it was indispensable for generating the coronavirus-specific activity that the OD values given by the mock-infected cell antigen were subtracted from those given by the virus-infected cell antigen.

Some *in vivo* subtle antigenic differences among FIPV, FECV and other heterologous coronaviruses doses have been described [4, 32–35]. Although recently developed competitive ELISA using type-specific mAbs may prove useful in distinguishing cats which are infected with virulent FIPV from cats infected with avirulent feline coronaviruses [36], none of the serologic assays routinely used has been specific enough to discern the virus to which a cat had been exposed [9, 26, 27]. The ELISA using CCV-infected cultured cells in the present report does not specifically determine the type of coronavirus exposure in cats, but the results indicate that it is routinely applicable as a very simple and easy serologic test for anti-coronavirus antibody detection in cats.

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REFERENCES

1. Siddell S., Wege H. and ter Meulen V. The biology of coronaviruses. *J. gen. Virol.* **64,** 761–776 (1983).
2. Horzinek M. C. and Osterhaus A. D. M. E. The biology and pathogenesis of feline infectious peritonitis. *Arch. Virol.* **59,** 1–15 (1979).
3. Jacobse-Geels H. E. L., Daha M. R. and Horzinek M. C. Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. *J. Immun.* **125,** 1606–1610 (1980).
4. Pedersen N. C., Black J. W., Boyle J. F., Evermann J. F., McKeirnan A. J. and Ott R. L. Pathogenic differences between various feline coronavirus isolates. *Adv. expl Med. Biol.* **173,** 365–380 (1984).
5. Pedersen N. C. and Boyle J. F. Immunologic phenomena in the effusive form of feline infectious peritonitis. *Am. J. vet. Res.* **41,** 868–876 (1980).
6. Weiss R. C. and Scott F. W. Antibody-mediated enhancement of disease in feline infectious peritonitis: comparison with dengue hemorrhagic fever. *Comp. Immun. Microbiol. infect. Dis.* **4,** 175–189 (1981).
7. Barlough J. E., Adsit J. C. and Scott F. W. The worldwide occurrence of feline infectious peritonitis. *Feline Pract.* **12,** 26–30 (1982).
8. Horzinek M. C. and Osterhaus A. D. M. E. Feline infectious peritonitis: a worldwide serosurvey. *Am. J. vet. Res.* **40,** 1487–1492 (1979).
9. Pedersen N. C., Boyle J. F., Floyd K., Fudge A. and Barker J. An enteric coronavirus infection of cats and its relationship to feline infectious peritonitis. *Am. J. vet. Res.* **42,** 368–377 (1981).
10. Fiscus S. A. and Teramoto Y. A. Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. *J. Virol.* **61,** 2607–2613 (1987).
11. Pedersen N. C., Ward J. and Mengeling W. L. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. *Arch. Virol.* **58,** 45–53 (1978).
12. Horzinek M. C., Lutz H. and Pedersen N. C. Antigenic relationships among homologous structural polypeptides of porcine, feline and canine coronaviruses. *Infect. Immun.* **37,** 1148–1155 (1982).
13. Barlough J. E., Stoddart C. A., Sorresso G. P., Jacobson R. H. and Scott F. W. Experimental inoculation of cats with canine coronavirus and subsequent challenge with feline infectious peritonitis virus. *Lab. Anim. Sci.* **34,** 592–597 (1984).
14. Reynolds D. J. and Garwes D. J. Virus isolation and serum antibody responses after infection of cats with transmissible gastroenteritis virus. *Arch. Virol.* **60,** 161–166 (1979).

15. Woods R. D. and Pedersen N. C. Cross-protective studies between feline infectious peritonitis and porcine transmissible gastroenteritis viruses. *Vet. Microbiol.* **4,** 11–16 (1979).
16. Barlough J. E., Jacobson R. H. and Scott F. W. Macrotiter assay for coronavirus-neutralizing activity in cats using a canine continuous cell line (A-72). *Lab. Anim. Sci.* **33,** 567–570 (1983).
17. Barlough J. E., Jacobson R. H., Downing D. R., Marcella K. L., Lynch T. J. and Scott F. W. Evaluation of a computer-assisted, kinetics-based enzyme-linked immunosorbent assay for detection of coronavirus antibodies in cats. *J. clin. Microbiol.* **17,** 202–217 (1983).
18. Eugster A. K. and Liauw H. Detection of antibodies to feline infectious peritonitis (FIP) virus in cats using transmissible gastroenteritis virus as antigen and an electronmicroscopic search for FIP virus. *Southwest. Vet.* **32,** 109–112 (1979).
19. Osterhaus A. D. M. E., Horzinek M. C. and Reynolds D. J. Seroepidemiology of feline infectious peritonitis virus infections using transmissible gastroenteritis virus as antigen. *Zen. Vet. Med.* **24B,** 835–841 (1977).
20. Toma par B., Duret C., Chappuis G. and Labadi J.-P. Péritonite infectieuse féline: étude des anticorps antivirus de la gastro-entérite transmissible du porc par séroneutralisation et hémagglutination passive. *Rec. Méd. vét.* **155,** 541–548 (1979).
21. Osterhaus A. D. M. E., Kroon A. and Wirahadiredja R. ELISA for the serology of FIP virus. *Vet. Q.* **1,** 59–62 (1979).
22. Reynolds D. J., Garwes D. J. and Gaskell C. J. Detection of transmissible gastroenteritis virus neutralizing antibody in cats. *Arch. Virol.* **55,** 77–86 (1977).
23. Mochizuki M., Sugiura R. and Akuzawa M. Micro neutralization test with canine coronavirus for detection of coronavirus antibodies in dogs and cats. *Jpn. J. vet. Sci.* **49,** 563–565 (1987).
24. Pedersen N. C. and Black J. W. Attempted immunization of cats against feline infectious peritonitis, using live virus or sublethal amounts of virulent virus. *Am. J. vet. Res.* **44,** 229–234 (1983).
25. Pedersen N. C., Boyle J. F. and Floyd K. Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. *Am. J. vet. Res.* **42,** 363–367 (1981).
26. Fiscus S. A., Teramoto Y. A., Mildbrand M. M., Knisley C. V., Winston S. E. and Pedersen N. C. Competitive enzyme immunoassays for the rapid detection of antibodies to feline infectious peritonitis virus polypeptides. *J. clin. Microbiol.* **22,** 395–401 (1985).
27. Ingersoll J. D. and Wylie D. E. Comparison of serologic assays for measurement of antibody response to coronavirus in cats. *Am. J. vet. Res.* **49,** 1472–1479 (1988).
28. Hayashi T., Yanai T., Tsurudome M., Nakayama H., Watabe Y. and Fujiwara K. Serodiagnosis for feline infectious peritonitis by immunofluorescence using infected suckling mouse brain sections. *Jpn. J. vet. Sci.* **43,** 669–676. (1981).
29. Loeffler D. G., Ott R. L., Evermann J. F. and Alexander J. E. The incidence of naturally occurring antibodies against feline infectious peritonitis in selected cat populations. *Feline Pract.* **8,** 43–47 (1978).
30. Johansson J., Fagraeus A. and Biberfeld G. Antibodies to calf serum as a cause of unwanted reaction in immunofluorescence tests. *J. immun. Meth.* **11,** 265–272 (1976).
31. Barlough J. E., Jacobson R. H., Pepper C. E. and Scott F. W. Role of recent vaccination in production of false-positive coronavirus antibody titers in cats. *J. clin. Microbiol.* **19,** 442–445 (1984).
32. McKeirnan A. J., Evermann J. F., Davis E. V. and Ott R. L. Comparative properties of feline coronaviruses *in vitro*. *Can. J. vet. Res.* **51,** 212–216 (1987).
33. Tupper G. T., Evermann J. F., Russell R. G. and Thouless M. E. Antigenic and biological diversity of feline coronaviruses: feline infectious peritonitis and feline enteritis virus. *Arch. Virol.* **96,** 29–38 (1987).
34. Woods R. D. Studies of enteric coronaviruses in a feline cell line. *Vet. Microbiol.* **7,** 427–435 (1982).
35. Reynolds D. J., Garwes D. J. and Lucey S. Differentiation of canine coronavirus and porcine transmissible gastroenteritis virus by neutralization with canine, porcine and feline sera. *Vet. Microbiol.* **5,** 283–290 (1980).
36. Fiscus S. A., Rivoire B. L. and Teramoto Y. A. Epitope-specific antibody responses to virulent and avirulent feline infectious peritonitis virus isolates. *J. clin. Microbiol.* **25,** 1529–1534 (1987).