Feline Infectious Peritonitis Virus Antibody Test Using Enzyme-linked Immunosorbent Assay
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Feline infectious peritonitis (FIP), a coronavirus disease of cats, is a complex disease involving systemic FIP virus (FIPV) infection and the host’s aberrant immune response to the agent [4, 9, 12]. Such a complex pathogenesis results in the concurrent presence of the virus and high-titered antibodies in the infected cats [8]. Therefore, serologic testing for demonstration of specific antibodies has been widely used not only in supporting the clinical diagnosis of FIP, but also in the investigational works on the pathogenesis of this disease. To measure antibody titers, indirect fluorescent antibody (IFA) tests have been employed using various antigens such as liver sections from experimentally infected cats [8], brain sections from infected suckling mice [2], or infected cell culture [10]. On the basis of the antibody testings on both clinical and experimental FIP cases, it is generally accepted that cats with the clinical disease show a positive titer usually 1:400 to 1:1600 or grater [2, 8], and the IFA testings thus have been proven a useful diagnostic aid. In the present study, by employing the same principle as in the IFA, we devised a simple enzyme-linked immunosorbent assay (ELISA) using FIPV-infected cell culture as the antigen.

Feline infectious peritonitis virus Yayoi strain which had been passaged in suckling mouse brains for 15 times (FIPV-Y15) [2] was inoculated to cat fcwf-4 cells [10]. After two blind passages in a growth medium (GM) consisting of minimum essential medium (MEM), 20% Leibovitz-15 (L-15) and 10% fetal calf serum (FCS), a focal cytopathic effect (CPE) characterized by cell rounding, aggregation and necrosis was noticed (Fig. 1). The infected cells were subsequently trypsinized and passaged with a supply of uninfected fcwf-4 cells, and the infected culture with a stable focal CPE was established. A FIPV nucleoprotein (NP) antigen was demonstrated in the cytoplasm of the infected cells in the CPE foci by IFA using a mouse monoclonal antibody to FIPV NP [1]. The freeze-thawed infected cells and the supernatant were combined and assayed on fcwf-4 cells seeded in a 96-well plate for infectivity, which gave a titer of $10^5$ TCID$_{50}$/ml on the basis of CPE development.

The infected cells were seeded in a 96-well culture plate along with approximately the same number of uninfected fcwf-4 cells, and the plate was incubated for 2 days before fixation with methanol. For antibody titration, test serum samples were serially diluted two-fold (1:25 to 1:51200) in a serum diluting buffer (Buffer III; 0.05M Tris, 0.001M EDTA, 0.15M NaCl, 0.1% bovine serum albumin, 0.05% Tween 20, pH 7.4) [7] using a separate 96-well plate and the diluted materials were transferred to the washed test plate with fixed cellular antigen. The plate was incubated at 37°C for 1 hr, washed three times with an ELISA wash (0.15M NaCl, 0.05% Tween 20) [7], and again incubated with a 1:400 dilution (Buffer III) of rabbit anti-cat IgG peroxidase conjugate (Cappel Laboratories, Cooper Biomedical, PA, U.S.A.) for another hour at 37°C. The plate was washed three times with the ELISA wash, and a substrate solution consisting of 50 ml 0.1M Tris, 50 mg diaminobenzidine and 15 μl 30% hydrogen peroxide was added. The wells were examined under an inverted microscope, and the wells containing brown-colored infected cells were scored positive. The reciprocal of the highest dilution giving the positive reaction was regarded as the antibody titer.

The IFA-positive sera on the infected mouse brain were examined in this ELISA system for comparison of the antibody titer. The positive reaction was distinct and easy to recognize. In the low power view, brown foci of infected cells were scattered in the positive well (Fig. 2). High power observation confirmed that the reaction is compatible to that in the IFA where the specific staining is confined to the infected cell cytoplasm (Fig. 3).
Fig. 1. A cytopathic effect observed in FIPV-Y15 infected fcwf-4 cells.

Fig. 2. A focus of FIPV-infected cells positively stained with anti-FIPV cat serum and anti-cat IgG peroxidase conjugate.
Fig. 3. A high power view of the positively stained FIPV-infected cells showing cytoplasmic staining.

Table 1. FIP antibody titer distribution among different category cat groups

<table>
<thead>
<tr>
<th>Antibody titers</th>
<th>Diseased</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FIP</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>1:51200&lt;sup&gt;«a&lt;/sup&gt;</td>
<td>3 (2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>1:25600</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>1:12800</td>
<td>7 (6)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>1:6400</td>
<td>3 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>1:3200</td>
<td>1 (2)</td>
<td>3 (2)</td>
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<tr>
<td>1:1600</td>
<td>1 (0)</td>
<td>2 (1)</td>
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<tr>
<td>1:800</td>
<td>3 (4)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>1:400</td>
<td>1 (1)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>1:200</td>
<td>1 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>1:100–25</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>1:25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 (6)</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

| Total           | 20    | 20    | 20     | 20   | 20  |

a) 51200 or greater.
b) Negative at 1:25.
c) Figures indicate number of cats with corresponding antibody titer in ELISA or IFA (parenthesis).

Table 1 shows antibody titer distribution of various cat serum samples. The ELISA antibody titer was almost identical to the IFA titer with a minimal variation within plus or minus one two-fold dilution. Twenty serum samples from cats with a confirmed diagnosis of FIP all showed
high antibody titers. The ELISA titer was greater than 3200 in most cases with the exception of four showing titers of 400 and 800, and there were three cases with the highest titer of 51200 or greater. All of 20 specific pathogen free (SPF) cat sera obtained from University of California, Davis, on the other hand, were negative at a serum dilution of 1:25. Since the lower dilutions of the SPF serum samples (1:5 or 1:10) sometimes gave nonspecific coloring of the antigen cells without any specific pattern of reaction confined to the CPE foci, 1:25 was chosen as the lowest dilution for the positive reaction.

In order to evaluate the clinical application of this assay system, 20 serum samples from cats in multiple-cat households with a history of losses due to confirmed clinical FIP cases, and another group of 20 samples from cats with chronic diarrhea were tested. In the former group of cats, the antibody titers varied from negative (<25) to 12800, and these titers were considered to be related to FIPV infection since all the cats in this group were living with cats with FIP possibly shedding the virus for some time. Therefore, the positive titers were interpreted as a result of either previous infection or ongoing inapparent infection.

The latter group also showed variations in titer with occasional high titers. Since FIPV has been known to cause enteric infection without causing clinical FIP [3], a low virulence FIPV infection may be responsible for some of them. Alternatively, however, feline enteric coronavirus (FECV) infection [11] is likely to play a part in raising the antibodies. Because of the shared antigen between the two coronaviruses, the ELISA detection of the antibodies can not distinguish the two, just as in the case with the IFA assay.

Another group of 20 healthy cats, usually kept inside the house, from single-cat households, were also tested for the antibodies. Most of these healthy cats were negative for the antibody, with only a few cases showing low titers below 400. Cats obtained from breeding catteries, where both FIPV and FECV infections are most likely to be common, were excluded from this group. Thus, these are the group of cats less likely to be exposed to the coronaviruses and the individual antibody titers were very low as compared to the other groups.

The specificity of the ELISA system was confirmed with a western blotting assay using the same infected cell antigens. The proteins from the FIPV-infected and uninfected fcwf-4 cells were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis, and were electrophoretically transferred to nitrocellulose paper as described previously [6]. Some of the positive and negative sera were incubated with the nitrocellulose paper strips, and the reaction was detected by anti-cat IgG peroxidase conjugate and the diaminobenzidine substrate.

As shown in Fig. 4, all antibody-positive sera tested were reactive against the NP p45, while negative sera failed to react with any of the proteins in the virus-infected cells. In addition, a
majority of sera from cats diagnosed FIP had a reactivity to an infected-cell protein with an approximate molecular weight of 95kd. Since this protein was not detected in the uninfected cells, and it has been suggested that FIPV peplomer protein E2 undergoes a proteolytic change in infected cells [5], this 95kd protein may be a monomeric form of E2. There was no reactivity, either positive or negative sera, to uninfected cell proteins.

From above results, a reasonable accuracy of the antibody detection system has been demonstrated. That is, all clinical FIP cases showed positive titers greater than 400, majority of exposed cats also had positive titers, a group of cats with diarrhea showed occasional high titers, and cats that have never been exposed were all negative. Although the sensitivity of the assay did not significantly differ from that of the well established IFA, a real advantage of this ELISA over IFA is the relative ease in assaying a large number of samples at a time and the simple method of preparation of the antigen plates. By using a 96-well plate, six samples and one positive and one negative controls with serial two-fold dilutions up to 1:51200 can be assayed. If two-fold dilutions from 1:100 to 1:12800 are used for a screening purpose, 10 samples can be run in a single plate, and it is not difficult to handle a number of plates at a time by a single person. By taking advantage of this simple methods, we are currently employing the FIP serology along with feline leukemia virus (FeLV) ELISA as a part of routine blood screening panel, and a study is in progress to relate FIP antibody titers and various clinical conditions.

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REFERENCES