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NOTE

Virology

Anti-feline panleukopenia virus serum neutralizing antibody titer in domestic cats with the negative or low hemagglutination inhibition antibody titer

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ABSTRACT. To evaluate the accuracy of hemagglutination inhibition (HI) test as the index of feline panleukopenia virus (FPV)-protective ability, sera from 153 FPV-vaccinated cats aged ≥7 months with HI titer of <1:10–1:40, were examined for serum neutralizing (SN) antibody. SN antibody was detected (≥1:10) in 33 (62.3%) of 53 HI antibody-negative cats, and ranged <1:10–1:160. This suggests that FPV-antibody detection sensitivity of HI test is lower than SN test, and SN test is more suitable for the assessment of FPV-vaccine effect than HI test especially in cats with negative or low HI titer. SN titer was 1:32, FPV-protective threshold, or higher in all cats with HI titers of ≥1:20, suggesting it may be appropriate to set protective HI threshold at 1:20.

KEY WORDS: cat, feline panleukopenia virus, hemagglutination inhibition test, serum neutralizing test

Feline panleukopenia virus (FPV) is a single-stranded DNA virus classified in *Carnivore protoparvovirus 1* species of the genus *Protoparvovirus*, together with canine parvovirus (CPV) and mink enteritis virus [2], and causes acute lethal infection with symptoms of enteritis and bone marrow suppression in domestic cats and wild felids. This virus invades the body through oral and nasal routes, infects pharyngolaryngeal lymphoid tissue, and disseminates throughout the body via the plasma phase [5]. Therefore, the presence of anti-FPV antibodies in blood is closely involved in prevention of this disease [9]. Vaccination is an important means to prevent the disease, but the vaccine failure may be led due to host factors, such as stress and maternal antibody, vaccine factors, and human errors [3]. As the method to estimate the immune status of FPV infection, measurement of the blood anti-FPV antibody titer is recommended [22].

The specificity of the serum neutralizing (SN) test, the gold standard serological test, is the highest among viral serological reactions, and the measured SN antibody is closely related to the infection-protective ability compared with antibodies detected by other test methods. However, since the SN test is high-cost and time-consuming, the hemaggutination inhibition (HI) test is clinically used frequently as a simple substitute method of the SN test [12, 16, 17], but its anti-FPV antibody detection sensitivity is lower than that of the SN test [8, 21]. Moreover, it has been reported that the correlation between HI and SN antibody titers is not necessarily high in CPV [18], belonging to the same viral species as FPV. Unexpectedly, results of comparisons between the anti-FPV HI and SN antibody titers are insufficient.

In this study, to assess the accuracy of the HI test as the index of FPV-protective antibody titer, the SN antibody titer was measured in cat sera negative for anti-FPV HI antibody or with a low antibody titer to compare the antibody titers by the two tests.

Serum samples were collected from 718 previously FPV-vaccinated and clinically healthy domestic cats aged 7 months or older brought to a veterinary clinic in Tokyo, Japan from January in 2013 to December in 2017 for a health checkup, vaccination, or spay/castration operation.

Anti-FPV HI antibody titer was examined by a slight modification of the previously reported methods [8, 15]. Twenty-five microliter of the heat-inactivated serum sample, which was treated with porcine erythrocytes and kaolin, was subjected to 2-fold serial dilutions started at 1:10 with phosphate buffered saline solution (PBSS) (pH 6.7) in a 96-well V-bottom microplate. Then, equal volume of the Som4 strain of FPV, which was isolated from a diarrheal domestic cat in Japan [6], containing four hemagglutination (HA) units was added into the serum dilution. Following one-hour incubation, 0.5% porcine erythrocytes were

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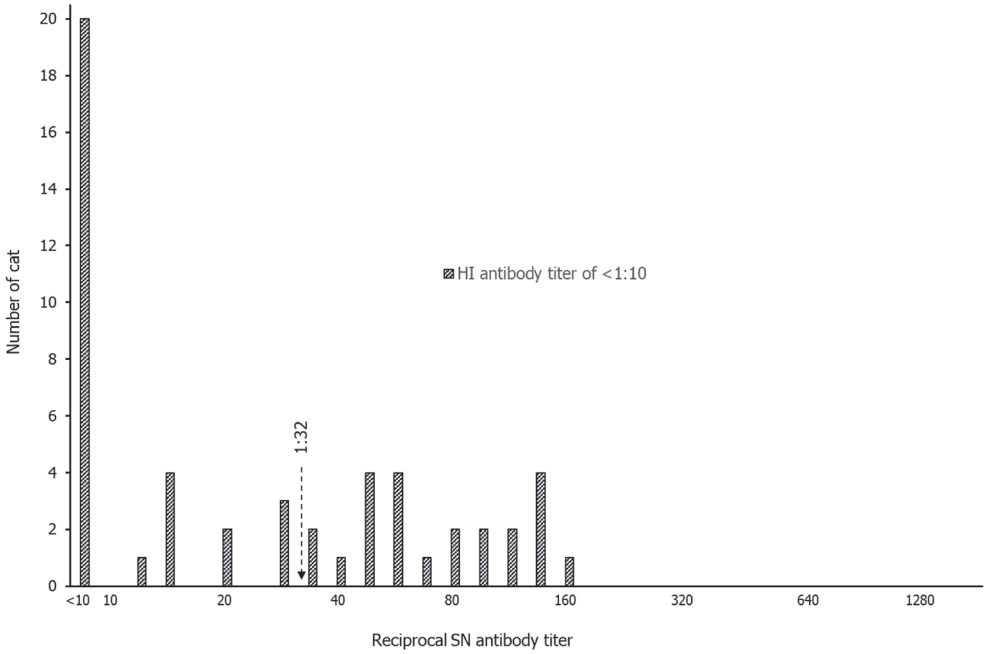

Fig. 1. Anti-FPV SN antibody titer in 53 HI antibody-negative cats (titer of <1:10). An arrow indicates the protective SN antibody titer threshold (1:32).

added to each plate well. After overnight incubation at 4°C, the antibody titer was expressed as the highest serum dilution with complete inhibition of hemagglutination.

Since determination of the endpoint for judgement of the antibody titer on the SN test is difficult because of a weak cytopathic effect of FPV-infected cultured cells, the infection in each well was examined based on the HA reaction [15, 23]. In brief, the antibody titer was measured in a 96-well tissue culture microplate. Quadruplicate 50 μl volumes of serum were diluted 2-fold serially started at 1:10, and mixed with 100 $TCID_{50}$ of the Som4 strain of FPV was added to each well. Following one-hour incubation, Crandell-Rees feline kidney cells were added to the serum-virus mixtures, and incubated overnight at 37°C. Then, the culture fluid was removed, and flesh culture fluid was added into each well. Following five-day incubation, the culture fluid was added into equal volume of 0.5% porcine erythrocytes in a 96-well V-bottom microplate. The antibody titer was calculated as the highest serum dilution at 50% inhibition of hemagglutination.

The antibody titer was analyzed statistically by the use of spreadsheet program (Microsoft Excel 2016).

Anti-FPV HI antibody was not detected (titer of <1:10) in 53 (7.4%) of a total of 718 cats, and the HI antibody titer was 1:10, 1:20, and 1:40 in 15, 28, and 57 cats, respectively. These 153 cats were also examined for anti-FPV SN antibody. As shown in Fig. 1, the SN antibody was detected (≥1:10) in 33 (62.3%) of the 53 HI antibody-negative cats. The SN antibody titer in the HI antibody-negative cats ranged from <1:10 to 1:160, and the geometric mean (GM) was 1:20.4. As shown in Fig. 2, the SN antibody was detected (≥1:10) in all cats with the HI antibody titers of 1:10, 1:20, and 1:40, and the SN antibody titer was distributed within ranges of 1:14–1:190 (GM: 1:70.4), 1:80–1:640 (GM: 1:267.6), and 1:130–1:1,500 (GM: 1:548.8), respectively. In analysis, an antibody titer of <1:10 was arbitrarily regarded as 1:5. The SN antibody titer exceeded 1:32, which is the highest among previously reported protective threshold, in all cats with the HI antibody titers of 1:20 or higher, whereas it was lower than this threshold in 56.6% (30/53) and 13.3% (2/15) of cats with the HI antibody titers of <1:10 and 1:10.

The averages and standard deviations of the logarithmic value of the reciprocal SN antibody titer in the groups with the HI antibody titers of <1:10, 1:10, 1:20, and 1:40 were 1.309 ± 0.548, 1.848 ± 0.296, 2.427 ± 0.237, and 2.739 ± 0.244, respectively, and the coefficients of variation (CV) were 41.9, 16.0, 9.8, and 8.9%, respectively (Fig. 3). The Pearson's correlation coefficient (R) was 0.860, showing a slightly high correlation between the two antibody titers, but the CV value clarified the presence of variation (41.9%) in cats with the HI antibody titer of <1:10. The gender, age, breed, and days from the most recent vaccination of the test cats were also investigated, but no significant difference was noted in any item (T test P>0.05) (Data not shown).

In CPV, the neutralizing epitope and hemagglutinin are located at different positions in the VP2 antigen, clarifying that the HI ability does not necessarily reflect the neutralizing ability [10]. No such finding has been reported for FPV, but it may have similar morphological properties because it is classified as the same viral species as CPV. Although a specific correlation was noted between the anti-FPV SN and HI antibody titers (R=0.860), both were not necessarily consistent, and CV of SN antibody titers was 41.9% in the group with the HI antibody titer of <1:10, showing their variation. Furthermore, the SN antibody was detected in 62.3% of HI antibody-negative cats, clarifying that the HI test is low-sensitive for anti-FPV antibody detection. This finding was consistent with those in previous reports [8, 21], and it may have been due to the virological properties described above.

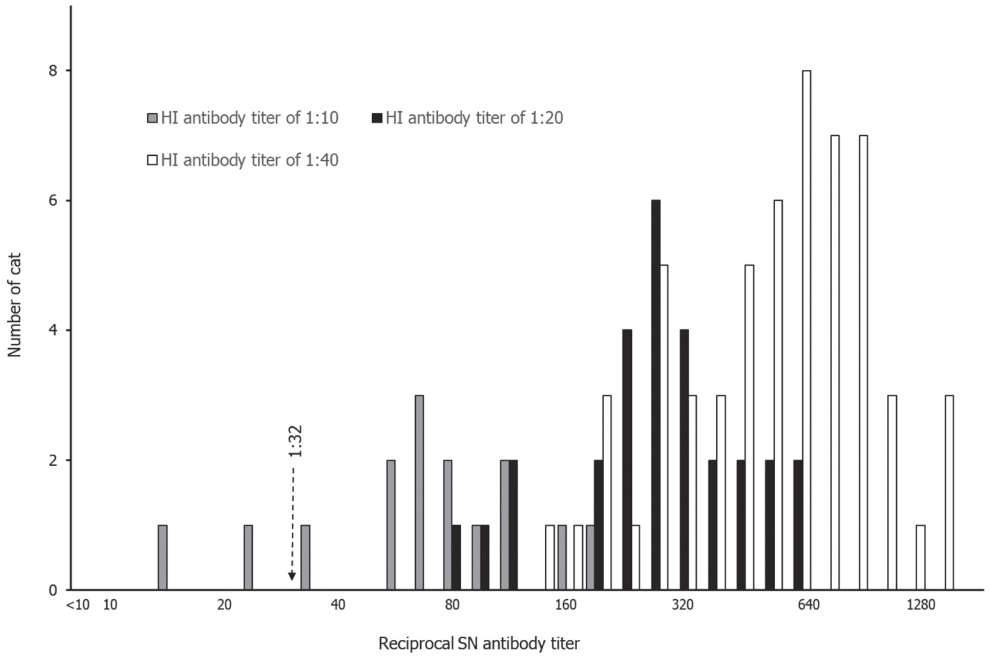

Fig. 2. Anti-FPV SN antibody titer in 15, 28, and 57 cats with the HI antibody titers of 1:10, 1:20 and 1:40, respectively. Gray, black, and white bars indicate the HI antibody titers of 1:10, 1:20 and 1:40, respectively. An arrow indicates the protective SN antibody titer threshold (1:32).

Although few studies have been done to investigate the rate of response to FPV vaccine, recently, Jakel *et al*. [7] and Mende *et al*. [14] reported interesting test findings concerning the vaccine effect, whereby no anti-FPV HI antibody was detected in 36.7% of cats vaccinated three times by 16 weeks old and 23% of cats vaccinated following the vaccination guidelines, based on which the performance of the current vaccine is suspected to be inadequate. In this study, the frequency of the HI antibody-negative cats was much lower (7.4%) than their finding, although simple comparison is not possible because the test was performed in randomly selected cats with previous vaccination. Furthermore, anti-FPV SN antibody was detected at a high rate (62.3%) of the HI antibody-negative cats. These results suggest that the performance of the FPV vaccine may have been underestimated in their findings, and combination with the SN test may be necessary to accurately investigate the immune response. However, the SN test is not clinically used widely and it is not appropriate as the routine laboratory test, since it is not simple. Thus, it may be necessary to typically use the HI test as a simple method, and the SN test may be used for cats without HI antibody response despite being vaccinated several times, e.g. low responder, and HI antibody-negative cats for which vaccination should be avoided due to immunosuppressive or cytotoxic therapy.

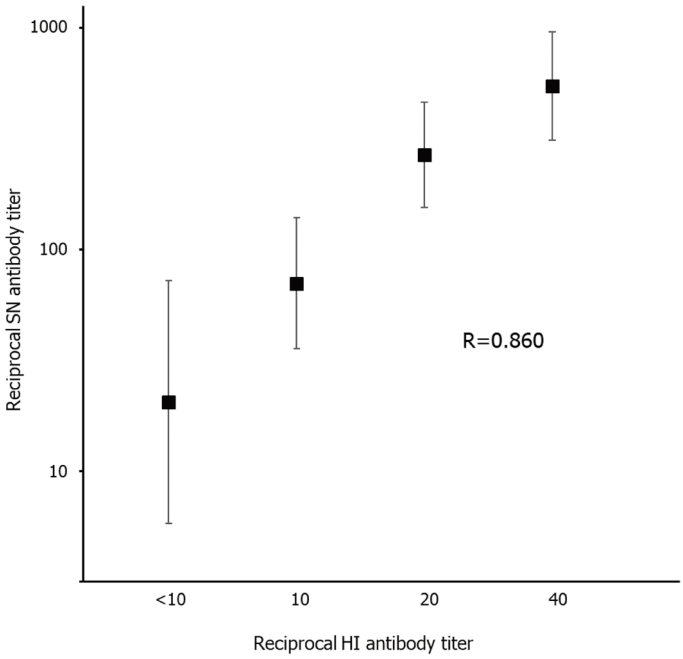

Fig. 3. Correlation between anti-FPV HI and SN antibody titers. Black squares and vertical lines indicate averages and standard variations of the logarithmic value of the reciprocal SN antibody titer, respectively.

From above findings, we concluded that the SN test is more suitable for the assessment of FPV-vaccine effect than the HI test especially in the cats with the negative or low HI antibody titer.

FPV-protective HI antibody titer thresholds of 1:20–1:40 have been recommended [11, 13, 16], but to our knowledge, the basis for setting these values is unclear, whereas the SN antibody titer thresholds for estimating the FPV-protective ability have been set at 1:10–1:32 based on the results of some experimental infections [4, 9, 19, 20]. In this study, the SN antibody titer was 1:32,

which was the highest among the previously reported SN antibody thresholds, or higher in all cats with the HI antibody titer of 1:20 or higher. Therefore, it may be appropriate to set the threshold at 1:20 when FPV-protective ability is diagnosed using the HI antibody titer as an index.

In this study, cats aged 7 months or older were used in consideration of the influence of maternal antibody on the test results. So, all detected anti-FPV antibodies were products by active immunity. It was surprising that antibodies undetectable by the HI test were present at such high rate. The presence of CPV with no HA activity property has been reported [1], although it has not been reported in FPV. Antibodies produced in response to infection with such viruses may be undetectable by the HI test because of the principle of the test. The inclusion of these cases in the present test cats also cannot be ruled out, although inconsistency between the two tests is likely to be simply due to the low detection sensitivity of the HI test. The consideration of this phenomenon may be necessary when the HI test is used.

Since the objective of this study was comparison between the HI and SN tests, performance of FPV vaccine was not evaluated, for which a survey and vaccination test should be performed based on detailed individual data, and a similar study should also be performed in dogs with regard to the accuracy of the HI test of CPV.

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