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NOTE

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Feline coronavirus antibody titer in cerebrospinal fluid from cats with neurological signs

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ABSTRACT. To investigate the utility of cerebrospinal fluid (CSF) anti-feline coronavirus (FCoV) antibody test for diagnosis of feline infectious peritonitis (FIP), the antibody titers were tested in CSF and sera from 271 FIP-suspected neurological cats. CSF antibody was detected in 28 cats, which were divided into 2 groups; 15 with CSF titer of 1:80 or lower and 13 with CSF titer of 1:640 or higher. In the latter group, reciprocal serum titer/reciprocal CSF titer was 8 or lower, which is extremely lower than normal range (256-2048), and FCoV RNA was detected in all of 11 CSF samples assayed by RT-PCR. Our findings indicate that CSF titer of 1:640 or higher may be served as a candidate for the index for diagnosing FIP.

KEY WORDS: antibody, CSF, feline coronavirus, RT-PCR

Feline infectious peritonitis (FIP) is an immune-mediated progressive systemic infectious disease that occurs in domestic cats and wild felids, and is caused by feline coronavirus (FCoV), a single-stranded RNA virus. FCoV is transmitted by the fecal-oral route and usually causes mild to inapparent enteritis, and FIP is considered to be induced by a virulent mutant of the enteric FCoV [16, 22]. It is divided into 2 clinical forms, effusive FIP, in which effusion is observed in body cavities, and non-effusive FIP [1, 2]. Non-effusive FIP lacks specific clinical signs, with lesions presented mainly in the eyes, central nervous system (CNS), and parenchymatous organs. Clinical signs associated with CNS disease in cases of FIP are observed in about 10% of all FIP cases and 25–33% of non-effusive FIP cases [1, 2, 9]. Recently, image diagnosis is often performed especially for neurological instances in veterinary medical field, and CSF sample can be collected at that time. Detection of FCoV-specific antibody in CSF, which is produced by local replication of FCoV in brain tissue, is useful for the ante-mortem diagnosis of neurologic-form FIP [9]. Several studies have reported the anti-FCoV antibody titers in CSF, but the number of clinical cases reported is small, and no diagnostic index of FIP has been proposed [4, 10, 20].

Substances with a molecular weight smaller than 160,000, such as immunoglobulin G antibodies and albumin, enter the brain relatively easily from circulating blood, and their levels reportedly range from 1/256 to 1/2,048 of that in serum in subjects with a normal blood brain barrier (BBB) [5, 11, 21]. Hence, CSF antibody should be simultaneously examined with serum antibody.

In this study, for determination of diagnostic criteria of FIP for CNS infected cases using the FCoV antibody test, we examined anti-FCoV antibody titers in CSF and sera from cats with neurological signs.

CSF and serum samples obtained from 271 clinically FIP-suspected cats with neurological signs under general anesthetic during image diagnosis, which had been submitted to a veterinary clinical laboratory (Marupi Lifetech, Osaka, Japan) from veterinarians between 2006 and 2016 for diagnosis of FIP, were examined for anti-FCoV antibody titers. All samples were stored at −35°C prior to testing. The antibody titers were performed employing an indirect fluorescent antibody method [15, 17]. Briefly, confluent monolayers of Crandell-Rees feline kidney cells were infected with FIP virus strain 79-1146 (American Type Culture Collection, Rockville, MD, U.S.A.) in a 96-well tissue culture plate and allowed to incubate for 20 hr, then the plate was fixed with a methanol-acetone solution, with CSF and serum samples subjected to 2-fold serial dilutions started at 1:10 and 1:80, respectively. Following incubation, the plate was washed and a fluorescein isothiocyanate-conjugated anti-cat IgG goat antibody (Cappel, Cochranville, PA, U.S.A.) was added to each well. After a second incubation period, the plate was washed and examined with a fluorescent microscope, and the antibody titer noted was expressed as the highest sample dilution with a positive reaction. In addition, CSF samples from 24 CSF antibody-positive and 21 CSF antibody negative cats were subjected to FCoV RT-PCR using the P205-P211 primer pair [13, 18] for definitive diagnosis of FIP, as the demonstration of FCoV RNA in CSF is highly suggestive of FIP [1, 8].

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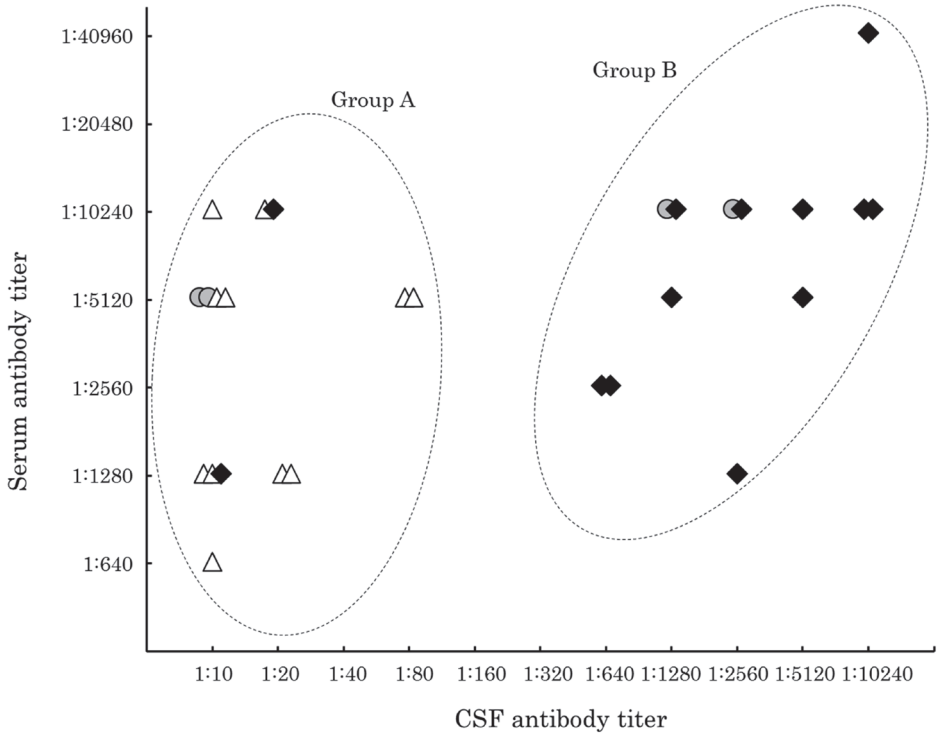

Fig. 1. Comparison between anti-FCoV antibody titers in CSF and serum samples obtained from 28 cats with neurological signs, positive for CSF antibody. White triangles and black squares represent CSF FCoV RNA-negative and positive cases, respectively.

The anti-FCoV antibody was detected in CSF samples from 28 cats (10.3%) (titer of 1:10 or higher). Figure 1 shows a comparison of antibody titers between CSF and serum samples from cats positive for the antibody in CSF. Two clusters of plot markers were observed and those cats were arbitrarily designated as Groups A (n=15) and B (n=13), which had CSF antibody titers ranging from 1:10 to 1:80 and from 1:640 to 1:10,240, respectively. CSF antibody titers of 1:160 and 1:320 were not detected. RT-PCR was performed using CSF samples from 13 Group A and 11 Group B cats, and RNA was detected in two (15.4%) and 11 samples (100%), respectively. In contrast, no RNA was detected in CSF antibody-negative cats. As shown in Fig. 2, the reciprocal serum antibody titer/reciprocal CSF antibody titer (S/C) in Group A cats ranged from 64 to 1,024 and that in Group B cats ranged from 0.5 to 8, showing that the groups could be also easily differentiated with use of that ratio.

Cats with neurological signs and with the anti-FCoV antibody in CSF were divided into 2 groups; those with a CSF antibody titer of 1:80 or lower (Group A) and 1:640 or higher (Group B). CSF FCoV RNA was detected in all of 11 Group B cats in which RT-PCR assay was performed. It is considered that when FCoV RNA is detected in CSF, that animal is very likely to have FIP [1, 8]. Furthermore, the S/C of Group B cats (8 or lower) was markedly lower than normal range (256–2,048) [5, 11, 21]. Based on above findings, we propose that a CSF antibody titer of 1:640 or higher is served as a candidate for the index of FIP diagnosis. Our results would suggest that FIP may be able to be often diagnosed by an antibody test, which allows relatively stable measurement, without genetic examinations such as RT-PCR, in which the contamination of amplicon and ribonuclease to a test sample can lead to any erroneous test result, and expensive equipment and a high level skill are required for performance.

However, method for CSF anti-FCoV antibodies in cats with neurological FIP have a low level of sensitivity [1], and FIP cases with neurological signs with a normal S/C have been reported [14, 20]. In this study, the RNA was detected in two Group A cats, even though the CSF antibody titer was low (1:10 and 1:20). These cases may have been at an early stage of neurologic-form FIP. Furthermore, no cases with CSF antibodies at titers of 1:160 and 1:320 were detected in this study, and we could not verify a relationship between the antibody titers and FIP in these cases. These findings suggest that the index proposed in this study may have a low negative predictive value for diagnosis of FIP. Therefore, it is considered that even if the antibody in the CSF is negative or low, FIP cannot be ruled out.

We were not able to obtain more detailed information, such as other examination results and clinical signs, from the attending veterinarians regarding the cats examined. Additional epidemiological investigations are necessary to more accurately and efficiently diagnose FIP using anti-FCoV antibody testing of CSF samples.

The S/C may also decrease due to a nonspecific collapse of the blood-brain barrier causing excess contamination of CSF with blood, even though the CNS is not infected [12]. In fact, it has been reported that non-FIP cases with CNS involvement showed marked reduced S/C [4, 14, 20]. A comparison of that ratio of antibodies against viruses other than the target virus or albumin may be useful to diagnose various viral infections [3, 5–7, 19]. It may be necessary to investigate the usefulness of addition of such reference tests when diagnosing FIP.

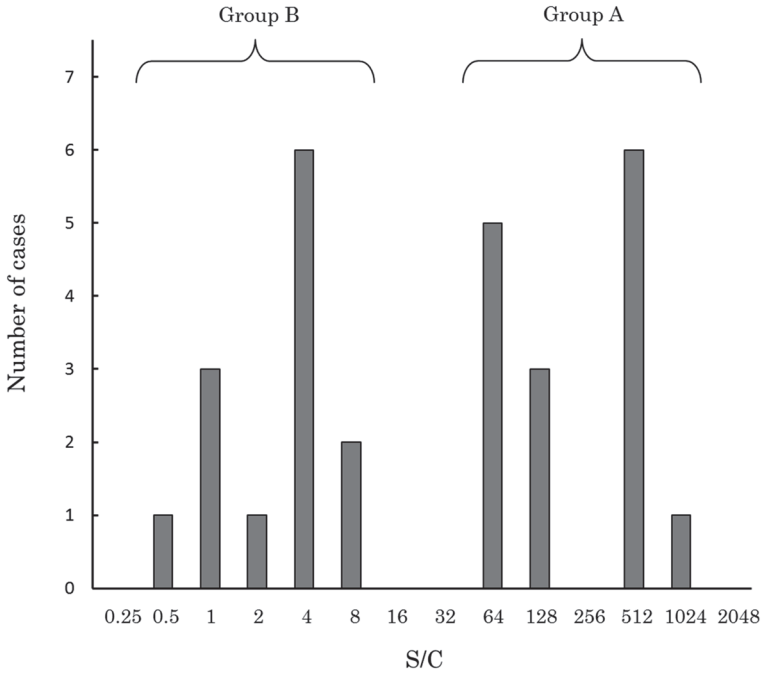

Fig. 2. Reciprocal serum anti-FCoV antibody titer/reciprocal CSF anti-FCoV antibody titer (S/C) in 28 cats with neurological signs, positive for CSF antibody.

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