

Detection of Feline Coronavirus Antibody, Feline Immunodeficiency Virus Antibody, and Feline Leukemia Virus Antigen in Ascites from Cats with Effusive Feline Infectious Peritonitis

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ABSTRACT. To investigate the usefulness of ascites as a material for viral tests in cats with effusive feline infectious peritonitis (FIP), we attempted to detect anti-feline coronavirus antibody, anti-feline immunodeficiency virus antibody, and feline leukemia virus antigen in ascites from 88 cats clinically suspected with effusive FIP. In each of these three viral tests, all cats positive for serum antibody/antigen were also positive for ascitic antibody/antigen, while cats negative for serum antibody/antigen were also negative for ascitic antibody/antigen. This finding indicates that ascites is useful for these viral tests.

KEY WORDS: ascites, FIP, viral test.

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Feline infectious peritonitis (FIP) is a progressive viral infectious disease caused by feline coronavirus (FCoV) infection in domestic and wild large cats [1]. As a diagnostic method of FIP, anti-FCoV antibody test has been reported in 1976 [8], and this test is now used as an important diagnostic method of FIP. Superinfection of FCoV-infected cats with feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV) has been reported as a factor inducing FIP [3, 9], and tests of these two viruses in addition to anti-FCoV antibody test are important for the diagnosis of FIP. FIP is classified into the effusive and non-effusive forms. The former is characterized by accumulation of ascites and thoracic effusion and the latter is characterized by multiple pyogranulomatous lesions. Generally, symptoms of the former acutely progress compared to the latter [1], and a sufficient amount of serum cannot be collected due to emaciation in many cases of the effusive FIP. In cases of the effusive FIP, immune complexes injure the blood vessel wall and cause leakage of plasma proteins and body fluid from the blood vessel into the body cavities [5]. Accordingly, certain amounts of serum viral antigens and antibodies may transfer to exudates. However, there has been no report of comparison of viral tests in serum and exudates.

In this study, to investigate the usefulness of peritoneal exudates (ascites) as a material for viral tests in cats with effusive FIP, we attempted to detect anti-FCoV antibody, anti-FIV antibody, and FeLV antigen in ascites from cats clinically suspected with effusive FIP, and compared with the serum levels.

As test materials, blood and ascites were collected from 88 pet cats clinically suspected of having FIP due to accumulation of ascites, and the supernatants after centrifugation at 3,000 rpm for 15 min were tested. Anti-FCoV antibody test was performed by indirect fluorescent antibody (IFA) assay as reported by Pedersen *et al.* [7]. Confluent mono-

layers of Crandell feline kidney (CRFK) cells (American Type Culture Collection (ATCC, U.S.A.) were infected with FIP virus 79-1146 strain (ATCC, U.S.A.) in a 96-well tissue culture plate. After 20-hr incubation, the plate was fixed with methanol-acetone solution (1:3). The sample that was diluted 1:100 and then serially 2-fold was added into the plate. After incubation, the plate was washed and fluorescein isothiocyanate (FITC)-conjugated anti-cat IgG goat antibody (Cappel, U.S.A.) was added into each well. After incubation, the plate was washed and examined with a fluorescent microscope. The antibody titer was expressed as the highest sample dilution with the positive reaction. Anti-FIV antibody test was performed by IFA assay as reported by Barr *et al.* [2]. FIV-chronically infected CRFK cells (ATCC, U.S.A.) were grown in a 96-well tissue culture plate. The plate was fixed with the methanol-acetone solution. The sample that was diluted 1:50 and then serially 2-fold was added into the plate. After incubation, the plate was washed and the FITC-conjugated anti-cat IgG goat antibody was added into each well. After incubation, the plate was washed and examined with the fluorescent microscope. The antibody titer was expressed as the highest sample dilution with the positive reaction. FeLV antigen test was performed by enzyme-linked immunosorbent assay (ELISA) as reported by Lutz *et al.* [6]. The sample diluted 1:8 was added into the 96-well ELISA plate coated with anti-p27 mouse monoclonal antibody (ATCC, U.S.A.). After incubation, the plate was washed and horseradish peroxidase-conjugated anti-p27 goat antibody (ViroStat, U.S.A.) was added into each well. After incubation, the plate was washed and the substrate buffer (0.05 M citric acid containing 0.2 mM 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] and 0.004 % H₂O₂, pH 4.0) was added into each well. After incubation, optical density (OD) value was determined at 405 nm.

When an anti-FCoV antibody titer of 1:100 or more was

regarded as positive, all 62 cats positive for serum antibody were also positive for ascitic antibody, while 26 cats negative for serum antibody were also negative for ascitic antibody (Table 1). Next, the serum and ascitic antibody titers were compared in the positive cats. The ascitic antibody titer was the same to the serum antibody titer in 48 cats. The ascitic antibody titers were 1/2 and 1/4 of the serum antibody titer in 12 and two cats, respectively (Table 2). When an anti-FIV antibody titer of 1:50 or more was regarded as positive, all 10 cats positive for serum antibody were also positive for ascitic antibody, while 78 cats negative for serum antibody were also negative for ascitic antibody (Table 1). The ascitic antibody titer was the same to the serum antibody titer in 7 cats. The ascitic antibody titers were 1/2 and 1/4 of the serum antibody titer in two and one cats, respectively (Table 2). When an FeLV antigen titer (OD value) of 0.100 or more was regarded as positive, all 16 cats positive for serum antigen were also positive for ascitic antigen, while 72 cats negative for serum antigen were also negative for ascitic antigen (Table 1). The mean and standard deviation of the ascitic OD value/serum OD value ratio were 1.45 ± 0.49 in the positive cats.

As described above, all cats positive for serum antibody/antigen were also positive for ascitic antibody/antigen, while cats negative for serum antibody/antigen were also negative for ascitic antibody/antigen in each of these three viral tests. This finding indicates that ascites is useful for the viral tests, and the viral tests using ascites can be performed in cats in which blood sampling is difficult. It has been reported that the IgG antibody level is slightly lower (about 70%) in exudates than in serum in humans [10]. The anti-FCoV and anti-FIV antibody titers were lower (1/2–1/4) in the ascites than in the serum in 22.6% and 30.0% of the antibody-positive cats, respectively, in the antibody tests that are IgG antibody measurement systems, in this study. In contrast, the FeLV antigen titer in the ascites was 1.45-fold higher on average than that in the serum in the antigen-positive cats. It has been reported that the ratio of exudates/serum proteins was inversely related to the molecular weight in humans [11]. The FeLV antigen test performed in this study detects p27 viral core antigen with a molecular weight of 27 k Dalton [6]. The leakage rate of this antigen may have been higher than that of IgG antibody with a molecular weight of 150 k Dalton [4]. It will be necessary to conduct further studies using a larger sample for a reliable comparison of the serum and ascitic titers in cats with FIP.

Exudates are generally more viscous than serum in FIP [12]. In this study, some ascites samples were very viscous, but the test systems of this study could readily measure the titers. In Japan, simple viral diagnostic kits have been commonly used as rapid tests in animal hospitals in recent years. In some kits, the test material is dripped on the kit without dilution, and accurate results might not be obtained from

Table 1. Comparison of viral test results in the ascites and serum from 88 cats suspected of having FIP

		Serum	
		+	-
FCoV antibody	+	62	0
	-	0	26
Ascites FIV antibody	+	10	0
	-	0	78
FeLV antigen	+	16	0
	-	0	72

+, Positive, -, Negative.

An anti-FCoV antibody titer of 1:100 or more, an anti-FIV antibody titer of 1:50 or more, and an FeLV antigen titer of 0.100 or more were regarded as positive.

Table 2. Ratio of antibody titers in the ascites and serum from the antibody-positive cats

Ascites/Serum ^{a)}	FCoV antibody	FIV antibody
1	48 (77.4%)	7 (70.0%)
1/2	12 (19.4%)	2 (20.0%)
1/4	2 (3.2%)	1 (10.0%)
Total	62 (100%)	10 (100%)

a) Ascites/Serum; Reciprocal of antibody titer in the ascites/reciprocal of antibody titer in the serum.

viscous materials. To increase the usefulness of exudates, it is necessary to investigate applicability of exudates to such diagnostic kits.

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