

# Use of anti-coronavirus antibody testing of cerebrospinal fluid for diagnosis of feline infectious peritonitis involving the central nervous system in cats

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**Objective**—To assess the use of measuring anti-coronavirus IgG in CSF for the diagnosis of feline infectious peritonitis (FIP) involving the CNS in cats.

**Design**—Prospective study.

**Sample Population**—CSF and serum samples from 67 cats.

**Procedures**—CSF and serum samples were allocated into 4 groups: cats with FIP involving the CNS (n = 10), cats with FIP not involving the CNS (13), cats with CNS disorders caused by diseases other than FIP (29), and cats with diseases other than FIP and not involving the CNS (15). Cerebrospinal fluid was evaluated for concentrations of erythrocytes, leukocytes, and total protein. Anti-coronavirus IgG was measured in CSF and serum by indirect immunofluorescence assay.

**Results**—CSF IgG (range of titers, 1:32 to 1:4,096) was detected in 12 cats, including 6 cats with neurologic manifestation of FIP, 4 cats with FIP not involving the CNS, and 2 cats with brain tumors. Cerebrospinal fluid IgG was detected only in cats with correspondingly high serum IgG titers (range, 1:4,096 to 1:16,384) and was positively correlated with serum IgG titers ( $r = 0.652$ ;  $P < 0.01$ ), but not with any other CSF parameter. Blood contamination of CSF resulted in  $\leq 333$  erythrocytes/ $\mu\text{L}$  in cats with CSF IgG.

**Conclusions and Clinical Relevance**—The correlation between serum and CSF IgG and the fact that CSF IgG was detected only in strongly seropositive cats suggested that CSF anti-coronavirus IgG was derived from blood. Measurement of anti-coronavirus IgG in CSF was of equivocal clinical use. (*J Am Vet Med Assoc* 2007;230:199–205)

Feline infectious peritonitis is a common cause of neurologic disease in cats.<sup>1-3</sup> Manifestation of FIP in the CNS is characterized by a pyogranulomatous meningoencephalitis and meningomyelitis.<sup>4-6</sup> A tentative diagnosis of neurologic FIP is often made on the basis of results of a combination of hematologic and serum biochemical findings, CSF analysis, and diagnostic imaging. Definite diagnosis requires detection of intracellular antigen in macrophages in samples obtained from effusions or histologic examination of organ biopsy specimens revealing characteristic perivascular pyogranulomatous inflammatory reactions or immune-mediated vasculitis<sup>7-9</sup>; however, biopsy

## ABBREVIATIONS

FIP	Feline infectious peritonitis
PPV	Positive predictive value
NPV	Negative predictive value

specimens may not be available in cats with disease restricted to the CNS.<sup>10</sup>

In 1 study,<sup>11</sup> it had been stated that the measurement of anti-coronavirus antibody titers in the CSF could be used to confirm the CNS manifestation of FIP, and this premise has been subsequently cited by others.<sup>12-17</sup> However, this finding was unexpected because the mere presence of anti-coronavirus antibody titers in serum had been found to be of little diagnostic relevance for FIP.<sup>9,18</sup> In addition, the former study used a small number of control cats and most cats with FIP not involving the CNS had been experimentally infected.

The purpose of the study reported here was, therefore, to provide additional data to assess the diagnostic use of measuring anti-coronavirus IgG in CSF for the diagnosis of FIP involving the CNS in cats. Special emphasis was placed on a large number of control cats, including cats naturally infected with FIP not involving the CNS, cats with CNS disorders caused by diseases other than FIP, and cats with diseases other than FIP and not involving the CNS.

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## Materials and Methods

**Sample collection**—Blood for serum and CSF samples were obtained nonselectively from cats that died or were euthanized at the Clinic of Small Animal Medicine, Ludwig-Maximilians-University of Munich, from 2001 to 2003. Only cats in which an extensive clinical workup and necropsy had been performed were included in the study. Gross and histologic examinations, including examinations of the CNS, were performed at the Department of Pathology and Neuropathology, Ludwig-Maximilians-University of Munich.

Samples from 67 cats were included in the study. Samples were allocated into the following 4 groups: samples from cats with FIP involving the CNS (group 1;  $n = 10$ ), samples from cats with FIP not involving the CNS (group 2; 13), samples from cats with CNS disorders caused by diseases other than FIP (group 3; 29), and samples from cats with diseases other than FIP and not involving the CNS (group 4; 15).

At necropsy, brain, spinal cord, and samples from all visceral organs were immediately immersed in neutral-buffered 10% formalin. Sections were processed routinely in an automatic tissue processor, embedded in paraffin, sectioned at 5 to 8  $\mu\text{m}$ , and stained with hemalaun-eosin. According to histopathologic appearance, certain samples underwent special neuropathologic staining and immunohistochemical investigation. All sections were placed on 0.1% polylysine-coated slides and were treated with a mounting medium.

Postmortem diagnoses of cats in group 1 were based on characteristic histopathologic features such as pyogranulomatous cell infiltration of leptomeninges, choroid plexuses, ependyma and superficial brain parenchyma, and Arthus-like inflammation of small cerebral blood vessels.<sup>4</sup> In cats in group 2, the CNS appeared histopathologically inconspicuous, but pyogranulomatous infiltrates with or without effusion were detected outside the CNS. Postmortem diagnoses of cats in groups 3 and 4 are summarized (Appendices 1 and 2, respectively).

Blood for serum was collected via venous puncture at the time of CSF collection and stored at  $-20^{\circ}\text{C}$  for 2 weeks, followed by storage at  $-70^{\circ}\text{C}$ . Cerebrospinal fluid was collected aseptically from the cerebellomedullary cistern in all but 1 cat in which a lumbar puncture was performed. Samples were processed within 30 minutes. Erythrocytes and leukocytes in CSF were counted by use of a Fuchs-Rosenthal chamber. Cerebrospinal fluid was centrifuged, and the supernatant was subsequently stored as described for serum. The total protein concentration in CSF was measured by nephelometry with trichloroacetic acid. As many as 3 leukocytes/ $\mu\text{L}$  and 0.3 g of protein/L in the CSF were considered normal. Similar reference limits have been reported in the literature.<sup>19-21</sup>

Anti-coronavirus IgG was measured in CSF and serum by use of an indirect immunofluorescence assay.<sup>a</sup> Feline coronavirus American Type Culture Collection VR-990 FIP virus strain WSU-1 146 was used as antigen and was grown in Crandell-Rees feline kidney cells. An antibody-conjugate consisting of fluorescein-labeled anti-cat IgG from goats<sup>b</sup> was used for detection of anti-coronavirus IgG by use of a fluorescence microscope.

Dilutions of samples from cats started at 1:32 in CSF and 1:128 in serum and ended at 1:16,384. The highest dilution in which fluorescence was detectable was considered as the final titer.

**Data analysis**—Age, breed, and sex; concentrations of erythrocytes, leukocytes, and total protein in CSF; and anti-coronavirus IgG titers in serum and CSF were analyzed for each group. Data were reported as median and range. Differences between groups were assessed by use of 1-way ANOVA and the Scheffé test for age and CSF parameters and by the Kruskal-Wallis test and Mann-Whitney  $U$  test for anti-coronavirus IgG antibody titers in CSF and serum. Correlations were calculated by use of the Spearman correlation coefficient. Sensitivity, specificity, PPV, and NPV described the diagnostic use of anti-coronavirus IgG in CSF. Values of  $P < 0.05$  were considered significant.

## Results

Cats with FIP (groups 1 and 2) were significantly ( $P < 0.01$ ) younger than cats with other diseases (groups 3 and 4). The median age of cats in groups 1 and 2 was 1 year (range, 0.3 to 5 years) and 2 years (range, 0.3 to 12 years), respectively, whereas cats in groups 3 and 4 had median ages of 14 years (range, 0.2 to 20 years) and 11 years (range, 0.6 to 19 years), respectively. The age of 2 cats was not known.

There were more male than female cats in groups 1, 2, and 4 (7/10, 9/13, and 9/15 cats, respectively). Fifty-four percent of cats in group 3 were females. Sex of 1 cat was not recorded.

Fifty-seven cats (86%) were domestic shorthair cats. Breeds of remaining cats included Russian Blue, Persian, British Shorthair, Angora cross, Devon Rex, Siamese, and Maine Coon. The proportion of pedigreed cats was highest in group 1 (3/10 cats) and lowest in group 2 (1/13 cats). Three of 28 cats in group 3 and 2 of 15 cats in group 4 were pedigreed cats. Breed of 1 cat in group 3 was not known.

Leukocytes were counted in CSF of 59 cats (Table 1). The highest leukocyte numbers in CSF were detected in cats with neurologic manifestations of FIP (group 1; 1 to 295 leukocytes/ $\mu\text{L}$ ; median, 28 leukocytes/ $\mu\text{L}$ ). The difference between this group and all other groups was highly significant ( $P < 0.01$ ). The leukocyte count in CSF of 2 cats in group 1 was not remarkable.

Erythrocytes were counted in CSF samples from 58 cats (Table 1). No significant differences were detected between groups. Most samples (38/58 [65%]) con-

Table 1—Median (range) number of erythrocytes and leukocytes and total protein concentration in CSF samples obtained from cats with FIP with (group 1;  $n = 10$ ) and without (group 2; 13) involvement of the CNS, with CNS disorders caused by diseases other than FIP (group 3; 29), and with diseases other than FIP and not involving the CNS (group 4; 15).

Group	Erythrocytes (RBCs/ $\mu\text{L}$ )	Leukocytes (WBCs/ $\mu\text{L}$ )	Protein (g/L)
1	12 (1–330)	28 (1–295)	0.26 (0.06–10.9)
2	5 (0–84)	0 (0–4)	0.08 (0.06–0.21)
3	35 (0–4,373)	4 (0–182)	0.24 (0.06–3.45)
4	3 (0–471)	1 (0–2)	0.10 (0.06–0.22)

tained  $< 33$  erythrocytes/ $\mu\text{L}$ . Erythrocyte numbers in CSF were positively correlated with leukocyte numbers ( $r = 0.625$ ;  $P < 0.01$ ) and total protein concentration ( $r = 0.447$ ;  $P < 0.01$ ) in CSF. No significant correlation was detected between the number of erythrocytes in CSF and anti-coronavirus IgG titers in CSF. None of the samples with a detectable anti-coronavirus IgG titer in CSF contained  $> 333$  erythrocytes/ $\mu\text{L}$ .

The total protein concentration in CSF was measured in 60 samples and was high only in cats with CNS disease (groups 1 and 3; Table 1). In group 1, only 4 of 10 cats had a protein concentration greater than the reference range (0.3 g/L). Statistically, there was no difference between groups.

All cats with neurologic manifestations of FIP (group 1) also had typical extracerebral lesions. Effusions were detected in 4 of 10 cats, whereas the non-effusive form of FIP was detected in the remaining cats (6/10). In each cat in group 1, microscopic inflammatory changes of the CNS appeared in more than 1 location or compartment or both. In particular, meningitis was detected in 9 cats, additional inflammation of the choroid plexuses was detected in 5 cats, additional ventriculitis was detected in 2 cats, and infiltration progressed to the neuroparenchyma in 5 cats. Hydrocephalus of the lateral ventricles and the fourth ventricle was identified in 1 cat. In those cats, infiltrations mainly resembled type B lesions, consisting of demarcated or confluent granulomas with or without a necrotic center.<sup>22</sup> These lesions were associated with remarkable inflammation of small veins and venules and had a pyogranulomatous character. Reactive microglia and astrocytes were commonly detected in neuroparenchymal lesions.

Three of 10 cats with histopathologic lesions in the brain (group 1) had no abnormalities on neurologic examination. In those cats, inflammatory lesions in the CNS were graded as mild. In one of those cats, the pathologic process was restricted to the temporal lobe of 1 side. In the remaining 7 cats in group 1, results of neurologic examination localized the lesion to the brainstem ( $n = 4$ ) because of gait dysfunction, altered proprioception, apathy, central vestibular signs, and cranial nerve deficits or to the forebrain (1) because of generalized seizures. Results of neurologic examination indicated multifocal intracranial disease in 2 cats.

In group 2, 11 of 13 cats had gelatinous serosanguinous exudate within pleural or peritoneal cavities. Histopathologic lesions of serosal surfaces in those cats were consistent with type A lesions consisting of diffuse serosal inflammation with fibrinous exudation, necrosis, perivasculitis, and vasculitis.<sup>22</sup> Two other cats had the non-effusive form of FIP with type B-like granuloma formation indicative of delayed-type hypersensitivity.<sup>22</sup> Histopathologic examination confirmed or completed the clinical diagnosis of cats in groups 3 and 4 (Appendices 1 and 2).

Anti-coronavirus IgG titers were measured in serum of 63 cats and in the CSF of 67 cats. Thirty-five (56%) cats had measurable serum anti-coronavirus IgG titers. Serum IgG titers ranged from 1:128 to 1:16,384. Anti-coronavirus IgG was detected in CSF samples from 12 (18%) cats. Anti-coronavirus IgG titers in CSF samples ranged from 1:32 to 1:4,096.

Six of the 12 cats with detectable anti-coronavirus IgG in CSF were in group 1 (6/10 cats), 4 cats were in group 2 (4/13), and 2 cats were in group 3 (2/29). In the latter group, brain tumors (meningioma and astrocytoma) were diagnosed. None of the cats in group 4 had measurable anti-coronavirus IgG in CSF. Anti-coronavirus IgG titers in CSF samples from cats in group 1 were significantly ( $P < 0.05$ ) higher than those in group 3, but there was no significant difference in anti-coronavirus IgG titers between groups 1 and 2.

Six of the 35 seropositive cats were in group 1 (6/9 cats tested), 12 cats were in group 2 (12/12), 11 cats were in group 3 (11/28), and 6 cats were in group 4 (6/14). Serum IgG titers were significantly higher in samples from cats in group 1 ( $P < 0.05$ ) and group 2 ( $P < 0.01$ ), compared with samples from cats in groups 3 and 4.

Anti-coronavirus IgG was detected only in CSF samples from seropositive cats. In addition, antibodies were detected only in CSF from cats with high serum IgG titers ranging from 1:4,096 to 1:16,384 (Figure 1). Anti-coronavirus IgG titers in CSF were positively correlated with serum IgG titers ( $r = 0.652$ ;  $P = 0.000$ ), but not with any other CSF parameter.

For all 67 cats, detection of anti-coronavirus IgG in CSF had a sensitivity of 60% and specificity of 90% for diagnosis of the CNS manifestation of FIP. The PPV and NPV were 50% and 93%, respectively. Because in a clinical setting one would perform CSF collection and analysis only in cats with neurologic disease, calculation was repeated for groups 1 and 3. Considering only the 39 cats with CNS alterations (groups 1 and 3), detection of anti-coronavirus IgG in CSF had a sensitivity of 60% and specificity of 93% for the diagnosis of FIP within the CNS. The PPV increased to 75%, and the NPV was 87%.

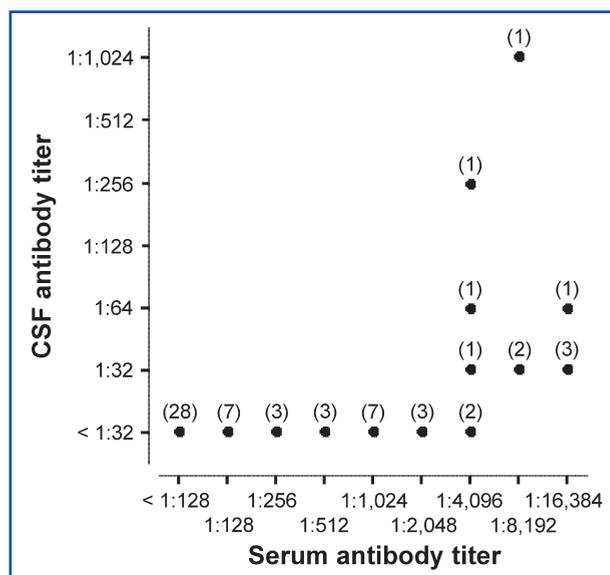


Figure 1—Corresponding pairs of anti-coronavirus antibody titers in CSF and serum from 63 cats obtained for assessment of the diagnostic use of anti-coronavirus antibody testing for detection of FIP. Number in parentheses is the number of cats with the same CSF-serum pair.

## Discussion

The purpose of the study reported here was to provide additional data to assess the diagnostic use of anti-coronavirus IgG titers in CSF for the diagnosis of FIP involving the CNS in cats. Anti-coronavirus IgG titers in CSF were measured simultaneously with serum IgG titers in 10 cats with neurologic manifestation of FIP and 57 control cats, including cats with FIP not involving the CNS, other diseases involving the CNS, and diseases other than FIP and not involving the CNS. Anti-coronavirus IgG titers were detected in CSF samples from 12 cats. Results of our study, which involved a comprehensive control group, were different from those in another study<sup>11</sup> and make recommendations to measure anti-coronavirus antibodies in CSF questionable.

In our investigation, 50% of the measurable anti-coronavirus IgG in CSF was detected in control cats. Anti-coronavirus IgG was detected in CSF samples from 6 of 10 cats with FIP involving the CNS, 4 of 13 cats with FIP not involving the CNS, 2 of 29 cats with CNS diseases other than FIP, and no cats with diseases other than FIP and not involving the CNS ( $n = 15$ ). No FIP-associated CNS lesions were detected via histologic examination in any control cats.

In general, anti-coronavirus IgG was detected only in CSF of cats with high serum anti-coronavirus IgG titers, and CSF titers were always lower than the corresponding serum antibody titers. Antibody titers in serum and CSF differed by a magnitude of 3 to 9 dilutions in a given cat. Cats with anti-coronavirus IgG in CSF had serum antibody titers  $\geq 1:4,096$ . A significant ( $r = 0.652$ ;  $P = 0.000$ ) correlation between serum and CSF antibody titers was detected. These findings suggest that anti-coronavirus IgG in CSF is not specifically synthesized in the CNS, but that most of the antibodies in CSF are derived from blood. These results are not in agreement with results of 1 study<sup>11</sup> and extensively cited<sup>12-17</sup> data. In the former study,<sup>11</sup> anti-coronavirus antibodies in CSF were detected in 15 of 16 cats with FIP-associated CNS disease and not in any control cats. Additionally, in that study, anti-coronavirus antibodies were detected in CSF of cats with low serum anti-coronavirus antibody titers and there was no correlation between serum and CSF antibody titers. In 2 cats, the anti-coronavirus antibody titer in CSF was equivalent to the serum antibody titer. These findings are indicative of intrathecal production of anti-coronavirus antibodies in cats in which the blood-brain barrier is intact.

The divergent results between our study and the study by Foley et al<sup>11</sup> cannot be explained easily and may be attributable to a more diverse and larger control group, as used in our study. The disease status of cats with FIP may have been different in both studies. Factors that influence course and severity of disease include dosage and virulence of the virus and the immune response and physical characteristics of infected cats.<sup>4,23</sup> Another explanation for the discrepancy between the 2 studies may be that the low number of cats in each group (affected vs control groups) is not representative for a specific disease in both studies. In addition, in the study by Foley et al,<sup>11</sup> control cats had considerably lower serum antibody titers than cats in our study, making comparison difficult. In that study, 5 of the 8 control cats with

FIP not involving the CNS had been experimentally infected and had a peracute course with low serum titers ( $\leq 1:400$ ) and no measurable CSF antibody titers. The beginning dilution and titration end point of CSF and serum antibody titers were not similar in both studies. One important limitation of the previous study<sup>11</sup> suggesting that CSF antibody titers are diagnostically useful for detection of CNS manifestation of FIP in cats is the lack of information on whether the CSF had been assessed for contamination with seropositive blood.

Results of the study reported here suggest that anti-coronavirus IgG in CSF is derived from seropositive blood. Antibodies in CSF can result from seropositive blood contamination during sample collection or leakage into the CSF from an impaired blood-brain barrier or altered CSF flow rate. Results of 1 study<sup>24</sup> indicate that contamination of 1 mL of CSF with 0.001  $\mu\text{L}$  of strongly seropositive blood can result in detectable antibodies in the CSF. In the study reported here, blood contamination resulted in  $\leq 333$  erythrocytes/L in cats that had measurable anti-coronavirus IgG titers in CSF, and  $< 33$  erythrocytes/L were detected in 50% of all samples. We consider blood contamination as an unlikely cause for the appearance of CSF antibodies in our study because serum IgG titers (1:256, 1:512, and 1:1,024) of the only 3 cats with 467 to 4,373 erythrocytes/ $\mu\text{L}$  of CSF did not result in detectable antibody titers in the CSF. In addition, there was no significant correlation between erythrocyte numbers and anti-coronavirus antibody titers in CSF.

In our study, an impaired blood-brain barrier or blood-CSF barrier could have resulted in detection of anti-coronavirus IgG in CSF. The blood-brain barrier in cats with FIP involving the CNS has not been specifically evaluated, but an impaired status is presumed.<sup>25</sup> Generally, in infection and inflammation of the CNS, cytokines attract leukocytes and adhesion molecules facilitate leukocyte migration into tissue. Release of metalloproteinases, nitric oxide, and other mediators leads to alterations of the tight junctions, basal membrane, and cerebrovascular endothelium.<sup>25-27</sup> In addition to morphologic barrier dysfunction, a reduced CSF flow rate is sufficient to explain increased protein concentrations in CSF without any structural alteration. The influx of proteins from blood follows the laws of diffusion. A reduced CSF flow rate results in higher molecular flux into the CSF, compared with normal CSF flow.<sup>28-30</sup>

Vasculitis is one of the prominent findings in cats with FIP.<sup>12,31</sup> It is caused by initial infiltration of monocytes<sup>32</sup> and subsequent deposition of immune complexes and fixation of complement, resulting in a pyogranulomatous inflammatory reaction.<sup>32-34</sup> In addition to these morphologic indicators of blood-brain barrier disruption, changes in CSF pressure and flow characteristics caused by inflammatory products are suspected in cats with FIP.<sup>5</sup>

Considering the pathogenesis of FIP, we postulate that an impaired blood-brain barrier or CSF flow rate influenced our results. The correlation between serum and CSF antibody titers and the fact that serum antibody titers were always higher than CSF antibody titers emphasizes a blood-brain or blood-CSF barrier dysfunction.

Alternatively, increased CSF antibody titers may result from local production of antibodies. Results of the study by Foley et al<sup>11</sup> indicate that anti-coronavirus antibodies

were produced intrathecally. Locally produced antibodies originate from B-lymphocytes that migrate into the CNS during an inflammatory reaction.<sup>35,36</sup> In cats with FIP, migration of B-lymphocytes into the CNS has not yet been determined, but can be assumed because coronavirus antigen<sup>11,33,37</sup> and plasma cells with coronavirus-specific antibodies<sup>33</sup> have been detected histopathologically in brains of cats with FIP. Additionally, sensitized lymphocytes may also migrate into the CNS and cause a specific immune response without the presence of the infectious agent.<sup>38</sup> The appearance of antibodies in the CSF may be part of any systemic immune response. Antibodies in CSF have been detected after parenteral application of ovalbumin,<sup>39</sup> in clinically healthy foals born to mares that were seropositive for *Sarcocystis neurona*,<sup>40</sup> and after vaccination of adult horses against *S. neurona*.<sup>41</sup> In the study reported here, anti-coronavirus IgG detected in the CSF may have also been produced in the CNS because the CNS may have participated in a systemic immune response. This may explain the detection of CSF antibodies in cats with FIP in which no histologic lesions were detected in the CNS.

In humans, calculation of the specific antibody index has been recommended for evaluation of local synthesis of antibodies in the CNS.<sup>42</sup> Use of this method is based on the assumption that a specific immunoglobulin has the same blood-brain barrier permeability as all other immunoglobulins of the same class. The CSF-to-serum ratio for a specific antibody is greater than the CSF-to-serum ratio of total IgG in cases of intrathecal production of this specific antibody. Local production is indicated with a specific antibody index  $> 1.5$  ( $> 4$  when titers are given).<sup>43,44</sup> Recently, the antibody index has been interpreted as the association between a specific antibody and total IgG in blood and CSF.<sup>45</sup> In veterinary medicine, specific antibody indices have been calculated in *Toxoplasma gondii* infection<sup>38,46</sup> and in canine distemper encephalitis,<sup>47,48</sup> but it has been determined that even increased specific antibody indices  $> 1$  do not necessarily imply active infection of the CNS.<sup>38</sup> The same has been detected in humans with multiple sclerosis, which is associated with a polyspecific immune response in the CNS and with increased specific antibody indices against multiple antigens without the antigen actually being present in the CNS.<sup>43,45,49</sup>

Histologic examination has been used as the gold standard for confirmation or exclusion of FIP in the CNS. Therefore, 3 cats in which results of neurologic examinations were considered normal were allocated into group 1 because of histopathologic lesions characteristic of FIP in the CNS. In clinical conditions, cats would be allocated into groups according to their clinical signs. Histologic examination was preferred because subclinical lesions could influence CSF parameters. In addition, cats with FIP not involving the CNS may not represent a true control group. Unexpectedly, in 4 of those cats (group 2), anti-coronavirus IgG was detected in low concentrations in CSF. This may result from migration of activated B-lymphocytes into the CNS as part of a systemic immune response without the coronavirus antigen being present in the CNS. However, the presence of small, scattered inflammatory lesions that may not have been detected during histologic examination of the CNS could have resulted in blood-brain barrier dysfunction or specific intrathecal antibody produc-

tion and cannot be ruled out. Use of immunohistochemistry or PCR assay for confirmation of FIP in mildly affected cats was not performed in our study.

Our study had a few limitations. Cats with FIP involving the CNS should have been compared with control cats with other inflammatory CNS diseases. In control cats with CNS disorders caused by diseases other than FIP (group 3), only 2 cats had CNS inflammation, of which 1 cat had protozoal meningoencephalitis and a meningioma and the other had polioencephalitis. Unfortunately, 1 of those 2 cats was seronegative, and the other had a low serum anti-coronavirus IgG titer. Neither cat had CSF IgG titers. In these cats, we do not know whether a high serum antibody titer would have resulted in CSF antibody titers because of inflammatory changes of the blood-brain barrier. There were only 2 cats with measurable CSF antibody titers in group 3. Those cats had serum anti-coronavirus IgG titers of 1:4,096 and 1:8,192, respectively, and had a meningioma and an astrocytoma. Four other cats in group 3 had serum anti-coronavirus IgG titers between 1:1,024 and 1:2,048, but no antibodies were detected in the CSF. In those cats, histologic changes indicated uremic encephalopathy, cortical and hippocampal necrosis, oculomotor tract degeneration, and an astrocytoma. Astrocytomas are known to have a variable effect on the blood-brain barrier in humans. Astrocytomas with low malignancy have little or no effect on the blood-brain barrier, whereas highly malignant forms lead to disruption.<sup>50,51</sup> Blood-brain barrier dysfunction has been described in cats with experimentally induced astrocytomas.<sup>52</sup> Similarly, meningiomas can break into the Virchow-Robin space and disrupt the blood-brain barrier.<sup>53</sup> Uremic encephalopathy, necrosis, and oculomotor tract degeneration are less likely to be associated with a disrupted blood-brain barrier than brain tumors and inflammatory disease; therefore, the absence of CSF antibodies is not unexpected in those cats.

Results of our study indicated that anti-coronavirus antibodies may be detected in CSF of cats with FIP involving the CNS, but not every cat with CNS manifestation of FIP had measurable CSF antibody titers. Alternatively, measurable anti-coronavirus antibodies were detected in CSF of control cats. Therefore, we conclude that the clinical use of CSF anti-coronavirus antibodies is as equivocal as that determined for serum antibodies.<sup>18,54,55</sup> The significant correlation between serum and CSF antibody titers and the fact that CSF antibodies were detected only in cats with high serum antibody titers suggest that CSF antibodies were derived from blood. However, on the basis of results of our study and another study,<sup>11</sup> the definite origin of anti-coronavirus antibodies in CSF of cats with FIP remains unknown. Future studies may therefore address the origin of anti-coronavirus antibodies in CSF by measurement of the albumin quotient for the assessment of the blood-brain and blood-CSF barriers and by calculation of the specific antibody index as an indicator of intrathecal antibody production.

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- ICN FITC goat anti-cat IgG, whole molecule, MP Biomedicals, Cappel, Aurora, Ohio.

## Appendix 1

Postmortem and histologic diagnoses for 29 cats with CNS disorders caused by diseases other than FIP (group 3) from which CSF samples were obtained for assessment of the diagnostic use of anti-coronavirus antibody testing for diagnosis of FIP involving the CNS.

Postmortem diagnosis (No. of cats)	Histologic diagnosis (No. of cats)
CNS neoplasia (11)	Meningioma (4) Astrocytoma (3) CNS manifestation of malignant lymphoma (3) Metastasis of hemangiosarcoma (1)
Unclassified, FIP excluded (10)	Degenerative encephalopathy (2) Uremic encephalopathy (1) Neuronal degeneration (1) Systemic final permeability alteration (1) Oculomotor tract degeneration (1) Condensation of vestibular neurons (1) Hypoxia (1) Unidentified, contrast enhancement detected during computed tomography (1) Spongiform degeneration (1)
Necrosis (3)	Cortical and hippocampal necrosis (2) Cortical necrosis (1)
Metabolic (2)	Hepatoencephalopathy (2)
Inflammatory (2)	Meningitis and polioencephalitis (FIP excluded; 1) Protozoal encephalitis and meningioma (1)
Neurovascular (1)	Subdural bleeding after thromboembolism in spinal cord (1)

## Appendix 2

Postmortem and histologic diagnoses for 15 cats with diseases not associated with FIP and not involving the CNS (group 4) from which CSF samples were obtained for assessment of the diagnostic use of anti-coronavirus antibody testing for diagnosis of FIP involving the CNS.

Postmortem diagnosis (No. of cats)	Histologic diagnosis (No. of cats)
Neoplasia (7)	Malignant lymphoma (3) Adenocarcinoma (3) Squamous cell carcinoma (1)
Cardiomyopathy (3) Hepatopathy (1) Enteropathy (1) Inflammatory (1) Endoparasitosis (1) Unclassified, FIP excluded (1)	Restrictive cardiomyopathy (2), not performed (1) Liver cirrhosis (1)* Catarrhal enteritis (1) Pyometra and peritonitis (1) Lung worms (1) Chronic myeloproliferative disease of bone marrow (1)

\*Esophageal malignant lymphoma was an incidental finding at necropsy.

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