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Utility of an immunocytochemical assay using aqueous humor in the diagnosis of feline infectious peritonitis

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Abstract

Objective In cats suffering from feline infectious peritonitis (FIP) without effusion, antemortem diagnosis is challenging. Uveitis is common in these cats. It was the aim of this study to evaluate sensitivity and specificity of an immunocytochemical assay (ICC) in aqueous humor of cats suspected of having FIP.

Animals studied The study included 26 cats with immunohistochemically confirmed FIP and 12 control cats for which FIP was suspected due to similar clinical or laboratory changes, but which suffered from other diseases confirmed via histopathology.

Procedures All aqueous humor samples were collected postmortem by paracentesis. ICC was carried out as avidin–biotin complex method. Sensitivity, specificity, and the overall accuracy including 95% confidence intervals (95% CI) were calculated.

Results Immunocytochemistry was positive in 16 of 25 cats with FIP and 2 of 11 control cats (one cat with lymphoma, one with pulmonary adenocarcinoma). Aqueous humor samples of one cat with FIP and of one control cat were excluded from statistical analysis. Sensitivity was 64.0% (95% CI: 42.5–82.0); specificity 81.8% (95% CI: 48.2–97.7); and overall accuracy 69.4% (95% CI: 51.9–83.7).

Conclusions As false-positive results occurred and specificity is most important in the diagnosis of FIP, the diagnostic utility of ICC in aqueous humor is limited. Further studies are required to clarify the origin of false-positive ICC results.

Key Words: FIP, immunocytochemistry, sensitivity, specificity, uveitis

INTRODUCTION

Feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV) are two distinct pathotypes of the feline coronavirus (FCoV).[1,2] FIPV can sustain replication within macrophages at high levels and can spread the infection to adjacent cells, ultimately causing feline infectious peritonitis (FIP).[3,4] Thus, in the past, it was believed that positive immunostaining of viral antigen within the cytoplasm of macrophages was diagnostic for FIP.[5] Therefore, immunostaining has been considered reference standard for diagnosing FIP in cats with effusion for a long time.[5] In the past 3 years, however, a number of studies suggested that immunostaining can be false-positive and this has questioned its usefulness.[6–8]

In cats without effusion, the definitive diagnosis of FIP currently can only be achieved by invasive procedures such as laparotomy or laparoscopy to obtain biopsy samples of affected tissue for immunostaining of macrophages.[9–12] Uveitis and neurological signs are often present in cats suffering from FIP without detectable body cavity effusions,[10] and FIP has been shown to be the most common infectious cause of uveitis in cats.[13] In cats suffering from FIP without significant effusions, ocular and/or neurological signs are present in about 60% of the affected cats. In contrast, if effusion is present, ocular and/or neurological signs occur in less than 9%.[10] More recently, however, it has been discussed that eye involvement might actually be underestimated, as 29% of cats confirmed to have FIP showed eye involvement.[11] The most common ocular signs seen in cats with FIP are uveitis and chorioretinitis.[14–16] A mixed uveal inflammatory cell infiltrate consisting of neutrophils and mononuclear inflammatory cells can usually be seen histologically.[17]

Aqueous humor is commonly obtained in cats with uveitis for cytology but is often nondiagnostic in cats with

infectious uveitis.[18–20] A mixed inflammatory cell population including macrophages is usually present within the anterior eye segment of cats suffering from uveitis secondary to FIP.[14–17,19,21]

Whereas usefulness of immunostaining of macrophages in the cerebrospinal fluid (CSF) has been reported recently,[8,22] immunocytochemical demonstration (ICC) of coronavirus antigen in macrophages in aqueous humor has not been evaluated before.

The hypothesis of the present study was that staining of coronavirus antigen in macrophages of aqueous humor could be a valuable diagnostic method to confirm FIP in cats with or without uveitis, especially in cats without body cavity effusions. Therefore, the aim of the study was to evaluate the sensitivity and specificity of an ICC assay in aqueous humor in cats suspected of having FIP. Additionally to ICC analysis, all samples were evaluated cytologically in order to validate ICC results.

MATERIALS AND METHODS

Animals

Overall, 38 cats suspected of having FIP based on clinical and/or laboratory signs were prospectively included in the study. The cats were presented either as patients of the Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universitaet, Munich, Germany ($n = 27$), or directly submitted for postmortem examination to the Institute of Veterinary Pathology, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universitaet, Munich, Germany ($n = 11$).

The FIP group ($n = 26$) consisted of cats definitively diagnosed with FIP. The diagnosis in all these cats was confirmed by histopathology plus positive immunohistochemical (IHC) staining of FCoV antigen in macrophages in FIP-typical lesions in tissue samples obtained at postmortem examination. IHC was performed as described before.[7] Histopathological examination and IHC included the eyes if macroscopic changes were present at postmortem examination, which was the case in three cats with FIP.

The control group ($n = 12$) consisted of cats for which FIP was considered an important differential diagnosis. An inclusion criterion therefore was the presence of one or more of the following signs consistent with FIP: effusion ($n = 7$), fever with not more than 20 000 white blood cells/μL and not more than 1000 band neutrophils/μL ($n = 1$), icterus ($n = 5$), or neurological signs ($n = 5$) (Table 1). Additionally, all control cats had to be definitively diagnosed with a disease other than FIP which explained the clinical signs, and in all cats, diagnoses were confirmed by full postmortem examination including histopathology after the cats were euthanized due to disease progression independent of the purpose of this study. In all control cats, IHC staining of affected tissues was performed and was always negative.

Table 1. Inclusion criteria, definitive diagnosis, and immunocytochemical (ICC) results for the 12 control cats. Diagnoses were confirmed by histopathology including negative immunohistochemical staining of affected tissues in all cats

Cat	Signs leading to inclusion	Diagnosis	ICC result
1	Pleural effusion	Bronchial carcinoma	Negative
2	Icterus	Systemic salmonellosis	Negative
3	Ascites, icterus, neurological signs	Cholangiohepatitis	Negative
4	Neurological signs	Globoid cell leukodystrophy	Negative
5	Neurological signs	Intracranial neoplasia	Negative
6	Pleural effusion	Pulmonary adenocarcinoma	Positive
7	Icterus, neurological signs	Meningoencephalitis	Negative
8	Ascites	Lymphoma	Negative
9	Pleural effusion	Lymphoma	Positive
10	Fever, icterus	Cholangiohepatitis	Negative
11	Icterus, neurological signs	Cholangitis and cholecystitis, degenerative polioencephalopathy	Negative
12	Pleural effusion	Pulmonary adenocarcinoma	n.d.

n.d., nondiagnostic (owing to the lack of cellular material).

Samples

In total, 38 aqueous humor samples were collected between 2012 and 2014. All aqueous humor samples were obtained postmortem by anterior eye segment paracentesis after the cats were euthanized or died naturally. In most cases, samples were collected within 24 h. A 22-G needle was inserted into the anterior eye segment at the limbus, above and parallel to the plane of the iris. Aqueous humor was then gently aspirated into a 2-mL syringe.

Aliquots of 100 μL of aqueous humor samples were cytocentrifuged (231 g for 5 min, then the supernatant was discarded, and the cell pellet was centrifuged at 643 g for 1 min) in a cytospin centrifuge (Universal 16R, Hettich), and slides were stored at −20°C until use. Two slides were prepared for each cat.

Cytological evaluation

One slide of each aqueous humor sample was stained with hematoxylin and eosin and semiquantitatively evaluated for cellularity, cellular composition, blood contamination/hemorrhage and erythrophagocytosis, protein, fibrin and aspiration of autochthonous material (e. g. melanin, fragments of punctured cornea). Considering these parameters, slides were classified as cytologically 'typical for FIP', 'compatible with FIP', or 'not indicative of FIP'. Pyogranulomatous inflammation with macrophagic engulfment of neutrophils and absence of microorganisms and giant cells was regarded as 'typical for FIP'. Mixed white blood cell populations containing macrophages were regarded as 'compatible with FIP'. Other cellular compositions, such

as exclusively neutrophilic or neoplastic cells, were regarded as 'not indicative of FIP' (Fig. 1).

ICC

Slide reading and interpretation of ICC results were performed by two independent investigators, who were blinded to all data of the cats, including their diagnoses.

Immunocytochemistry of aqueous humor samples was performed as described previously for effusion samples.[7]

All immunostained aqueous humor samples were evaluated for cellularity and positivity of the immunostaining using light microscopy. Only samples with more than 50 nucleated cells in total, including mononuclear cells, were included in the statistical analysis. Samples lacking unimpaired cellular material were excluded from statistical analysis. If positive immunostaining was present within a sample, cells with a positive signal were further assessed considering cell type, signal pattern, and intensity of the staining. A positive sample was defined if containing macrophages that revealed brown, intensely stained and granulated cytoplasm independent of possible pigmentophagia (Fig. 1). A negative sample was defined as containing macrophages without any immunostaining or cells with nonspecific immunostaining. Immunostaining of lymphocytes, plasma cells, neutrophils, or erythrocytes was considered nonspecific. Additionally, diffuse and/or light cytoplasmic staining was considered nonspecific and was classified as negative on ICC. All ICC-positive samples were categorized semi-quantitatively regarding their yield of true positive cells (low-yield positive: few single macrophages with strong positive signal; medium-yield positive: about half of the macrophages with strong positive signal; high-yield positive: majority of macrophages with strong positive signal).

Statistical evaluation

To evaluate the diagnostic value of the ICC in aqueous humor in the diagnosis of FIP, sensitivity, specificity, and overall accuracy were determined. To quantify uncertainty, 95% confidence intervals (95% CI) were calculated. Statistical analysis was performed using MS Excel (Microsoft Inc., Redmond, Washington, USA) and Prism Version 5.04 (GraphPad Software, Inc., La Jolla, California, USA).

RESULTS

Ocular signs and histopathology of the eye

In all cats that were presented to the Clinic of Small Animal Medicine (n = 27), routine physical examination was performed and did not reveal clinical signs of uveitis.

In total, three cats with confirmed FIP had ocular changes on postmortem examination. Twenty-two of the 26 cats with FIP had effusions; two of them also had postmortem changes consistent with uveitis. Macroscopic postmortem changes included conjunctivitis, white miliary foci within the iris, and turbidity within the anterior chamber. Histopathological changes included lymphocytic–plasmacytic infiltration of the iris, multifocal lymphocytic conjunctivitis, plasmacytic and macrophagic infiltration of the anterior chamber, and accumulation of fibrin within the anterior chamber.

None of the control cats had ocular signs on physical or macroscopic postmortem examination.

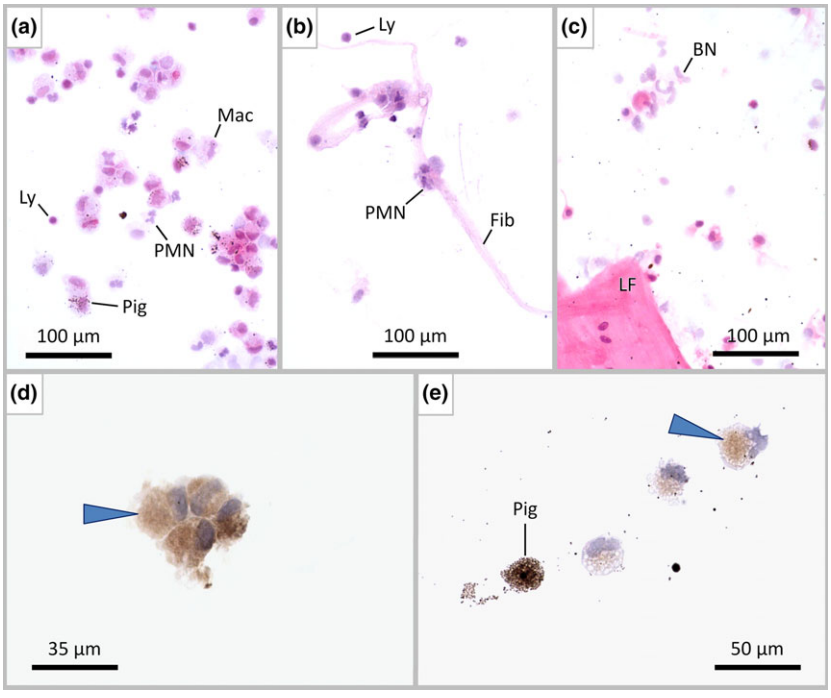

Fig. 1. Cytological and immunocytochemical (ICC) findings. (a) Feline infectious peritonitis (FIP)-typical cytology showing pyogranulomatous inflammation including neutrophils (PMN), macrophages (Mac), rare lymphocytes (Ly), and macrophages containing melanin pigment (Pig). (b) Cytology compatible with FIP showing mixed white blood cells and fibrin threads (Fib). (c) Cytology not indicative of FIP in a case of phacoclastic uveitis containing lense fragments (LF) and band neutrophils (BN). (d) Immunopositive macrophages in a cat with FIP (arrowhead). (e) Autochthonous pigmented cells (Pig) should not be confused with macrophages (arrowhead).

Cytological evaluation

Results of the cytological evaluation of the hematoxylin–eosin-stained aqueous humor samples are shown in Table 2. A 100% inter-rater agreement was achieved through robust criteria. Overall, cellular composition was either predominantly neutrophilic with (n = 1) or without necrosis (n = 3), lymphomonocytic (n = 14), monocytic/macrophagic (n = 2), lymphoplasmacytic (n = 2), pyogranulomatous (n = 8), or neoplastic (n = 1). An equivocally mixed white blood cell population comprising neutrophils, monocytes/macrophages, and lymphocytes was present in five aqueous humor samples. Two hematoxylin–eosin-stained aqueous humor samples did not contain inflammatory cells.

ICC

Positive ICC results were obtained in 18 (16 with FIP, two controls) of the 38 cats (Table 3, 4). The two false-positive results occurred in a cat with lymphoma and in a cat with pulmonary adenocarcinoma diagnosed at full postmortem examination including histopathology. Only one of the 18 cats with positive aqueous humor samples had gross postmortem changes consistent with uveitis. Two immunostained aqueous humor samples of one cat with FIP and of one control cat were considered nondiagnostic owing to the lack of cellular material. These samples were excluded from statistical analysis. The ICC result of two aqueous humor samples of control cats was questionable, as dark brown immunostaining was present within macrophages, but the typical granular pattern was missing. As these samples did not fulfill the inclusion criteria for a positive ICC result, they were considered ICC-negative.

Diagnostic sensitivity, specificity, and overall accuracy are shown in Table 5.

Table 2. Cytological evaluation of hematoxylin- and eosin-stained aqueous humor samples

Group		FIP	Controls	Total
Number of samples		26	12	38
Cellularity	Low	11	5	16
	Medium	12	6	18
	High	2	1	3
	No cells	1	0	1
Blood contamination/ hemorrhage	+	7	3	10
	++	3	1	4
	+++	0	0	0
	Negative	14	7	21
	Not evaluable	2	1	3
Erythrophagocytosis	+	4	0	4
	++	0	0	0
	+++	0	0	0
	Negative	20	11	31
	Not evaluable	2	1	3
Protein	+	11	7	18
	++	4	0	4
	+++	4	0	4
	Negative	6	4	10
	Not evaluable	1	1	2
Fibrin	+	6	5	11
	++	8	2	10
	+++	4	0	4
	Negative	5	5	10
	Not evaluable	3	0	3
Aspiration of melanin	+	12	5	17
	++	5	1	6
	+++	2	3	5
	Negative	6	3	9
	Not evaluable	1	0	1
Cytological signs for FIP	Typical	9	0	9
	Compatible	9	1	10
	Not indicative	8	11	19

FIP, feline infectious peritonitis.

Table 3. Cellularity and results of immunocytochemical staining (ICC) of aqueous humor samples

Cat	Cellularity*	Immunostaining†	Group
1	++	Nonspecific ‡	FIP
2	+++	Neg.	FIP
3	+++	+++	FIP
4	+++	Neg.	FIP
5	+	+	FIP
6	+	++	FIP
7	+++	+++	FIP
8	++	Neg.	FIP
9	++	Neg.	FIP
10	+++	+	FIP
11	+++	+	FIP
12	+	+	FIP
13	++	Neg.	FIP
14	+	Neg.	FIP
15	++	+++	FIP
16	++	+	FIP
17	–	Acellular	FIP
18	+++	+++	FIP
19	+	Neg.	FIP
20	++	+	FIP
21	++	+++	FIP
22	++	+++	FIP
23	+	+	FIP
24	+	+	FIP
25	++	Neg.	FIP
26	+++	+	FIP
27	+++	Nonspecific‡	Control
28	+++	Neg.	Control
29	++	Neg.	Control
30	+	Neg.	Control
31	+	Neg.	Control
32	+++	+++	Control
33	++	Nonspecific	Control
34	+	Neg.	Control
35	++	+++	Control
36	+	Nonspecific	Control
37	+++	Neg.	Control
38	–	Acellular	Control

FIP, feline infectious peritonitis. *+, low cellular; ++, medium cellular; +++, high cellular; – lack of cellular material, nondiagnostic. †+, low-yield positive immunostaining; ++, medium-yield positive immunostaining; +++, high-yield positive immunostaining; neg., negative immunostaining; acellular samples were regarded as nondiagnostic. ‡Nonspecific staining was defined as diffuse and/or light cytoplasmic staining of macrophages. Samples with nonspecific staining were regarded as ICC-negative.

Table 4. Results of immunocytochemistry (ICC) in 36 aqueous humor samples (two samples were considered nondiagnostic and were therefore excluded from statistical analysis)

	FIP	Control	Total
Positive ICC	16	2	18
Negative ICC	9	9	18
Total	25	11	36

FIP, feline infectious peritonitis.

Table 5. Sensitivity, specificity, and overall accuracy of immunocytochemistry (ICC) in 36 aqueous humor samples (two samples were considered nondiagnostic and were therefore excluded from statistical analysis)

	ICC in aqueous humor
Sensitivity % (95% CI)	64.0 (42.5–82.0)
Specificity % (95% CI)	81.8 (48.2–97.7)
Overall accuracy % (95% CI)	69.4 (51.9–83.7)

95% CI, 95% confidence interval.

DISCUSSION

The aim of this study was to determine the diagnostic value of an ICC assay in aqueous humor in cats suspected of having FIP. This study is the first to evaluate the use of aqueous humor for ICC analysis in the diagnosis of FIP.

In cats without effusion, antemortem diagnosis of FIP is still difficult. Reverse transcription polymerase chain reaction (RT-PCR) is often applied to establish a diagnosis in various diagnostic samples such as effusion.[23–26] In the absence of effusion, however, there is a need for other appropriate material. Whereas sensitivity of RT-PCR is very good in effusions,[24–27] blood has proven not to be suitable due to a very low virus load. Even though cell-associated viremia was detected in all three cats experimentally infected with an enteric strain of FCoV in a recent study,[28] FCoV RNA could be detected in the whole blood, plasma, or white cell fraction of none of 20 cats in which FIP was experimentally induced in a different study.[27] In addition, sensitivity of realtime RT-PCR was also very low in PBMC, serum, or plasma of naturally infected cats with FIP.[24,25] A recent study examined RT-PCR in CSF, which showed a slightly better but still insufficient sensitivity.[23] Specificity of a RT-PCR detecting FCoV in blood is only 20–88% and healthy cats can be positive by blood RT-PCR.[29–31] Thus, a discriminative RT-PCR specifically able to distinguish FECV and FIPV could have improved specificity, but further studies are required to determine its diagnostic value, especially in material other than effusion. Therefore, evaluation of other diagnostic possibilities, such as ICC, is important to further facilitate the diagnosis of FIP.

Diagnostic specificity of the FCoV ICC in aqueous humor samples was only 81.8%. This is unexpected, as many previous studies evaluating immunostaining of FCoV in macrophages in effusion described an excellent specificity of 100%,[31–34] giving the impression that false-positive results do not occur. Two more recent studies, however, also found false-positive cats in immunofluorescence or ICC staining of effusion macrophages, and specificity was even slightly lower than in the present study (71% and 72%, respectively).[6,7] Additionally, false-positive results have also been reported for ICC in CSF, and specificity was comparable to the present study (83%).[8]

It is possible that nonspecific staining of cells other than macrophages was misclassified in the present study as positive upon microscopic evaluation of the slides, leading to a false interpretation of the ICC results in these two cats. Viral replication of FIPV is restricted to monocytes/macrophages[4,35–37] and therefore only immunostaining within the cytoplasm of macrophages should be considered ICC-positive. Nonspecific staining of other cells (e.g., red blood cells, neutrophils) might result from binding of the antibody to cellular structures other than the FIPV nucleocapsid. Nevertheless, as macrophages usually can easily be differentiated, this possibility seems unlikely, especially as two different investigators independently interpreted the false-positive results as positive.

In 9/12 aqueous humor samples of control cats, melanin was present to variable extents (Table 2). The two samples which were false-positive in ICC also were melanin-positive. Given the very similar colors of pigment and the chromogen diaminobenzidine-tetrahydrochloride (DAB), there is a small likelihood of confusion between these two, and the identification of ICC-positive macrophages becomes more difficult. Melanin was also present in 19/25 samples of cats with FIP for which cytological data were available. Twelve of these 19 were ICC-positive. It could be argued that some of these could also be false-positive due to confusion of melanin with DAB. Thus, the use of a different chromogen for aqueous humor samples should be considered in the future. Nevertheless, the isometric and even distribution of FCoV antigen within the cytoplasm usually allows a definitive identification of ICC-positive macrophages, even in the presence of melanin.

Contamination might have been a problem during the staining procedure as well, as slides were kept in close proximity during all washing and incubation steps. Negative control slides could help to potentially identify this problem in future studies.

The two cats with false-positive ICC results suffered from lymphoma and pulmonary adenocarcinoma. In both cases, the diagnosis was confirmed by full postmortem examination including histopathological examination of tissue material. However, these two cats with false-positive results in the present study were severely ill. Similarly, all cats with false-positive immunostaining in previous studies suffered from terminal diseases, such as lymphoma, carcinoma, end-stage cardiac disease, or meningoencephalitis.[6–8] These cats were certainly immunocompromised due to their diseases, and therefore were rather susceptible to

developing FIP. Thus, there is the possibility that the two cats with false-positive staining in the present study suffered from two diseases, their tumors and early-stage FIP, but still without histopathological lesions. Nevertheless, neither of the two cats with false-positive ICC results had 'FIP-typical' aqueous humor cytology.

In contrast to what was believed previously,[38,39] replication of FECV is not restricted exclusively to the intestinal tract. As a consequence, FECV can be detected in monocytes in the blood of clinically healthy cats.[29,30,40,41] Subsequently, FECV can spread systemically,[42] and it might be possible that it enters the aqueous humor with other blood components in the presence of inflammation. The two cats that were tested false-positive suffered from end-stage neoplasia. Possibly, they were infected with a 'harmless' FECV that was able to cross the blood–aqueous barrier and enter the aqueous humor in the presence of their systemic disease. As a consequence, ICC might then have detected FECV, which could explain the false-positive results.

Overall, this lack of specificity, however, is a serious problem, because a very high specificity of a diagnostic test is required for a deadly disease like FIP. False-positive results can lead to euthanasia of cats suffering from potentially treatable diseases. Further studies are required to answer the question of false-positive staining.

Diagnostic sensitivity of the ICC assay in aqueous humor was 64.0%. Previous studies on ICC or immunofluorescence using effusion or CSF have reported higher sensitivities of 85–100%.[6–8] One explanation that not all cats with FIP were positive in ICC is a low number of macrophages in the examined specimens. Especially in cats without uveitis the number of macrophages in the aqueous humor can be low, but could be much higher if inflammation is present. Given the fact that only one of the cats with positive ICC results on aqueous humor samples showed clinical signs or histopathological changes indicative of uveitis, the sensitivity appears rather high when compared to sensitivity of immunostaining in other material, such as effusion. Nevertheless, aqueous humor cytology was abnormal in all of the cats with FIP for which cytological data was available and in 11/12 of the control cats, regardless of whether they showed signs of uveitis. All of the control cats suffered from severe diseases, such as neoplasia, commonly associated with inflammation, which can explain their abnormal aqueous humor. Apparently, inflammatory and neoplastic cells originating from other parts of the body are able to cross the blood–aqueous barrier, even without the presence of uveitis. A sufficient number of macrophages was detected cytologically in 8/9 cats with FIP with false negative ICC results. Thus, lack of cellularity is unlikely responsible for the false-negative results.

All aqueous humor samples were obtained postmortem after the cats were euthanized or died due to disease progression, which is a limitation to the present study. It is possible that antemortem paracentesis would lead to a better diagnostic utility of the ICC. Some degradation of macrophages might have occurred at the time of sampling. Antemortem paracentesis therefore might lead to a higher sensitivity of the ICC. Further explanations for a low sensitivity are a competitive binding of FCoV in immune complexes by circulating antibodies. Also the use of a monoclonal antibody in the staining protocol could be a reason, and a polyclonal antibody conjugate might have increased sensitivity (but concurrently decreased specificity).

Two aqueous humor samples were considered nondiagnostic, as adequate evaluation was not possible owing to the absence of a sufficient number of cells on the slides. It is possible that the aqueous humor already did not contain a large number of cells upon sampling. Additionally, processing of the samples during ICC staining could have further decreased the number of adequate cells upon the slides. Potentially, fixation of the cellular material on the slides could increase cellularity of the ICC slides and further studies should compare and evaluate different fixation methods to avoid low cellularity.

As aqueous humor was collected postmortem in the present study, a 22-G needle was used for paracentesis to increase the amount of sample material and facilitate the collection. If performed antemortem, 27- to 29-G needles are usually used to reduce the risk of ocular damage.[20] The use of the larger needle could have resulted in improved cell retrieval and reduced cell disruption which might be a problem in antemortem samples. This is a limitation of the present study. Another limitation is the fact that no control cats with uveitis were included, and future studies are required to compare this new method in cats with and without uveitis by taking antemortem samples. Additionally, ophthalmological examination was not routinely performed in all of the cats, and only a relatively small number of cats was included in each group. Another limitation of this study is the fact that only four cats with FIP did not have effusions. Therefore, the usefulness of ICC in the diagnosis of FIP in cats without effusion cannot be determined and further studies should be performed in cats without effusion. Finally, the present study would have been enhanced by the inclusion of a RT-PCR assay using aqueous humor samples. Possibly, RT-PCR would have been useful in the clarification of the origin of the positive immunostaining in the two cats without histopathologically confirmed FIP and in the equivocal samples. This comparison should be investigated in future studies.

The present study for the first time evaluated an ICC assay in the diagnosis of FIP using aqueous humor samples. The lack of specificity, however, is disappointing, as a high specificity would be required to confirm a diagnosis of FIP.

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