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Detection of feline coronavirus in cerebrospinal fluid for diagnosis of feline infectious peritonitis in cats with and without neurological signs

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Abstract

Objectives The objective of this study was to evaluate the sensitivity and specificity of a real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) detecting feline coronavirus (FCoV) RNA in cerebrospinal fluid (CSF) of cats with and without neurological and/or ocular signs for the diagnosis of feline infectious peritonitis (FIP).

Methods This prospective case-control study included 34 cats. Nineteen cats had a definitive histopathological diagnosis of FIP (seven of these with neurological and/or ocular signs), and 15 cats had other diseases but similar clinical signs (three of these with neurological and/or ocular signs). Real-time RT-PCR was performed on the CSF of all cats, and sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) were calculated.

Results Real-time RT-PCR of CSF showed a specificity of 100% in diagnosing FIP, a sensitivity of 42.1%, a PPV of 100% and an NPV of 57.7%. The sensitivity of the real-time RT-PCR of CSF in cats with neurological and/or ocular signs was 85.7%.

Conclusions and relevance Although it is known that RT-PCR can give false positive results, especially if performed using serum or plasma, this real-time RT-PCR detecting FCoV RNA in CSF can be considered a reliable specific tool for the diagnosis of FIP. If only cats with neurological involvement are evaluated, the sensitivity of this real-time RT-PCR in CSF is also high.

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Introduction

Feline infectious peritonitis (FIP) is a globally occurring fatal disease caused by feline coronaviruses (FCoVs).[1] FCoV infection is common among cats, particularly in catteries, in which up to 100% of cats are infected, but only approximately 5–10% develop FIP.[2–4] In these cats, FIP is caused by mutation of the generally harmless FCoV, which is sometimes also called feline enteric coronavirus (FECV).[5–7] When specific mutations occur, the virus can then effectively replicate in macrophages, which is considered the key event in the pathogenesis of FIP.[7,8] The virus replicating in macrophages is sometimes called feline infectious peritonitis virus (FIPV). However, it is important to realise that FECV and FIPV are only two biotypes that are almost identical in their genome and thus cannot be differentiated by routine reverse transcriptase polymerase chain reaction (RT-PCR). Among all cats with FIP, approximately 10% develop neurological signs,[9,10] which occur as a result of virus-induced pyogranulomatous meningoencephalitis and

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meningomyelitis.[10,11] Commonly reported neurological clinical signs are ataxia, seizures, nystagmus, hyperaesthesia and cranial nerve deficits.[12–15] Most often, a multifocal location is suggested, but, occasionally, focal signs can also occur.[13,16] Ocular manifestations consist predominantly of uveitis and chorioretinitis with associated fibrinous exudate in the anterior ocular chamber, which are common in neurological forms of FIP.[11,17]

The median survival time of cats with FIP is 9 days,[18] and the diagnosis of FIP usually leads to euthanasia. Therefore, a reliable diagnostic tool is needed to confirm the diagnosis. A definitive diagnosis of FIP remains challenging, especially if no effusion is present, and requires histological examination of biopsy specimens of affected organs,[3] but this approach is limited in cats with FIP restricted to the central nervous system (CNS). RT-PCR on blood samples is sometimes used to support a diagnosis of FIP; however, both sensitivity and specificity are too low to allow a definitive diagnosis or to rule out FIP.[3,19,20] So far, there has been only one study that looked into the diagnostic value of RT-PCR detecting FCoV in cerebrospinal fluid (CSF).[15]

The aim of the present study was to determine sensitivity and specificity of a real-time RT-PCR in CSF to diagnose FIP in cats with and without neurological and/or ocular signs, comparing cats with confirmed FIP with control cats with clinical signs similar to FIP but other confirmed diagnoses.

Materials and methods

Animals

This study was designed as a case-control study, and included 34 cats. The cats were presented to the Clinic of Small Animal Internal Medicine, LMU University of Munich, Germany (n = 28), or to private veterinarians (n = 6). The FIP group (n = 19) consisted of animals with a definitive diagnosis of FIP (Table 1). FIP diagnosis was established in all 19 cats by post-mortem examination, including full body necropsy with histopathological examination. FIP diagnosis was confirmed by typical

Table 1 Cats with feline infectious peritonitis (FIP), clinical signs, method of confirmation of the diagnosis of FIP, presence of neurological and/or ocular signs, and threshold cycle (Ct) values of the tested cerebrospinal fluid (CSF) sample

Cat	Signs for inclusion	Diagnosis	Confirmation	Method of confirmation	Neurological and/or ocular signs	Ct values CSF
1	Thoracic effusion, icterus	FIP	Post mortem	Histopathology	Seizures	No Ct
2	Thoracic effusion, fever	FIP	Post mortem	Histopathology	–	36.1 (positive)
3	Thoracic effusion	FIP	Post mortem	Histopathology	–	No Ct
4	Ascites, icterus, neurological signs	FIP	Post mortem	Histopathology	Seizures	32.1 (positive)
5	Ascites, icterus	FIP	Post mortem	Histopathology	–	No Ct
6	Thoracic effusion	FIP	Post mortem	Histopathology	–	No Ct
7	Ascites	FIP	Post mortem	Histopathology	–	No Ct
8	Thoracic and pericardial effusions	FIP	Post mortem	Histopathology	–	31.7 (positive)
9	Ascites, neurological signs	FIP	Post mortem	Histopathology	Paresis, ataxia, anisocoria, inability to control urination and defecation	32.6 (positive)
10	Ascites, fever, icterus, neurological and ocular signs	FIP	Post mortem	Histopathology	Paresis, uveitis	32.0 (positive)
11	Ascites, icterus	FIP	Post mortem	Histopathology	–	No Ct
12	Ascites, icterus	FIP	Post mortem	Histopathology	–	No Ct
13	Ascites, icterus	FIP	Post mortem	Histopathology	–	No Ct
14	Thoracic effusion and ascites, fever	FIP	Post mortem	Histopathology	–	No Ct
15	Fever, icterus, neurological signs	FIP	Post mortem	Histopathology	Ataxia	26.5 (positive)
16	Fever, ocular signs	FIP	Post mortem	Histopathology	Uveitis	32.0 (positive)
17	Thoracic effusion, fever, ocular signs	FIP	Post mortem	Histopathology	Uveitis	29.9 (positive)
18	Thoracic effusion and ascites, fever	FIP	Post mortem	Histopathology	–	No Ct
19	Ascites, fever, icterus	FIP	Post mortem	Histopathology	–	No Ct

Table 2 Cats in the control group with signs for inclusion, confirmed diseases, method of confirmation, presence of neurological and/or ocular signs, and the threshold cycle (Ct) values of the tested cerebrospinal fluid (CSF) sample

Cat	Signs for inclusion	Diagnosis	Confirmation	Method of confirmation	Neurological and/or ocular signs	Ct values CSF
1	Thoracic effusion	Lymphoma	Ante-mortem	Cytology	–	No Ct
2	Ascites	Lymphoma	Post mortem	Necropsy	–	No Ct
3	Ascites	Lymphoma	Post mortem	Necropsy	–	No Ct
4	Thoracic effusion	Lymphoma	Post mortem	Necropsy	–	No Ct
5	Ascites	Neoplasia close to the liver, probably adenocarcinoma of the biliary tract	Post mortem	Necropsy	–	No Ct
6	Thoracic effusion	Adenocarcinoma, lung	Post mortem	Necropsy	–	No Ct
7	Thoracic effusion	Bronchial carcinoma, metastasising	Post mortem	Necropsy	–	No Ct
8	Thoracic effusion	Sarcoma, metastatsising	Post mortem	Necropsy	–	No Ct
9	Thoracic effusion	Decompensated cardiac disease	Ante-mortem	Echocardiography	–	No Ct
10	Thoracic effusion, fever, neurological signs	Pulmonary fibrosis with thoracic effusion	Post mortem	Necropsy	Ataxia	No Ct
11	Thoracic effusion	Chylothorax with fibroplastic pleuritis	Post mortem	Necropsy	–	No Ct
12	Icterus	Lymphoma	Post mortem	Necropsy	–	No Ct
13	Icterus	Lymphoma	Post mortem	Necropsy	–	No Ct
14	Neurological signs	Lymphoma, mild encephalomyelitis, high-grade cellulitis, fascitis, myositits (interstitially) in the lumbar part of the spine	Post mortem	Necropsy	Ataxia, paralysis of the tail, inability to control urination and defecation	No Ct
15	Neurological signs, icterus	Hepatoencephalopathy due to severe hepatolipidosis	Post mortem	Necropsy	Status epilepticus	No Ct

morphology (surface-bound multisystemic pyogranulomatous and fibrinonecrotic disease with venulitis with or without high-protein exudate). In the control group (n = 15), cats for which FIP was considered as a differential diagnosis because of 'FIP typical' clinical signs were included. Cats with one or more of the following clinical signs were included: effusion (n = 11), a rectal temperature of ⩾40°C (with ⩽20,000 white blood cells/µl and ⩽1000 banded neutrophils/µl; n = 1), icterus (n = 3) or neurological signs (n = 3). Cats were only included in the control group if they were definitively diagnosed with diseases other than FIP that explained the clinical signs. These other diseases were confirmed either at necropsy (n = 13) or ante-mortem (n = 2). One of the two cats diagnosed ante-mortem had effusion caused by lymphoma, which was confirmed by cytological examination of thoracic effusion and fine-needle aspiration of lymph nodes. The other cat had thoracic effusions caused by a decompensated cardiac disease confirmed by echocardiography (Table 2).

Of the 19 cats with FIP, seven had neurological (n = 5) and/or ocular (n = 3) signs (Table 1). Of the 15 cats with other diseases, three had neurological (n = 3) and/or ocular (n = 0) signs (Table 2).

Samples

CSF was collected immediately after cats were euthanased with a 19 G needle from the cerebellomedullary cistern. Cell-free CSF was stored at –80°C in a 1.5 ml Eppendorf Safe-Lock microcentrifuge tube.

Table 3 Results of real-time reverse transcriptase polymerase chain reaction (RT-PCR) of cerebrospinal fluid of all cats with feline infectious peritonitis (FIP) and of all cats with other diseases, and of cats with FIP and other diseases with and without neurological and ocular involvement

	Real-time RT-PCR positive	Real-time RT-PCR negative	**Total**
Cats with FIP (n = 19)	**8**	**11**	**19**
Cats with neurological and/or ocular signs (n = 7)	6	1	**7**
Cats without neurological and without ocular signs (n = 12)	2*	10	**12**
Cats with other diseases (n = 15)	**0**	**15**	**15**
Cats with neurological and/or ocular signs (n = 3)	0	3	**3**
Cats without neurological and without ocular signs (n = 12)	0	12	**12**
Total	**8**	**26**	**34**

*Post-mortem examination identified microscopic involvement of the central nervous system in one of these cats

Table 4 Sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of real-time reverse transcriptase polymerase chain reaction (RT-PCR) in cerebrospinal fluid to diagnose feline infectious peritonitis (FIP) and the prevalence of FIP

	All cats (n = 34)	Cats with neurological and/or ocular signs (n = 10)
Sensitivity	42.1 (20.3–66.5)	85.7 (42.1–99.6)
Specificity	100.0 (78.2–100.0)	100.0 (29.2–100.0)
PPV	100.0 (63.1–100.0)	100.0 (54.1–100.0)
NPV	57.7 (36.9–76.7)	75.0 (19.4–99.4)
Prevalence (%)	55.9	70.0

Values are given as % (95% confidence interval) unless otherwise indicated

RNA extraction

Viral RNA was isolated from cell-free CSF using a QIAamp Viral Mini RNA Kit (Qiagen). Briefly, 140 µl aliquots of samples were lysed under highly denaturing conditions to inactivate RNases and isolate the intact viral RNA. Adjusted buffering conditions yielded an optimal binding of the viral RNA on the silica membrane of the QIAamp Mini spin column. After being washed with two wash buffers, the RNA was eluted with 60 µl of RNase-free buffer and stored at –80°C.

Real-time RT-PCR

The detection of FCoV was performed using a real-time RT-PCR.[21] A QuantiTect Probe RT-PCR Kit (Qiagen) was used for this one-step real-time RT-PCR. Five microlitres of RNA template was added to 12.5 µl Master Mix, 0.25 µl RT Mix, 5.25 µl RNase-free water and 2 µl primer probe mix. All primers were used in a concentration of 0.8 µM, and 5′FAM/3′BHQ-1 labelled TaqMan probes were used in a concentration of 0.3 µM. The following temperature profile was chosen: reverse transcription at 50°C for 30 mins, reverse transcriptase inactivation and polymerase activation at 95°C for 15 mins, 42 cycles of denaturation for 30 s at 95°C, and annealing and elongation for 60 s at 60°C. A Stratagene Mx3005P (Thermo Scientific) was used for the fluorescence measurement.

Data analyses

The sensitivity and specificity, as well as the positive (PPV) and negative predictive values (NPV), were calculated for the whole group, as well as only for cats with neurological and/or ocular signs. Ninety-five percent confidence intervals were determined. Data analyses were performed using a two-sided Fisher's exact test with GraphPad Prism Version 5.0 and a significance threshold of 0.05.

Results

Results of the real-time RT-PCR in cats with neurological and/or ocular signs, and all cats are shown in Table 3. Threshold cycle (Ct) values of all real-time RT-PCR results are shown in Tables 1 and 2. Sensitivity, specificity, and PPV and NPV of real-time RT-PCR of CSF are shown in Table 4. None of the specimens were false positive in the real-time RT-PCR of CSF, leading to a specificity of 100%. Sensitivity was only 42.1% when looking at all cats, but was better when the results of only cats with neurological and/or ocular signs were evaluated (Table 4).

Discussion

The purpose of this study was to determine sensitivity and specificity of a real-time RT-PCR on CSF samples in order to assess the diagnostic feasibility of this method for the ante-mortem diagnosis of FIP.

FCoV can be detected in the CSF if the virus replicates in CSF macrophages in cats with FIP with neurological involvement, or if a spillover of infected blood monocytes occurred during the disease or the tap. The entry route for FCoV into the CSF is unknown but the virus probably trespasses the blood–brain barrier (BBB) cell-bound within macrophages. As in other parts of the body, macrophages also resemble the principal target cells for FCoV in the CNS.[17] The other possibility for the presence of FCoV in the CNS, and therefore in CSF, is a non-targeted way through an impaired BBB or blood–CSF barrier that could be disrupted non-specifically during virtually any inflammation of the CNS.[22] Generally, in inflammatory states involving the CNS, mononuclear cells can enter both by opened tight junctions between endothelial cells and via diapedesis through endothelial cells.[23–26] Even if not specifically investigated in FIP, the brain endothelium produces inflammatory mediators, adhesion molecules and matrix metalloproteinases, which lead to a disruption of the tight junction complex allowing particles to cross the barriers.[22]

The specificity of the real-time RT-PCR in CSF in this study was 100%. While RT-PCR is commonly used in serum and plasma for the diagnosis of FIP, it is not a reliable tool for confirmation because specificities range only between 20% and 90%.[3,19,27–29] False positive RT-PCR results in serum and plasma can be caused by the fact that intestinal infection with harmless FCoV is accompanied by viraemia.[30,31] Recent studies determined mutations in different parts of the FCoV genome.[32,33] Detecting mutations in the putative fusion peptide of the spike protein of FCoV seems to be a more reliable tool for the diagnosis of FIP,[32] but large studies confirming specificity are still missing. The reason for the high specificity of the real-time RT-PCR used in the present study, which did not specifically detect the mutated virus, can be explained by an absence of FCoV in CSF if no inflammation and an intact BBB are present, and FCoV is not produced within the CNS. The presence of viral RNA therefore seems to be more reliable in diagnosing FIP than the presence of antibodies; in a previous study anti-coronavirus antibodies were detected in CSF of cats with FIP without neurological involvement, but also in the CSF of cats with neurological diseases other than FIP.[34] In this previous study it was postulated that the anti-coronavirus antibodies were derived from antibody-containing blood and did not necessarily indicate intrathecal antibody production and the presence of FCoV in the CNS. As many cats are FCoV antibody-positive in blood,[2,35] antibodies can easily cross the BBB in cats with any disruption of the BBB due to various diseases that impair the BBB or CSF flow. Thus, a method, like RT-PCR, detecting the pathogen itself instead of antibodies in CSF seems to be more specific. The results of the present study are in accordance with the only previous study that investigated RT-PCR in CSF. In this previous study, similar to the present one, only three cats with neurological disease other than FIP were investigated,[15] but the present study included a large number of non-neurological controls.

While the real-time RT-PCR in CSF in this study showed an excellent specificity, sensitivity was not as high (42.1%). Failure to detect FCoV in CSF in this real-time RT-PCR was most likely caused by the absence of CNS inflammation and FCoV-infected macrophages in the CSF. In the cats with neurological signs in which FCoV was detected in the CSF, Ct values were relatively high (mean Ct 30.8; range 26.5–32.6). This indicates that the FCoV numbers were relatively low, even if the CNS is involved. Nevertheless, the sensitivity of real-time RT-PCR in CSF, even when looking at all cats (with and without neurological involvement), was even higher in the present study than in a previous study with a sensitivity of 31% in cats with neurological involvement.[15]

In the present study, two cats with FIP with ocular but without neurological signs had detectable virus. As ocular signs often co-occur with neurological FIP,[11,17] these two cats could have had the beginning of neurological involvement, without clinical signs and pathological lesions. As the CNS and eyes are in close proximity to each other, a spillover of infected monocytes into CSF or monocyte homing might also be possible. Another two cats with FIP but without clinically obvious neurological or ocular signs had positive results in real-time RT-PCR in CSF. In one of these two cats, histological examination of the brain showed inflammatory infiltration with macrophages, granulocytes, plasma cells and lymphocytes, as well as necrotic lesions, which were obviously too mild to cause clinical signs. In the other cat, no visible gross or histological lesions were found in the CNS. However, it might be that this cat also had the beginnings of neurological involvement of FIP without visible changes of tissue.

One limitation of this study was the relatively low sample size, especially of cats with neurological and/or ocular involvement. Another limitation was the assignment of cats to the control group. Cats were assigned to the control group if a disease other than FIP was confirmed that explained the observed signs. There is a low probability that a cat in the control group suffered from both another disease as well as FIP; however, this situation was not likely as 13/15 cats were examined at necropsy and no false positive real-time RT-PCR result was observed in the present study.

Conclusions

This study evaluated the sensitivity and specificity of a real-time RT-PCR detecting FCoV in CSF to diagnose FIP in cats with and without neurological involvement. The study found an excellent specificity, indicating that real-time RT-PCR in CSF is a reliable tool for diagnosing FIP. The sensitivity of this approach was fairly high, at least in cats with neurological and/or ocular signs, making this an interesting tool for the diagnosis of neurological FIP.

Conflict of interest The authors do not have any potential conflicts of interest to declare.

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