

## Comparison of Different Tests to Diagnose Feline Infectious Peritonitis

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Clinical data from 488 cats (1979–2000) with histopathologically confirmed feline infectious peritonitis (FIP) and 620 comparable controls were evaluated retrospectively to assess the value of several diagnostic tests frequently used in the evaluation of cats with suspected FIP. Diagnostic utility of serum albumin to globulin ratio for the diagnosis of FIP was greater than of the utility of serum total protein and  $\gamma$ -globulin concentrations. Diagnostic utility of these variables was higher when performed on effusion. On effusion, positive and negative predictive values of Rivalta's test, a test that distinguishes between exudates and transudates (0.86 and 0.97), anti-coronavirus antibody detection (0.90 and 0.79), and immunofluorescence staining of coronavirus antigen in macrophages (1.00 and 0.57) were investigated. The positive and negative predictive values of presence of anti-coronavirus antibodies were 0.44 and 0.90, respectively, antibody concentrations (1 : 1,600) were 0.94 and 0.88, presence of immune complexes measured by a competitive enzyme-linked immunosorbent assay were 0.67 and 0.84, and detection of viral RNA by serum reverse-transcriptase polymerase chain reaction (RT-PCR) were 0.90 and 0.47. Effusion RT-PCR was performed in 6 cats; it was positive in all 5 cats with FIP and negative in the cat with another disease. Diagnostic assays on the fluid in cats with body effusion had good predictive values. Definitive diagnosis of FIP on the basis of measurement of various variables in serum was not possible. Serum tests can only be used to facilitate the decision for more invasive diagnostic methods.

**Key words:** Cat; Diagnosis; FCoV; Feline coronavirus; FIP.

**F**eline infectious peritonitis (FIP) is a common disease and a frequent reason for referral; approximately 1 of every 200 new feline cases presented to American Veterinary Teaching Hospitals represent cats with FIP.<sup>1</sup> FIP is a fatal, immune-mediated disease, triggered by infection with a feline coronavirus (FCoV).<sup>2</sup> FCoV belongs to the family *Coronaviridae*, a group of enveloped, positive-stranded RNA viruses that are frequently found in cats. Coronavirus-specific antibodies are present in 80–90% of cats in catteries and in 10–50% of those in single-cat households.<sup>3–6</sup> However, only 5–10% of FCoV-infected cats develop FIP in a cattery situation; a much lower incidence occurs in single-cat households.<sup>3,7</sup> FIP is a deadly disease with no effective long-term management. A rapid and reliable diagnosis is critical for prognostic reasons to lessen suffering

of affected patients while avoiding euthanasia of unaffected cats. Difficulties in definitively diagnosing FIP arise from nonspecific clinical signs, lack of pathognomonic hematological and biochemical abnormalities and low sensitivity and specificity of tests routinely used in practice. Diagnostic value of most of the described variables is only known in experimental settings, and some tests have not been widely used in clinical patients. The objective of this study was to determine the sensitivity and specificity, as well as positive and negative predictive values, of different clinicopathologic variables and virological methods used to diagnose FIP in clinical cases.

### Materials and Methods

#### Animals

The study was designed as a case control study. The study population included 1,108 cats that were examined for suspected FIP at the I. Medizinische Tierklinik, Munich, Germany, from 1979 to 2000. From this population, "cases" were defined by histopathologic diagnosis of FIP at postmortem examination (n = 488). "Controls" were cats with a definitive alternative diagnosis at postmortem examination or cats that survived longer than 12 months after the time point of presentation (n = 620). Because the prevalence of FIP and diagnostic evaluation differ on the basis of presenting clinical signs, cases were divided into 2 groups: cats with effusion (391/488; 80.1%) and cats with no visible fluids in the body cavities (either antemortem on radiographs or ultrasound or at postmortem examination) (97/488; 19.9%). Controls were assigned to either the effusion group (375/620; 60.5%) or the group without effusions (245/620; 39.5%).

In the study population (n = 1,108), prevalence of FIP in the cats with effusion (n = 766) was 51% (391 cases; 375 controls); prevalence of FIP in the cats without effusion (n = 342) was 28% (97 cases; 245 controls).

All available data of the study population were examined. Clinical variables measured in these cats varied over time but were not influenced by stage of disease. Variables that were statistically evaluated included (1) total protein concentration,  $\gamma$ -globulin concentration, and albumin to globulin ratio in serum and in effusion; (2) Rivalta's test, presence of anti-FCoV antibodies, and immunofluorescence staining of FCoV antigen in macrophages in effusion (qualitative parameters performed in effusion); and (3) presence of anti-FCoV antibodies (any

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titer), presence of highest anti-FCoV antibody titer (1:1,600), antigen antibody complexes, and nested FCoV reverse transcriptase polymerase chain reaction (RT-PCR) in serum (qualitative parameters performed in serum).

### ***Histopathologic Diagnosis of Feline Infectious Peritonitis***

Histopathologic confirmation of FIP was used to define cases and as the “gold standard” for diagnostic test comparisons. Diagnosis of FIP was achieved in cats with effusions or yellow to white foci or nodules in different organs plus presence of typical histologic lesions, including plasmacellular perivasculitis, accumulation of plasma cells with a necropurulent center, or both. Typical lesions consisted of an arteriole or venule surrounded by a central area of necrosis that, in turn, was surrounded by proliferation macrophages and lymphocytes, plasma cells, and neutrophils.<sup>8</sup>

### ***Total Protein and Protein Fractions in Serum and Effusion***

**Total Protein Concentration.** Total protein concentration was determined in serum of 314 cats (230 cases, 84 controls) and in effusion of 64 cats (34 cases, 30 controls). Total protein was measured by an automatic analyzer (1979–1996: Hitachi 705<sup>a</sup>; 1996–2000: Hitachi 717<sup>b</sup>) by the Biuret method.

**$\gamma$ -Globulin Concentration.**  $\gamma$ -Globulin concentration was determined in serum of 133 cats (86 cases, 47 controls) and in effusion of 64 cats (34 cases, 30 controls). Protein electrophoresis was performed by cellulose acetate electrophoresis. Proteins were separated (23 minutes, 240 volts) in an electrophoretic chamber;<sup>c</sup> subsequently stained for 10 minutes in Red Ponceau<sup>d</sup> (0.5 g in 100 mL of 5% trichloroacetic acid), destained in several steps (3 minutes each) with acetic acid solutions, and dried for 10 minutes at 120°C. Protein fractions were calculated with a photometer.<sup>e</sup>

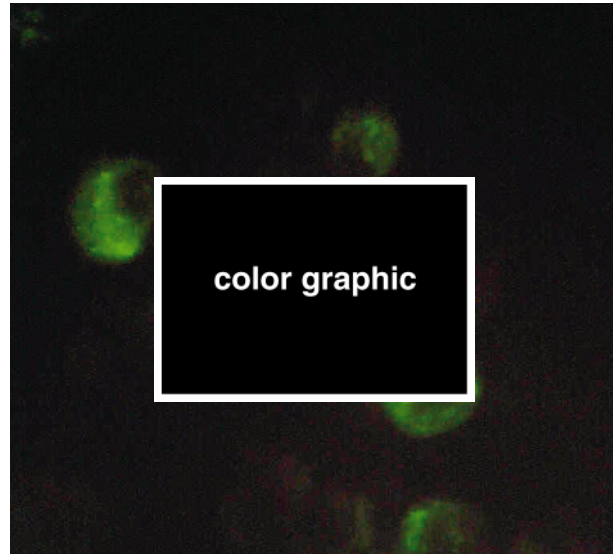
**Albumin to Globulin Ratio.** Albumin to globulin ratio was determined in serum of 118 cats (85 cases, 33 controls) and in effusion of 64 cats (34 cases, 30 controls). Ratio was calculated by dividing the albumin concentration by the concentration of all globulins; both were measured electrophoretically.

### ***Anti-FCoV Antibodies in Serum and Effusion***

Anti-FCoV antibody test was performed in serum of 342 cats without effusion (97 cases, 245 controls) and in effusion of 193 cats (119 cases, 74 controls). Antibodies were detected by an immunofluorescence assay (IFA) that had been developed by Osterhaus et al.<sup>9</sup> Porcine thyroid cells infected with a transmissible gastroenteritis virus (TGEV) strain (Purdue strain) were used as antigen. Fluorescein isothiocyanate-labeled rabbit anti-cat IgG immunoglobulin preparation<sup>f</sup> was used with a fluorescence microscope to detect the presence of anti-FCoV antibodies. The result (“titer”) was consistent with the highest dilution (1:25, 1:100, 1:400, 1:1,600) in which fluorescence was detectable.

### ***Rivalta's Test in Effusion***

Rivalta's test was performed in effusion of 285 cats (163 cases, 122 controls). The Rivalta's test can be used to differentiate transudates from exudates.<sup>10</sup> A reagent tube was filled with 5 mL distilled water; 1 drop of acetic acid (98%) was added, and the tube content was thoroughly mixed. On the surface of this solution, 1 drop of the effusion fluid was carefully layered. If the drop disappeared and the solution remained clear, the Rivalta's test was defined as negative. If the drop retained its shape, stayed attached to the surface, or slowly floated down to the bottom of the tube (drop- or jelly fish-like), the Rivalta's test was defined as positive.



**Fig 1.** Positive immunofluorescent staining of FCoV antigen in macrophages of peritoneal fluid.

### ***Immunofluorescent Staining of FCoV Antigen in Macrophages in Effusion***

Immunofluorescent staining of intracellular FCoV antigen in macrophages was performed in effusion of 171 cats (109 cases, 62 controls). This method is similar to a test described by Parodi et al.<sup>11</sup> Effusion fluid was centrifuged at  $1,500 \times g$ , and cell-rich pellet material was placed on a slide. Presence of FCoV antigen in the smears was detected by immunofluorescent staining. Smears were dried at room temperature and fixed in acetone at 20°C for 30 seconds. Smears were covered with a conjugate solution containing anti-FCoV antibodies. Antibodies had been obtained through precipitation with polyethylene glycol of filtered feline ascitic fluid (with an anti-FCoV titer of 1:1,600). Purified antibodies were coupled with fluorescein isothiocyanate. Evaluation of the smears was performed with a fluorescence microscope. Samples were positive if a granular cytoplasmic fluorescence in macrophages was present (Fig 1).

### ***Antigen Antibody Complex Detection in Serum***

Antigen antibody complex detection was performed in serum of 112 cats without effusion (29 cases, 83 controls) with a competitive enzyme-linked immunosorbent assay (ELISA). Cat sera were treated with polyethylene glycol in order to precipitate potentially present antigen antibody complexes. Supernatant was removed, and polyethylene glycol in a phosphate buffer was added a 2nd time to resuspend the immune complexes. Buffered solution was pipetted into a microtiter plate containing a FIP-producing FCoV strain (DF-2 WT FIPV) and was incubated. DF-2 WT FIPV was originally isolated from the liver of a cat with FIP and had been passaged 1st in several specific pathogen-free cats and subsequently in Norden Laboratories feline kidney cells at 39°C (passage 1–60) and at 31°C (passage 61–99).<sup>12</sup> A monoclonal antibody directed against the glycoprotein of the DF-2 WT FIPV was added that competed with the antibodies of the complexes in the plate wells. After a washing step, a peroxidase-labeled antibody (goat anti-mouse IgG) binding to the monoclonal antibodies was added to the wells. Extinction of the color reaction was measured photometrically. The more antigen antibody complexes had been in the serum, the less monoclonal antibodies were bound to the antigen and the weaker the color reaction was. A weak color reaction (a low photometric extinction) was consistent with a positive test result; a strong color reaction (high photometric extinction) was interpreted as a neg-

**Table 1.** Specificity, sensitivity, and optimum cutoff value of different total protein concentrations (n = 314),  $\gamma$ -globulin concentrations (n = 133), and albumin to globulin ratios (n = 118) in serum.

Total Protein (g/dL)	Total Protein		$\gamma$ -Globulin			Albumin to Globulin Ratio		
	SP	SE	$\gamma$ -Globulin (g/dL)	SP	SE	Albumin to Globulin Ratio	SP	SE
5.0	0.05	0.96	0.5	0.28	0.98	0.5	0.92	0.50
6.0	0.15	0.88	0.1	0.57	0.80	0.6	0.87	0.75
7.0	0.33	0.76	1.5	0.72	0.72	0.7	0.85	0.77
8.0 <sup>a</sup>	0.60	0.62	2.0	0.82	0.71	0.8 <sup>a</sup>	0.82	0.80
9.0	0.83	0.38	2.5 <sup>a</sup>	0.86	0.70	0.9	0.78	0.85
10.0	0.91	0.24	3.0	0.93	0.45	1.0	0.68	0.90
11.0	0.96	0.12	3.5	0.95	0.40			
12.0	0.97	0.10	4.0	0.97	0.35			
			4.5	0.99	0.15			

SP, specificity; SE, sensitivity.

<sup>a</sup> Optimum cutoff value as determined by differential positive rate analysis.

ative test result. A test was positive if a binding of monoclonal antibodies was prevented by at least 35% because of the presence of antigen antibody complexes.

### *Polymerase Chain Reaction (Nested RT-PCR) in Serum and Effusion*

Nested RT-PCR was performed in serum of 25 cats without effusion (17 cases, 8 controls) and in the effusion of 6 cats (5 cases, 1 control). To detect RNA by PCR, serum and effusion samples were stored at  $-20^{\circ}\text{C}$ . Effusion fluid was diluted 1:100 in phosphate-buffered saline (PBS). Viral RNA from 100  $\mu\text{L}$  serum or diluted effusion fluid was concentrated by guanidinium thiocyanate-silica ( $\text{SiO}_2$ ) with a protocol described by Boom et al.<sup>13</sup> and the modification of 20  $\mu\text{L}$  of the 50%  $\text{SiO}_2$  suspension instead of 40  $\mu\text{L}$ . The RNA was eluted from the silica pellet with 25  $\mu\text{L}$  of TE (10 mM Tris-HCl [pH 8.0], 0.5 mM edetic acid) by a 10-minute incubation at room temperature followed by a 10-minute incubation at  $65^{\circ}\text{C}$ . The silica was pelleted by centrifugation, and the supernatant fraction was used in the RT reaction. The nested RT-PCR with the use of oligonucleotide primers from the highly conserved 3'-untranslated region (3'-UTR) of the FCoV genome has been described by Herrewegh et al.<sup>14</sup> Samples showing a band of 177 base pairs after the nested PCR were considered positive for FCoV RNA.

### *Statistical Analysis*

Characteristics used to compare the diagnostic utility of the different clinicopathologic parameters and virological methods were the diagnostic sensitivity (proportion of positive test results in infected animals), the diagnostic specificity (proportion of negative test results in noninfected animals), the positive predictive value (PPV; probability that a test-positive animal is infected), and the negative predictive value (NPV; probability that a test-negative animal is noninfected). The gold standard for diagnosis of FIP was the histopathology result. Because the pretest likelihood of FIP diagnosis was different between cats with effusion and those without, PPV and NPV were corrected and calculated for the appropriate population (prevalence of 0.51 for cats with effusion; prevalence of 0.28 for cats without effusion) for appropriate comparison ( $\text{PPV} = \text{sensitivity} \cdot \text{prevalence} / [\text{sensitivity} \cdot \text{prevalence} + (1 - \text{specificity})(1 - \text{prevalence})]$ ;  $\text{NPV} = \text{specificity}(1 - \text{prevalence}) / [\text{specificity}(1 - \text{prevalence}) + \text{prevalence}(1 - \text{sensitivity})]$ ).

To evaluate the optimum cutoff and diagnostic utilities of total protein,  $\gamma$ -globulin and albumin to globulin ratio (in serum and effusion) measurements, receiver operating characteristics (ROC) curve analysis was used. Calculation of the optimum cutoff values for total protein,

$\gamma$ -globulin, and albumin to globulin ratio (in serum and effusion) to differentiate between cats with FIP and cats with other diseases was performed with the use of a differential positive rate (DPR) curve ( $\text{DPR} = \text{sensitivity} - [1 - \text{specificity}]$ ).<sup>15</sup> When the optimum cutoff value for diagnosis was identified, likelihood ratios (LRs) were determined for this cutpoint ( $\text{LR} = \text{proportion of true positive results} / \text{proportion of false-positive results}$ ) on each test to compare the diagnostic utility of the tests.<sup>16</sup> Likelihood ratios were also used to compare the diagnostic utility of specific tests on serum and effusion.

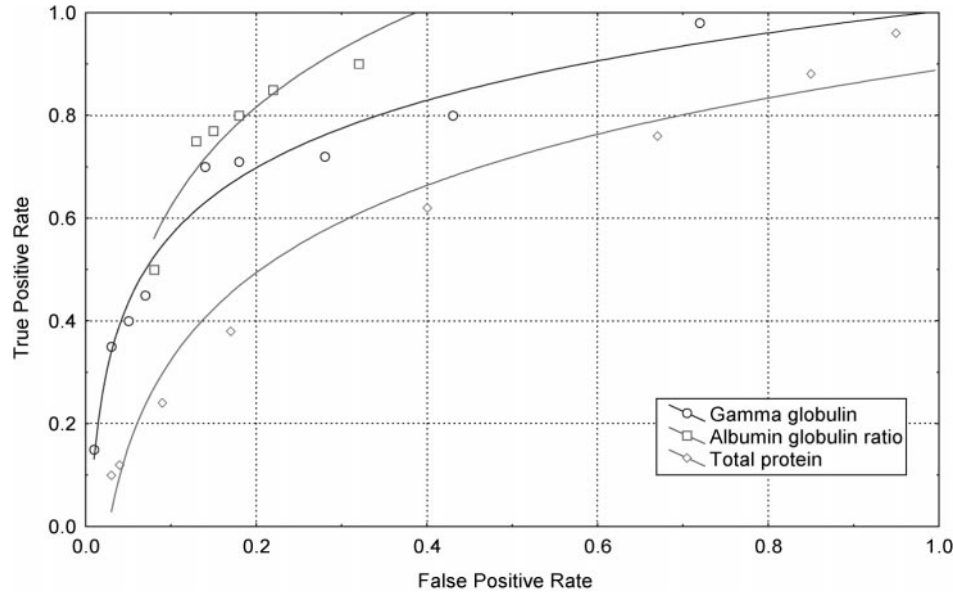
## **Results**

### *Total Protein and Protein Fractions in Serum and Effusion*

Total protein concentration in serum was determined in 314 cats with a FIP prevalence of 0.73 (230 cases, 84 controls). Serum total protein was more likely recorded in cats with FIP than in cats without FIP, and this altered the observed FIP prevalence in this group. Concentration of  $\gamma$ -globulin was determined in 133 cats (FIP prevalence 0.65; 86 cases, 47 controls) and albumin to globulin ratio in 118 cats (FIP prevalence 0.72; 85 cases, 33 controls). Specificity and sensitivity of total protein concentrations from 5.0 to 12.0 g/dL, of  $\gamma$ -globulin concentration from 0.5 to 4.5 g/dL, and of albumin to globulin ratios from 0.5 to 1.0 differed (Table 1), with the albumin to globulin ratio having the highest diagnostic value (Fig 2).

Predictive values of diagnostic tests are influenced by the pretest likelihood of disease (prevalence) in the tested population. Predictive values for total protein concentrations,  $\gamma$ -globulin concentrations, and albumin to globulin ratios differed depending on the pretest likelihood of disease (Table 2).

Specificity, sensitivity, PPV and NPV of total protein concentration,  $\gamma$ -globulin concentration, and albumin to globulin ratio were determined in effusions from 64 cats (FIP prevalence 0.53; 34 cases, 30 controls; Table 3). DPR curves determined an optimum cutoff value for the total protein concentration of 8.0 g/dL in serum (DPR 0.22) and in effusion (DPR 0.45). Optimum cutoff values are those values that, if used as a cutoff, have the highest number of correct predictions (true positive and true negative predictions). Optimum cutoff values for the  $\gamma$ -globulin concen-



**Fig 2.** Calculated ROC curves for total protein concentration,  $\gamma$ -globulin concentration, and albumin to globulin ratio in serum.

tration were 2.5 g/dL in serum (DPR 0.56) and 1.0 g/dL in effusion (DPR 0.65). The albumin to globulin ratio had optimum cutoff values at 0.8 in serum (DPR 0.68) and 0.9 in effusion (DPR 0.60).

Likelihood ratios calculated with the use of the optimal cutoff values determined by DPR analysis revealed that the diagnostic utility of protein and protein fraction tests were different between serum and effusion. In serum, the albumin to globulin ratio had the greatest diagnostic utility (LR 3.9) in the study population, followed by  $\gamma$ -globulin (LR 3.0) and total protein (LR 1.6) concentration. A serum albumin to globulin ratio  $>0.8$  g/dL was nearly 4 times more likely among cats with FIP than among those without. In contrast, total protein measures on effusion had the highest diagnostic utility (LR 5.5). Similarly, a  $\gamma$ -globulin concentration  $>0.1$  g/dL in effusion was nearly 5 times more likely (LR 4.8) among cases compared to controls. In effusion, the albumin to globulin ratio had the lowest diagnostic utility (LR 3.3).

### Specific Tests in Effusion

Diagnostic tests performed in effusion samples included presence of anti-FCoV antibodies in the effusion, Rivalta's test, immunofluorescent staining of FCoV antigen in macrophages of the effusion, and RT-PCR. Effusion RT-PCR was performed in 6 cats; it was positive in all 5 cats with FIP, and it was negative in the cat diagnosed with a different disease (malignant lymphoma).

Results of analysis of anti-FCoV antibodies in the effusion, Rivalta's test, and immunofluorescent staining of FCoV antigen in macrophages of the effusion are shown in Table 4. The diagnostic utility of the anti-FCoV antibodies (LR 5.7) was slightly greater than the diagnostic utility of the Rivalta's test (LR 4.9). Both of these tests had a higher proportion of correct predictions (0.90 and 0.85, respectively) than the immunofluorescent staining of intracellular FCoV antigen in macrophages. However, specificity and, therefore, positive predictive value reached 1.0 in the an-

**Table 2.** Positive predictive values of different total protein concentrations,  $\gamma$ -globulin concentrations, and albumin to globulin ratios in serum in populations with different FIP prevalences.

Total Protein (g/dL)	Total Protein			$\gamma$ -Globulin (g/dL)	$\gamma$ -Globulin			Albumin to Globulin Ratio	Albumin to Globulin Ratio		
	PPV (0.25)	PPV (0.50)	PPV (0.75)		PPV (0.25)	PPV (0.50)	PPV (0.75)		PPV (0.25)	PPV (0.50)	PPV (0.75)
5.0	0.25	0.50	0.75	0.5	0.31	0.58	0.80	0.5	0.68	0.86	0.96
6.0	0.26	0.51	0.76	0.1	0.38	0.65	0.85	0.6	0.66	0.85	0.95
7.0	0.27	0.53	0.77	1.5	0.46	0.72	0.89	0.7	0.63	0.84	0.94
8.0	0.34	0.61	0.82	2.0	0.57	0.80	0.92	0.8	0.60	0.82	0.93
9.0	0.43	0.69	0.88	2.5	0.63	0.83	0.94	0.9	0.56	0.79	0.92
10.0	0.47	0.73	0.91	3.0	0.68	0.87	0.95	1.0	0.48	0.74	0.89
11.0	0.50	0.75	0.92	3.5	0.73	0.89	0.96				
12.0	0.53	0.77	0.93	4.0	0.80	0.92	0.97				
				4.5	0.83	0.94	0.98				

FIP, feline infectious peritonitis; PPV, calculated positive predictive value in a population with a FIP prevalence of 0.25, 0.50, or 0.75.

**Table 3.** Specificity, sensitivity, positive predictive value, negative predictive value, and optimum cutoff value of different total protein concentrations,  $\gamma$ -globulin concentrations, and albumin to globulin ratios in effusion (n = 64; prevalence 0.53).

Total Protein (g/dL)	Total Protein				$\gamma$ -Globulin (g/dL)	$\gamma$ -Globulin				Albumin to Globulin Ratio	Albumin to Globulin Ratio			
	SP	SE	PPV	NPV		SP	SE	PPV	NPV		SP	SE	PPV	NPV
5.0	0.10	1.00	0.56	1.00	0.5	0.47	0.94	0.67	0.87	0.5	0.89	0.62	0.86	0.76
6.0	0.33	0.88	0.60	0.71	0.1 <sup>a</sup>	0.83	0.82	0.84	0.80	0.6	0.85	0.67	0.83	0.70
7.0	0.53	0.82	0.66	0.72	1.5	0.93	0.65	0.91	0.70	0.7	0.82	0.69	0.81	0.71
8.0 <sup>a</sup>	0.90	0.55	0.78	0.62	2.0	0.97	0.44	0.94	0.61	0.8	0.79	0.78	0.80	0.68
9.0	0.93	0.32	0.84	0.55	2.5	0.99	0.35	0.98	0.57	0.9 <sup>a</sup>	0.74	0.86	0.79	0.82
10.0	0.95	0.23	0.85	0.52	3.0	1.00	0.26	1.00	0.55	1.0	0.65	0.94	0.75	0.91
11.0	0.98	0.12	0.87	0.50										
12.0	0.99	0.07	0.89	0.49										

SP, specificity; SE, sensitivity; PPV, positive predictive value; NPV, negative predictive value.

<sup>a</sup> Optimum cutoff value as determined by differential positive rate analysis.

tigen staining (meaning that if this test is positive, the likelihood of FIP is 100%).

**Specific Tests in Serum**

Anti-FCoV antibody test was performed in the serum of 342 cats with a FIP prevalence of 0.28 (Table 5). Sensitivity, specificity, PPV, and NPV of the presence of anti-FCoV antibodies (independent of their concentration) were evaluated (any titer was defined as positive; if there were no detectable antibodies it was defined as negative). In addition, the diagnostic utility of using the highest titer measured in the assay (1 : 1,600) as the cutpoint for diagnosis was determined (a titer of 1 : 1,600 was defined as positive; no antibodies or any titer <1 : 1,600 was defined as negative). Low and medium titers (1 : 25, 1 : 100, 1 : 400) were not of any diagnostic value. Antigen antibody complex detection was performed in serum of 112 cats with a FIP prevalence of 0.26. Nested RT-PCR was performed in serum of 25 cats with a FIP prevalence of 0.68.

The presence of the highest (1 : 1,600) antibody titer had

**Table 4.** Comparison of diagnostic tests performed in effusion samples (presence of anti-FCoV antibodies in the effusion, Rivalta’s test, and immunofluorescent staining of FCoV antigen in macrophages of the effusion).

	Presence of Antibodies	Rivalta’s Test	Antigen Staining in Macrophages
Number of cats	193	285	171
Prevalence	0.62	0.57	0.64
Portion of correct test results	0.85	0.90	0.73
Specificity	0.85	0.80	1.00
Sensitivity	0.86	0.98	0.57
Positive predictive value	0.90	0.86	1.00
Negative predictive value	0.79	0.97	0.57
PPV (0.51)	0.86	0.84	1.00
NPV (0.51)	0.85	0.97	0.69

FCoV, feline coronavirus; FIP, feline infectious peritonitis; PPV (0.51), calculated positive predictive value in a population with a FIP prevalence of 0.51; NPV (0.51), calculated negative predictive value in a population with a FIP prevalence of 0.51.

significantly better diagnostic utility than any other test evaluated (LR 33.5). The diagnostic utility of the presence of antigen antibody complexes and serum RT-PCR was similar (LR 5.3 and 4.4, respectively). The presence of antibodies (any titer) was the least useful of these tests (LR 2.0). The highest specificity and PPV values were reached by the presence of the highest (1 : 1,600) antibody titer, followed by antigen antibody complex detection and serum RT-PCR.

**Discussion**

Feline infectious peritonitis is often misdiagnosed.<sup>17</sup> Many times, its general clinical signs (eg, chronic fever, weight loss, anorexia, and malaise) are nonspecific. Clinicopathologic changes in FIP (lymphopenia, neutrophilia, anemia, hyperproteinemia, and hypergammaglobulinemia)<sup>18,19</sup> are not pathognomonic. Other clinicopathologic parameters (liver enzyme activities, bilirubin, BUN, creatinine) can be variably increased depending on degree and localization of organ damage<sup>20,21</sup> but are not helpful in making an etiologic diagnosis. In many clinical cases, definitively diagnosing FIP antemortem can be difficult. The aim of this study was to determine sensitivity and specificity as well as PPV and NPV of different clinicopathologic parameters and virologic methods by retrospectively evaluating data of 1,108 cats that had been worked up for FIP, including 488 cats with histopathologically confirmed FIP and 620 representative controls, in order to help clinicians interpret tests results.

Of 488 cats with FIP, 80.1% had effusions. This percentage is comparable to the results of Walter and Rudolph<sup>22</sup> who found effusion in 84% of their cats with FIP, but is higher than in a study of Lutz et al.<sup>23</sup> in which effusion was only found in 60% of cats with FIP that were investigated at postmortem examination. The percentage of 80.1% might not reflect the actual situation in clinical practice, because only postmortem examination confirmed cases of FIP were included in the study. Clinically evident effusion raises the probability of FIP. Thus, cases of FIP without visible effusion might be underrepresented.

In this study, cats with FIP were compared to control

**Table 5.** Comparison of diagnostic tests performed in serum samples of cats without effusions (presence of anti-FCoV antibodies [any titer], highest anti-FCoV antibody titer [1:1600], antigen antibody complexes, and serum RT-PCR to detect FCoV RNA in serum).

	Presence of Antibodies	Antibody Titer 1:1,600	Antigen Antibody Complexes	PCR
Number of cats	342	342	112	25
Prevalence	0.28	0.28	0.26	0.68
Portion of correct test results	0.65	0.89	0.80	0.64
Specificity	0.57	0.98	0.91	0.88
Sensitivity	0.85	0.67	0.48	0.53
Positive predictive value	0.44	0.94	0.67	0.90
Negative predictive value	0.90	0.88	0.84	0.47
PPV (0.28)	0.44	0.94	0.67	0.63
NPV (0.28)	0.90	0.88	0.82	0.83

FCoV, feline coronavirus; FIP, feline infectious peritonitis; PPV (0.28), calculated positive predictive value in a population with a FIP prevalence of 0.28; NPV (0.28), calculated negative predictive value in a population with a FIP prevalence of 0.28; RT-PCR, reverse transcriptase polymerase chain reaction.

groups in which, as a result of history and clinical findings, FIP was seriously considered as differential diagnosis by the clinician, but in which postmortem examination revealed other diseases or that survived longer than 12 months after presentation. The rationale for including surviving cats in the control group was the fact that a cat suffering from clinically evident FIP will not live for 12 months, as there is no effective treatment<sup>24</sup> and the course of disease results in death within this time period. In previous retrospective studies to identify serologic, biochemical, and hematological markers of FIP, there were differences in the way the data were evaluated and in the prevalence of disease in the populations examined.<sup>18,20,25–28</sup> In this study, cases in which FIP was considered a major differential diagnosis were selected as control for comparison. As a consequence, the prevalence might be different than in other studies, a fact that dramatically alters the predictive values. The purpose of this selection was to identify predictive values that mimic the real situation in which a clinician has FIP as a differential diagnosis in mind. This is more representative than studies in which the whole clinic population, or healthy cats, or cats with 1 specific disease other than FIP are used as control groups.

It has been shown in previous studies that the most consistent clinicopathologic finding in cats with FIP is an increase in total serum protein concentration, a finding in about 50% of the cats with effusion and 70% of cats without effusion.<sup>27</sup> This increase in total protein is caused by increased globulins, mainly  $\gamma$ -globulins, and is characterized by a decrease in the albumin to globulin ratio.<sup>25,26</sup> In experimental infections, an early increase of  $\alpha_2$ -globulins was reported,<sup>29</sup> whereas  $\gamma$ -globulins and antibody titers increase just before appearance of clinical signs.<sup>3,29–31</sup> The characteristically high concentrations of  $\gamma$ -globulins<sup>32</sup> and the increased antibody titers<sup>3,33,34</sup> invite the conclusion that hypergammaglobulinemia is due to a specific anti-FCoV immune response. Antibody titers and hypergammaglobulinemia show a linear correlation, but the wide variation in anti-FCoV titers at a given concentration of  $\gamma$ -globulins indicates that additional (immune-mediated) reactions occur in the pathogenesis of FIP<sup>34</sup> and that other mechanisms like

IL-6-induced activation of plasma cells contribute to the hypergammaglobulinemia.<sup>35</sup>

In this study, comparison (ROC curve analysis and LR) of total serum protein concentration,  $\gamma$ -globulin concentration, and albumin to globulin ratio revealed that the albumin to globulin ratio has better diagnostic utility than the 2 other variables. An optimum cutoff value (maximal efficiency) of 0.8 was determined for the albumin to globulin ratio (DPR curve analysis). Other studies suggest similar cutoff values. In a study of Shelly et al.,<sup>25</sup> a cutoff of 0.8 also was proposed, whereas Duthie et al.<sup>28</sup> determined a cutoff of 0.7. Although the cutoff established by DPR curve analysis gives the optimum discrimination value (on the basis of both specificity and sensitivity), in FIP, high PPV is more important than high NPV because, in most cases, diagnosis of FIP leads to euthanasia. A decrease of the cutoff value for the albumin to globulin ratio could increase the PPV (eg, if the albumin to globulin ratio is  $<0.5$ , the likelihood of FIP is 68, 86, and 96% in populations with a FIP prevalence of 0.25, 0.50, and 0.75, respectively) but would decrease the NPV.

For total protein, the optimum cutoff determined was 8.0 g/dL in serum; however, the DPR (0.22) and LR (1.6) values are low for this test. Serum total protein concentration is not a good diagnostic test for FIP. Even if the serum protein concentration is  $\geq 12$  g/dL, the likelihood of FIP is only 53, 77, and 93% (in populations with a FIP prevalence of 0.25, 0.50, and 0.75, respectively). Cats in this study with serum protein concentrations of 12 g/dL and higher that did not have FIP suffered from severe chronic stomatitis (probably because of chronic antigen stimulation in chronic calicivirus infection), chronic upper respiratory disease or multiple myeloma. The  $\gamma$ -globulin concentrations did not have higher diagnostic values than total protein concentrations. Optimum cutoff value determined for  $\gamma$ -globulin was 2.5 g/dL.

In effusion, the diagnostic utility of the total protein concentration, the  $\gamma$ -globulin concentration, and the albumin to globulin ratio tended to be higher than for serum. The optimum cutoff value for total protein in effusion was 8.0 g/dL as well (with a higher DPR and LR and, therefore, better

diagnostic value than in serum); it was 1.0 g/dL for the  $\gamma$ -globulin concentration and 0.9 for the albumin to globulin ratio.

Variables with a very high PPV are the most important tools because they predict the likelihood that this cat actually has FIP, and this is what the clinician needs to know. However, the PPV is dependent on the prevalence of disease in the population being tested, and this is markedly altered by the clinical presentation and diagnostic judgement of the attending clinician. Therefore, PPVs were calculated for different theoretical cat populations (Table 2). This illustrates the point that, in populations with different disease prevalences, the same total protein concentration has a completely different meaning. For example, a serum total protein value of 8.0 g/dL in a population with a FIP prevalence of 0.25 predicts FIP with a PPV of 0.34 (likelihood of FIP 34%); in a population with a FIP prevalence of 0.75, the same protein concentration has a PPV of 0.82 (likelihood of FIP 82%).

Although effusions of clear yellow color and sticky consistency are often called “typical,” the presence of fluid in body cavities alone is not diagnostic. Only about half of the cats with effusions suffer from FIP.<sup>36</sup> Even if the fluid seems to be typical, other diseases (eg, heart failure) cannot always be easily excluded. A simple test, the so-called Rivalta’s test, has been used to differentiate transudates from exudates.<sup>10</sup> This test, however, is not sensitive to detect exudates in dogs.<sup>37</sup> It is not only the high protein content, but high concentrations of fibrin and inflammatory mediators that induce a positive reaction. Therefore, this test seems to be useful in cats to differentiate between effusion from FIP and effusions caused by other diseases. In this study, Rivalta’s test had a PPV of 0.86 and a NPV of 0.97, both very high. There were some false-positive results in cats with bacterial peritonitis. Those effusions, however, are usually easy to differentiate (through macroscopic examination, cytology, and bacterial culture). Some cats with malignant lymphoma also reacted positively in the Rivalta’s test, but many of them can also be differentiated cytologically by the presence of malignant lymphocytes. Overall, Rivalta’s test is an easy, inexpensive method that does not require special laboratory equipment and can be easily done in private practice. It provides very good predictive values and therefore is a helpful diagnostic parameter.

Antibody titers measured in serum are an extensively used diagnostic tool.<sup>4,38</sup> However, in view of the fact that a large percentage of the healthy cat population is FCoV antibody-positive, that high and rising titers are frequently found in asymptomatic cats, and that most of those cats will never develop FIP, antibody titers must be interpreted extremely critically.<sup>5,6,18</sup> From the time the 1st FIP antibody test was described more than 2 decades ago<sup>3</sup> to the present, the inadequacies and pitfalls of the test have been the topic of continuous discussions and controversy. Meanwhile, the so-called FIP test is referred to as “feline coronavirus antibody test,” emphasizing that the latter more correctly describes the detection of antibodies that react with a large group of closely related coronaviruses.<sup>31</sup>

One purpose of this study was to examine whether anti-FCoV antibodies have any diagnostic value. It is suggested that medium titers do not have any value to diagnose FIP,

as was confirmed in this study in which low and medium titers (1:25, 1:100, 1:400) had low predictive value and were therefore not statistically evaluated. However more specifically, the likelihood that a negative titer excludes FIP was investigated. Also, the question was raised whether there is any predictive value if the highest measurable titer is reached (which was 1:1,600 in this study). And finally, diagnostic value of antibody detection in fluids other than serum (eg, effusion) was determined.

If antibodies (any titer) were measurable, 44% of the cats actually had FIP (PPV 0.44). This is not a very high predictive value given the fact that it is close to the prevalence of 0.28 (which means that 28% of the tested population had FIP irrespective of their titer result). If the anti-FCoV antibody test was negative, 90% of the cats did not have FIP. This means, that 10% of the cats without antibodies actually had FIP. In these cats, antibodies were probably bound in antigen antibody complexes and therefore were not detectable. It has been shown, for instance, that in cats with fulminant FIP, titers decrease terminally.<sup>31</sup> Therefore, even negative test results do not exclude FIP. In addition, it is important to mention that the tests that are used in a specific laboratory will affect the results markedly. The antigen used in a test can play an important role in test sensitivity and specificity. In this study, the antigen was derived from TGEV, a pig virus, and this could lower the sensitivity of the test compared to tests in which a feline antigen is used. Thus, it is essential that antibody results interpreted and compared by the clinician are always obtained with the same method performed by the same laboratory, and it is essential to use antibody (and RT-PCR) tests validated by the scientific community.

If an antibody titer of 1:1,600 was present, the probability of FIP was 94% (PPV 0.94). Concerning the proportion of correct test results, the presence of the highest (1:1,600) antibody titer had the greatest diagnostic utility compared to total protein,  $\gamma$ -globulin, albumin to globulin ratio, the presence of antibodies (any titer), antigen antibody complex detection, or serum RT-PCR. It was shown in this study that low and medium titers are of no diagnostic value, negative titers have limited value, but at least the highest titer (if present) raises the probability of FIP considerably. However, highest titers were only present in 36 cats in this study.

Some studies evaluated the diagnostic utility of antibody detection in fluids other than the serum (eg, in CSF<sup>39</sup> or effusions).<sup>40</sup> Although coronavirus antibody detection in CSF seems to be of diagnostic value,<sup>39</sup> Kennedy et al.<sup>40</sup> found antibody titers in body effusions not helpful because all cats in their study had medium antibody titers irrespective of whether they had FIP or not. In this study, however, detection of anti-FCoV antibodies (any titer) in effusion had both a high PPV (0.90) and a high NPV (0.79). Magnitude of titer was not correlated with the presence of FIP. There was almost no difference between the diagnostic utility of the presence of anti-FCoV antibodies and the Rivalta’s test in effusion.

Compared with serology, RT-PCR provides the obvious advantage of directly detecting the ongoing infection rather than documenting a previous immune system encounter with a coronavirus. Because the critical mutation does not

always occur at the same location, although in a specific area of the FCoV genome, it is not possible to distinguish between a mutated and a nonmutated virus by PCR.<sup>41</sup> In this study, the PPV of serum RT-PCR was 0.90, the NPV was 0.47 (if corrected for a disease prevalence of 0.28, PPV was 0.63 and NPV was 0.83). Serum RT-PCR was not better (LR 4.4) than antigen antibody complex detection (LR 5.3). There are several plausible explanations for false-negative RT-PCR results. First, serum was used in this study. A higher success rate might have been obtained if plasma had been used instead. A dilution experiment in which plasma and serum samples from the same cat were compared showed that RT-PCR with plasma is more sensitive.<sup>14</sup> Although a direct comparison of RT-PCR in serum or plasma versus whole-blood or peripheral blood mononuclear cells has not been published, it could be argued that this would have given even better results. Second, the assay requires reverse transcription of viral RNA to DNA before amplification of DNA, and degradation of RNA could be a potential problem because RNases are virtually ubiquitous. Third, there may be sufficient strain and nucleotide sequence variation such that the target sequence chosen for this assay might not detect all strains of FCoV. However, the PCR assay used was targeted to the 3'-UTR of the viral genome. Because the nucleotide sequence of this region is highly conserved among various FCoV isolates,<sup>42-44</sup> the RT-PCR should detect most FCoV strains circulating in the cat population. There is also a number of explanations for false-positive serum RT-PCR results. First, the assay does not distinguish between "virulent" and "avirulent" FCoV strains, nor will it discriminate FCoV from canine coronavirus (CCV) and TGEV. Although the role of these viruses in the field is unknown, cats can be experimentally infected with CCV and TGEV<sup>45-47</sup>; these infections could result in a positive PCR result. Second, recent studies support the hypothesis that viremia not only occurs in cats with FIP but also in healthy carriers. FCoV RNA could be detected in the blood of cats with FIP but also in healthy cats that did not develop FIP for a period of up to 70 months.<sup>14,48-50</sup> In a study of Gunn-Moore et al.,<sup>30</sup> it was shown that in households in which FCoV is endemic, up to 80% of the cats can be viremic, irrespective of their health status, and that the presence of viremia does not appear to predispose the cats to the development of FIP. Therefore, the results of serum RT-PCR tests must be interpreted in conjunction with other clinical findings and cannot be used as the sole criteria for determining FIP. It also has to be considered that although the cats of the control groups in the present study did not show signs of FIP at postmortem examination, there is no reliable means to prove that they did not have early subclinical infection with a mutated FCoV. It is possible that the cats were infected before clinical evidence of FIP and that the stress of another chronic illness allowed sufficient viral replication to be detected in the assay.

RT-PCR of effusion samples can give better results, as indicated in a paper published by Egberink et al.<sup>48</sup> In this study, only 6 effusion samples were tested by RT-PCR. All results correctly diagnosed or ruled out FIP; the number of samples in this study, however, was too low to give a definitive answer or to compare with other tests. The results indicate, however, that this approach might be more prom-

ising than serum RT-PCR and that further field studies are needed to confirm or deny the statement of Egberink et al. that effusion RT-PCR is diagnostic.<sup>48</sup>

Another method to detect the virus is searching for the presence of FCoV antigen. In a study by Parodi et al.,<sup>11</sup> immunofluorescent assays detecting intracellular FCoV antigen in cells of effusion seemed to be useful; however, the number of cats enrolled in that study was limited. Hirschberger et al.<sup>36</sup> detected FCoV antigen in 34 of 34 samples from cats with FIP-induced effusions. Hök<sup>51</sup> was able to demonstrate FCoV antigen in the membrana nicticans of cats with FIP. Tammer et al.<sup>52</sup> used immunohistochemistry to detect intracellular FCoV antigen in paraffin-embedded tissue of euthanized cats and found FCoV antigen only in macrophages of cats that had FIP and not in control cats.

In this study, immunofluorescent staining of intracellular FCoV antigen in macrophages of the effusion was performed. Although the other tests carried out in effusion (Rivalta's test and anti-FCoV antibodies) had higher numbers of correct predictions (0.90 and 0.85, respectively), specificity and PPV of immunofluorescent staining of intracellular FCoV antigen in macrophages reached 1.00. There were no false-positive results. This means that if this staining test is positive, it predicts 100% that the cat has FIP. Unfortunately, the NPV was not very high (0.57). Cases that stained negative (although the cats had FIP) can be explained by the possibly insufficient number of macrophages on the effusion smear. Another explanation is a potential masking of the antigen by competitive binding of FCoV antibodies in the effusion that displace binding of fluorescent antibodies.

Because FIP is an immune-mediated disease and antigen antibody complexes play an important role, detecting circulating complexes in serum and effusions has been suggested.<sup>34,53</sup> Data to provide information about the usefulness of this approach as a diagnostic tool have not been published so far. In this study, detection of antigen antibody complexes with the use of a competitive ELISA was performed. The PPV of this test was 0.67; the NPV was 0.84. If calculated PPV and NPV in a (theoretical) population with a FIP prevalence of 0.28 were compared, values of the antibody antigen complex detection were very close to the values of the serum RT-PCR (PPV 0.67 and 0.63, respectively; NPV 0.82 and 0.83, respectively). This is considerably less than the PPV of the highest (1:1,600) antibody titer (PPV 0.94); this titer, however, was only present in 36 cats.

In effusion, all 3 tests compared in this study provide useful tools to diagnose FIP. If immunofluorescent staining of intracellular FCoV antigen is positive, it is diagnostic for FIP. The Rivalta's test is simple and inexpensive and should be performed in any case of effusion in cats. Anti-FCoV antibody test in effusion also had high PPV and NPV and can be recommended. In addition, RT-PCR in effusion showed promising preliminary results. Therefore, if a clinician suspects FIP, the 1st diagnostic step should always be to look for effusion radiographically and sonographically before submitting expensive blood tests. Diagnostic tests that can be performed in effusion have higher predictive values than tests that can be performed in blood. Unfortunately, if no effusion is present, diagnostic options are lim-



ited. Serum RT-PCR cannot be considered a useful tool to diagnose FIP because it is very sensitive and detects low-grade viremia in cats that don't have FIP and will never develop FIP. Antigen antibody complex detection is a very interesting approach, but unfortunately not specific and sensitive enough in a clinical setting. Anti-FCoV antibodies should be tested in a cat with signs of FIP but should be interpreted very critically because negative titers don't exclude FIP. Only quantitative titer assays should be used and only the highest titer has useful predictive values. All the tests (serum RT-PCR, antigen antibody complexes, anti-FCoV antibodies) should never be the sole criteria for diagnosing FIP and the decision of euthanasia, but they can help facilitate the decision for more invasive diagnostic methods like exploratory laparotomy or laparoscopy and histology of organ biopsies that might lead to the final diagnosis.

Definitive diagnosis is desired in a fatal and fulminant disease like FIP. The only way to definitively diagnose FIP is histopathology or the detection of intracellular FCoV antigen by immunofluorescent or immunohistochemistry staining. It was shown in this study that a positive staining of macrophages in effusion predicts FIP 100%. The same seems to be true for immunohistochemical staining of tissue macrophages,<sup>52</sup> although not evaluated in a high number of clinical cases. This tool should be used whenever possible. If no effusion is present, the statement that Barlough<sup>54</sup> established more than 15 years ago that "organ biopsy is the only test procedure that can be used to definitely diagnose FIP in the living animals" is still true despite the many new tests and molecular biology methods that are available.

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### Footnotes

- <sup>a</sup> Hitachi 705<sup>®</sup> autoanalyzer; Boehringer Mannheim, Mannheim, Germany  
<sup>b</sup> Hitachi 717<sup>®</sup> autoanalyzer; Boehringer Mannheim, Mannheim, Germany  
<sup>c</sup> Elphor-Mikro-Rapid-Kammer<sup>®</sup>, Elpho-Vario V<sup>®</sup>; Vogels, Helmbrechts, Germany  
<sup>d</sup> Red Ponceau; Bender und Hobein, Karlsruhe, Germany  
<sup>e</sup> Elscript 3<sup>®</sup>; Vogels, Helmbrechts, Germany  
<sup>f</sup> Anti-cat IgG [H+L]-FITC; KPL, Gaithersburg, MD
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