

## ORIGINAL RESEARCH

# Diagnostic accuracy of the Rivalta test for feline infectious peritonitis

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## Key Words

Cat, diagnosis, effusion, FIP, sensitivity, specificity

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**Background:** The Rivalta test has been used routinely in Europe to diagnose feline infectious peritonitis (FIP) in cats with effusions, but its diagnostic accuracy is uncertain.

**Objectives:** The objectives of this study were to calculate sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values of the Rivalta test for FIP and to identify correlations between a positive Rivalta test and variables measured in effusion fluid and peripheral blood.

**Methods:** In this retrospective study, medical records of cats with effusions were reviewed, and cats with conclusive results for the Rivalta test were included. The prevalence of FIP in this population was determined, and sensitivity, specificity, and PPV and NPV of the Rivalta test were calculated. Variables measured in effusion fluid and peripheral blood were compared between cats that had positive or negative Rivalta tests using the Mann-Whitney *U*-test and multivariate analysis.

**Results:** Of 851 cats with effusions, 782 had conclusively positive or negative results for the Rivalta test. A definitive final diagnosis was made in 497 of these cats. Prevalence of FIP in cats with effusion and a conclusive Rivalta test result was 34.6%. The Rivalta test had a sensitivity of 91.3%, specificity of 65.5%, PPV of 58.4%, and NPV of 93.4% for the diagnosis of FIP. These values increased when cats with lymphoma or bacterial infections were excluded, or when only cats  $\leq 2$  years were considered. Increased effusion cholesterol concentration and specific gravity as well as decreased serum albumin:globulin ratio and hyperbilirubinemia were positively correlated with positive Rivalta test results.

**Conclusions:** Sensitivity, specificity, and PPV of the Rivalta test for the diagnosis of FIP were lower than previously reported except when used in young cats. The components in effusions that lead to a positive Rivalta test remain unknown, but the positivity is not simply related to high total protein concentration.

## Introduction

Feline infectious peritonitis (FIP) is a common disease, especially in young cats. Clinical signs are variable, depending on which organs are affected,<sup>1,2</sup> and it is often difficult to make a definitive diagnosis of FIP, even though there are many tests for evaluating blood and body cavity effusions.<sup>3,4</sup> In Germany and many other countries, a simple assay termed *the Rivalta test* has been performed on effusions of cats suspected of having FIP. The test was developed by the Italian

physician Fabio Rivalta, who published a report of the method in 1895, to differentiate exudates from transudates in human body cavity effusions.<sup>5-7</sup> Since then, the Rivalta test has been used mostly in Germany, Poland, Russia, and France.<sup>8</sup> The principle of the test is the formation of a precipitate when a sample of the effusion fluid is added to a solution of acetic acid. The test is inexpensive and can be performed quickly in a private practice setting. The test is no longer used in human medicine, because other analytical methods, including Light's criteria (pleural lactate

dehydrogenase [LDH] activity, pleural fluid:serum LDH ratio, and pleural fluid:serum protein ratio), have replaced it. For cats, the test was demonstrated to have high sensitivity and specificity for the diagnosis of FIP in 2 previous studies<sup>3,9</sup>; however, relatively low numbers of cats were evaluated.<sup>3,9</sup> In dogs, the Rivalta test has not been demonstrated to have diagnostic value.<sup>10</sup>

The objectives of this study were to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Rivalta test to diagnose FIP in a large cohort of cats with a definitive diagnosis. In addition, correlation of results of the Rivalta test with variables measured in blood and effusion fluid was investigated.

## Materials and Methods

### Study design and selection of cats

The study was conducted according to the Standards for Reporting of Diagnostic Accuracy (STARD),<sup>11,12</sup> which standardize the conduct and reporting of results for studies of diagnostic accuracy. This retrospective analysis included data from cats with effusions presented to the Clinic of Small Animal Medicine of the Ludwig Maximilian University of Munich, Germany from January 1999 through May 2010. Age of the cats, variables measured in blood and effusion fluid, and the definitive diagnosis, when available, were retrieved from the medical records. Inclusion criteria were the presence of effusion as detected by ultrasonographic examination and performance of the Rivalta test. The only exclusion criterion was a questionable Rivalta test result, defined as formation of slightly cloudy swirls in solution with neither distinct dissolution nor distinct precipitation. For determination of the prevalence of FIP and the diagnostic accuracy of the Rivalta test, only cats with a definitive diagnosis were included (Figures 1–3). For correlation of Rivalta test results with variables in blood or effusion fluid, all cats with a conclusively positive or negative Rivalta test result were included (Figure 4).

A definitive diagnosis of FIP was either confirmed by positive immunofluorescent staining of macrophages in effusion fluid for feline coronavirus (FCoV) antigen,<sup>9</sup> by identifying histologic changes diagnostic for FIP in tissues,<sup>13,14</sup> or by immunohistochemical staining of tissue macrophages for FCoV antigen at post-mortem examination.<sup>15</sup> For cats with effusions that were not caused by FIP, diagnoses were made by echocardiography for heart disease<sup>16,17</sup>; cytologic, histologic, or gross necropsy examination for neoplasia<sup>18</sup>; identification of bacteria by culture or cytologic exami-

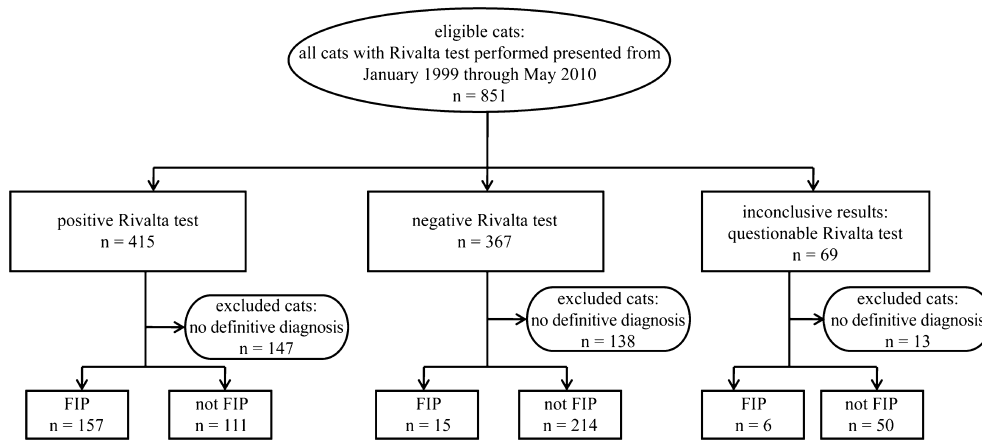
nation for bacterial infections<sup>19,20</sup>; ultrasonographic examination, laparotomy, or necropsy for cholangiohepatitis and intussusception<sup>21,22</sup>; serum feline pancreatic lipase immunoreactivity (fPLI) concentration, ultrasonographic examination, laparotomy, or necropsy, or a combination of these methods, for pancreatitis<sup>23,24</sup>; history of anuria and physical and ultrasonographic examination for uroperitoneum; comparison of PCVs of effusion fluid and blood with PCV of fluid  $\geq$  PCV of blood for hemorrhage<sup>25</sup>; and Doppler-guided measurement of blood pressure for systemic hypertension.<sup>26</sup> When a diagnosis of FIP was made, the effusion was attributed to this disease and cats were placed in the FIP group. If cats had FIP and concurrent diseases, eg, diabetes mellitus or infection with feline immunodeficiency virus (FIV), these other diseases were not specifically evaluated because they were not considered causes for effusion.

### Analysis of effusion fluid

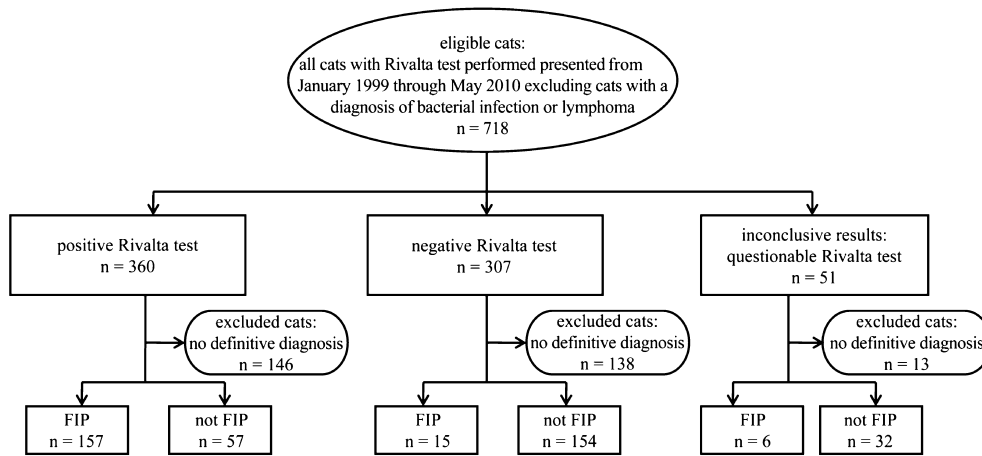
Thoracocentesis or abdominocentesis had been performed using ultrasound guidance, often without sedation. Using aseptic technique, a 19- or 21-gauge butterfly needle connected to a closed system using a 3-way-stopcock and a 10-mL syringe was used to carefully collect 0.5–800 mL of effusion fluid from the body cavity. Fluid was placed in tubes containing EDTA or in plain serum tubes (Sarstedt AG & Co, Nümbrecht, Germany). Fluid was analyzed and the Rivalta test was performed on the day of collection.

To perform the Rivalta test, 7–8 mL of distilled water were placed in a 10-mL plastic tube (Sarstedt AG & Co). One drop (20–30  $\mu$ L) of acetic acid (98–100%) was added using a disposable pipette (Merck, Darmstadt, Germany), and the solution was mixed thoroughly. Using a second disposable pipette, 1 drop (20–30  $\mu$ L) of effusion fluid was carefully placed on top of the acetic acid solution. If a precipitate developed and remained attached to the surface, retained its shape, or slowly floated to the bottom of the solution, the Rivalta test was considered positive (Figure 5). If the drop of effusion fluid dissipated and the solution remained clear, the Rivalta test was considered negative. When slightly cloudy swirls appeared that neither formed a distinct precipitate nor dissipated completely, the Rivalta test was considered questionable, and the cat was excluded from the study.

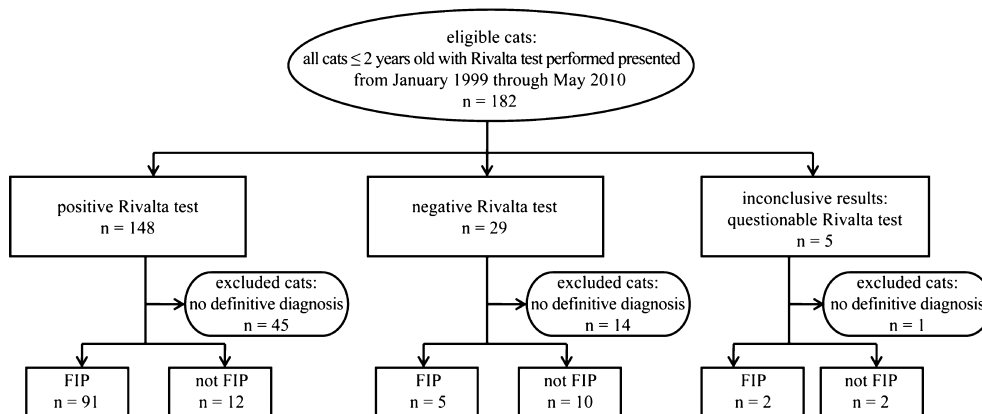
Analysis of the effusion fluid included measurement of specific gravity (SG) by refractometry (Atago Company Ltd, Tokyo, Japan), total nucleated cell counts (TNCC) and red blood cell (RBC) counts (Cell-Dyn 3500; Abbott Laboratories, Abbott Park, IL, USA),



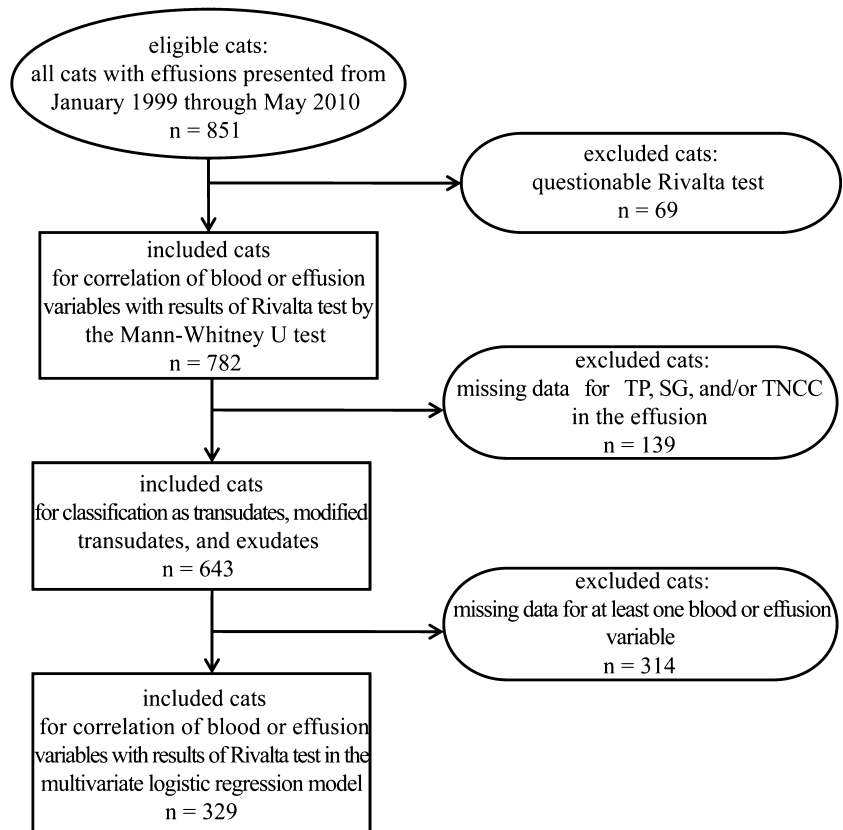
**Figure 1.** Population of cats evaluated to determine the prevalence of feline infectious peritonitis (FIP) and the diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of the Rivalta test for the diagnosis of FIP.



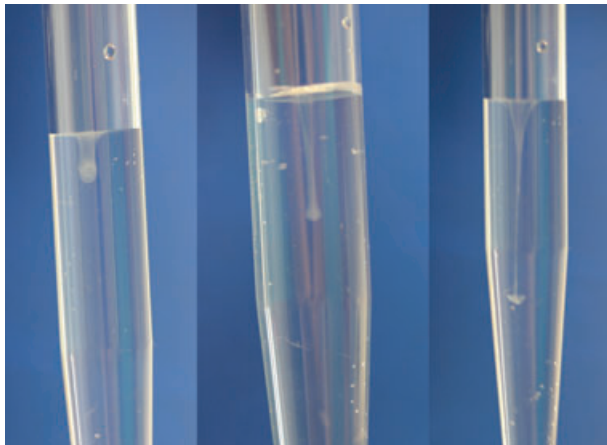
**Figure 2.** Population of cats evaluated to determine the prevalence of feline infectious peritonitis (FIP) and the diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of the Rivalta test for the diagnosis of FIP when cats with a diagnosis of bacterial infection or lymphoma were excluded.



**Figure 3.** Population of cats  $\leq 2$  years old evaluated to determine the prevalence of feline infectious peritonitis (FIP) and the diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of the Rivalta test for the diagnosis of FIP.



**Figure 4.** Population of cats evaluated to identify correlations between a positive Rivalta test and variables measured in effusion fluid and peripheral blood using the Mann-Whitney *U*-test and a multivariate logistic regression model, and for classification of effusions as transudates, modified transudates, and exudates. TP, total protein; SG, specific gravity; TNCC, total nucleated cell count.



**Figure 5.** Positive Rivalta test. A precipitate forms on the surface of the acetic acid solution and slowly floats to the bottom of the reaction tube. (Left) After 1 second; (Center) after 3 seconds; (Right) after 7 seconds.

and concentrations of total protein (TP), triglycerides, glucose, creatinine, albumin, and cholesterol and activities of LDH and  $\alpha$ -amylase (1999–2000: Hitachi 717; 2000–2010: Hitachi 911; Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany). Globulin concentrations were calculated (TP concentration

minus albumin concentration). Effusions were classified according to SG, TP concentration, and TNCC as transudates (SG < 1.018, TP < 25 g/L, and TNCC < 1500/ $\mu$ L), modified transudates (SG 1.018–1.025, TP 25–30 g/L, and TNCC 1500–7000/ $\mu$ L), or exudates (SG > 1.025, TP > 30 g/L and TNCC > 7000/ $\mu$ L). Fluids with characteristics of both transudates and exudates (eg, TP > 30 g/L and TNCC < 1500/ $\mu$ L) also were classified as modified transudates.

### Analysis of blood

Blood samples had been collected from the cephalic, saphenous, or jugular veins using a 20-gauge needle. Blood was collected in plastic tubes containing EDTA, in plastic serum tubes without anticoagulant, and in tubes containing lithium-heparin (Sarstedt AG & Co). Analysis of whole blood in EDTA included measurements of RBC count, hemoglobin concentration, hematocrit (HCT), and white blood cell (WBC) count, segmented neutrophil, band neutrophil, lymphocyte, and monocyte counts (Cell-Dyn 3500; 100-cell manual differential counts). Measurements in serum or plasma included concentrations of bilirubin, TP, albumin (and calculation of globulin concentrations as well as the albumin:globulin [A:G] ratio), urea,

creatinine, glucose, phosphorus, sodium, potassium, chloride, and total and ionized calcium and activities of alanine aminotransferase and alkaline phosphatase (1999–2000: Hitachi 717; 2000–2010: Hitachi 911; for ionized calcium, GEM Premier 3000; Instrumentation Laboratory GmbH, Kirchheim, Germany).

### Statistical analysis

The diagnostic utility of the Rivalta test was evaluated by calculating diagnostic sensitivity (proportion of cats among all cats with FIP with positive Rivalta test results), diagnostic specificity (proportion of cats among all cats with diseases other than FIP with negative Rivalta test results), PPV (probability of a cat with a positive Rivalta test result to have FIP), and NPV (probability of a cat with a negative Rivalta test result to not have FIP). These values were calculated for all cats with a definitive diagnosis and for 2 subpopulations of cats: (1) all cats except those with lymphoma or bacterial infections, because these diagnoses are usually easily differentiated from FIP by cytologic examination or bacterial culture<sup>9</sup> and (2) cats  $\leq$  2 years old, because most cats with FIP are young.<sup>1,3,27–29</sup>

To investigate the correlation between variables measured in effusion fluid and blood with Rivalta test results and classifications of the effusions as transudates, modified transudates, or exudates, statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Based on visual inspection of boxplots, most variables were not normally distributed; thus, the Mann–Whitney *U*-test was used to evaluate differences between variables measured in effusion fluid and blood of cats with positive or negative Rivalta test results. All variables for which significant differences in the univariate analysis were found were then analyzed in a multivariate logistic regression model, using backward selection (likelihood ratio). Not all variables were available for all cats. For the logistic regression model, only those samples with complete data could be analyzed. Variables were retained in the model if the *P*-value was  $< .05$ . The significance of a variable was greater, the more the odds ratio (OR), a descriptive statistic that describes the strength of association between 2 variables, varied from 1.000.

## Results

### Diagnoses in cats with effusions

Based on examination of medical records, 851 cats with effusions were identified from January 1999

through May 2010. A conclusive result for the Rivalta test was obtained in 782 of these cats (Figure 1). A definitive diagnosis was made in 553 of all cats and in 497 cats with a conclusive Rivalta test result (Table 1). Of these 497 cats, 172 had FIP and 325 had effusions from other causes (156, neoplasia; 93, heart disease; 45, bacterial infection; 11, pancreatitis; 3, cholangiohepatitis; 2, intussusception; 1, systemic hypertension; 4, hemorrhage; and 10, effusion secondary to obstruction or lesions of the urinary tract). Effusions in 643 of the 782 cats could be classified as transudates, modified transudates, or exudates; effusions from 139 cats could not be classified because measurements for SG, TP concentration, or TNCC were not available (Figure 4). Of the effusions for which the Rivalta test was positive, a small percentage were classified as transudates, and most were modified transudates and exudates; of the effusions for which the Rivalta test was negative, 11.5% were exudates (Table 2).

### Rivalta test results

Based on results of the 497 cats with a definitive diagnosis, the prevalence of FIP in the study population was 34.6%. Sensitivity, specificity, PPV, and NPV of the Rivalta test in the diagnosis of FIP were calculated for the whole population and the 2 subpopulations (Figures 1–3; Table 3). Of 118 cats  $\leq$  2 years with a conclusive Rivalta test, 96 (81.4%) had FIP, and the prevalence of FIP and the sensitivity and PPV of the Rivalta test in this subgroup were higher than for the overall population.

### Correlation of effusion fluid and blood variables with results of the Rivalta test

After exclusion of effusions with a questionable Rivalta test result, 782 effusions were included (Figure 4) in the analysis. Significant differences were found for most variables measured in fluid and blood between cats with positive vs negative Rivalta test results

**Table 1.** Results of the Rivalta test in 497 cats with effusions caused by feline infectious peritonitis (FIP) and other diseases.

Rivalta Test	All Cats <i>n</i>	Cats with FIP		Cats with Other Diseases	
		<i>n</i>	%	<i>n</i>	%
Positive	268	157	58.6	111	41.4
Negative	229	15	6.6	214	93.4

Of 497 cats, 172 (34.6%) had effusions caused by FIP, and 325 (65.4%) had effusions caused by diseases other than FIP.

**Table 2.** Rivalta test results for 643 effusions classified as transudates, modified transudates, and exudates.

	Transudate		Modified Transudate		Exudate		Total <i>n</i>
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Rivalta-positive	18	5.7	140	44.6	156	49.7	314
Rivalta-negative	72	21.9	219	66.6	38	11.5	329
All effusions	90	14.0	359	55.8	194	30.2	643

See text for definitions of transudates, modified transudates, and exudates based on specific gravity, total protein concentration, and total nucleated cell counts of effusions.

**Table 3.** Prevalence of feline infectious peritonitis (FIP) in a population of 497 cats with effusions presented to the Clinic of Small Animal Medicine of the Ludwig Maximilian University of Munich, Germany from January 1999 through May 2010 and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Rivalta test to diagnose FIP.

	<i>n</i>	Prevalence of FIP, %	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, %	NPV, %
All cats with a definitive diagnosis	497	34.6	91.3 (86.1–94.6)	65.5 (60.2–70.5)	58.4	93.4
Cats without lymphoma or bacterial infections	383	45.0	91.3 (86.1–94.6)	73.0 (66.6–78.5)	73.4	91.1
Cats ≤ 2-years-old	118	81.4	94.8 (88.4–97.8)	45.5 (26.9–65.3)	88.4	66.7

CI, confidence interval.

(Tables 4 and 5) using the Mann–Whitney *U*-test. When the multivariate logistic regression model was used to evaluate 329 effusions, only 4 variables (SG and concentrations of cholesterol, triglycerides, and glucose) measured in the effusion fluid and 2 variables (A:G ratio and bilirubin concentration) in the blood remained significant factors in the model (Tables 4 and 5); their significance was greater, the more their OR varied from 1.000.

## Discussion

The prevalence of FIP in cats with effusion in this study was lower than 41–51% reported previously, and sensitivity, specificity, and PPV of the Rivalta test for the diagnosis of FIP were also lower than previously reported.<sup>3,9</sup> It is unknown whether there has been a real decrease in overall prevalence. It is possible that when FIP is the primary differential diagnosis, cats are

**Table 4.** Correlation of results of the Rivalta test with variables measured in feline effusions.

Variables	<i>n</i>	Mann–Whitney <i>U</i> -test				<i>P</i> -value	Multivariate Analysis	
		Rivalta-Negative		Rivalta-Positive			<i>P</i> -value	Odds Ratio (95% CI)
		Median	2.5-97.5th Percentiles	Median	2.5-97.5th Percentiles			
Globulins g/L	499	15.6	1.5–43.6	28.7	6.3–66.7	<.001	NS	-
LDH U/L	489	102	16–7304	773	50–6337	<.001	NS	-
Specific gravity	655	1.022	1.008–1.040	1.030	1.014–1.046	<.001	.002	1.131 (1.044–1.224)
Total protein g/L	608	31.8	3.0–69.7	48.0	8.7–84.8	<.001	NS	-
α-amylase U/L	492	613	113–2164	1029	225–3296	<.001	NS	-
TNCC × 10 <sup>9</sup> /L	690	1.05	0.02–34.04	4.17	0.07–129.75	<.001	NS	-
Cholesterol mmol/L	502	1.83	0.10–5.94	2.68	0.45–5.75	<.001	.031	1.534 (1.041–2.262)
Creatinine μmol/L	502	114	40–715	80	27–449	<.001	NS	-
Glucose mmol/L	492	6.70	0.11–11.75	5.16	0.05–12.47	<.001	.008	0.896 (0.826–0.972)
Albumin g/L	500	15.4	0.9–32.1	17.2	3.4–29.4	.001	NS	-
RBC × 10 <sup>6</sup> /μL	671	0.006	0.000–1.699	0.015	0.000–2.583	.001	NS	-
Triglycerides mmol/L	501	0.44	0.11–11.75	0.37	0.11–16.73	.010	.013	0.954 (0.919–0.990)

Variables are ordered by ascending *P*-values for the Mann–Whitney *U*-test; multivariate analysis was based on a multivariate logistic regression model.

*n* indicates the number of cats included in the analysis; CI, confidence interval; LDH, lactate dehydrogenase; NS, not significant; TNCC, total nucleated cell count.

**Table 5.** Correlation of results of the Rivalta test with variables measured in feline blood.

Variables	Reference Intervals*	Mann–Whitney <i>U</i> -test					Multivariate Analysis		
		<i>n</i>	Rivalta-Negative		Rivalta-Positive		<i>P</i> -value	<i>P</i> -value	Odds Ratio (95% CI)
			Median	2.5-97.5th Percentiles	Median	2.5-97.5th Percentiles			
A:G ratio		607	0.88	0.30–1.52	0.56	0.24–1.16	<.001	.013	0.025 (0.001–0.460)
Creatinine $\mu\text{mol/L}$	0–169	637	121	42–825	85	27–362	<.001	-	NS
Globulins g/L	31–38	606	34.6	17.9–68.5	42.1	22.3–82.5	<.001	-	NS
Urea mmol/L	5.00–11.30	634	11.42	4.33–66.79	8.10	3.59–38.79	<.001	-	NS
Albumin g/L	26.0–56.0	608	28.8	14.5–43.1	23.96	14.1–38.6	<.001	-	NS
ALP U/L	0–94	533	30	7–460	19	3–139	<.001	-	NS
Band neutrophils $\times 10^9/\text{L}$	0.00–0.60	577	0.19	0.00–4.66	0.50	0.00–9.23	<.001	-	NS
Bilirubin $\mu\text{mol/L}$	0.00–4.74	555	2.90	0.50–110.86	6.72	0.46–137.03	<.001	.002	1.019 (1.007–1.031)
ALT U/L	0–114	547	55	12–858	38	10–618	<.001	-	NS
Sodium mmol/L	146–165	567	148	128–158	146.5	122–158	<.001	-	NS
Glucose mmol/L	3.70–6.90	586	7.58	3.39–29.89	6.72	2.28–19.54	<.001	-	NS
Hemoglobin mmol/L	5.6–9.3	668	7.04	2.5–11.9	6.3	2.5–10.9	<.001	-	NS
HCT L/L	0.30–0.44	668	0.34	0.13–0.54	0.30	0.12–0.50	<.001	-	NS
Total protein g/L	57.0–94.0	606	65.4	35.3–95.2	69.7	39.9–102.6	<.001	-	NS
RBC $\times 10^6/\mu\text{L}$	5.00–10.00	670	7.84	2.54–12.80	7.38	2.59–11.74	.007	-	NS
Total calcium mmol/L	2.30–3.00	293	2.24	1.65–3.00	2.19	1.35–2.71	.008	-	NS
Potassium mmol/L	3.5–5.6	570	4.1	2.7–6.2	4.0	2.5–5.9	.029	-	NS
WBC $\times 10^9/\text{L}$	6.00–11.00	671	14.00	3.32–43.32	15.60	1.76–46.73	.039	-	NS
Chloride mmol/L	100–124	531	115	98–128	114	90–127	.050	-	ND
Segmented neutrophils $\times 10^9/\text{L}$	3.00–11.00	576	11.47	0.71–39.39	12.64	1.27–40.91	.084	-	ND
Lymphocytes $\times 10^9/\text{L}$	1.00–4.00	576	1.06	0.07–7.10	1.05	0.00–7.19	.211	-	ND
Phosphorus mmol/L	0.97–2.36	533	1.66	0.60–4.46	1.74	0.58–2.75	.255	-	ND
Monocytes $\times 10^9/\text{L}$	0.04–0.50	577	0.28	0.00–2.96	0.24	0.00–2.52	.268	-	ND
Ionized calcium mmol/L	1.20–1.35	356	1.19	0.91–1.47	1.17	0.83–1.42	.318	-	ND

Variables are ordered by ascending *P*-values for the Mann–Whitney *U*-test; multivariate analysis was based on a multivariate logistic regression model.

\*Source of reference intervals: Referenzbereiche in der Labordiagnostik der Katze (Reference intervals for laboratory diagnostics in the cat; dissertation), Katrin Hartmann, Munich, 1990.

*n* indicates the number of cats included in the analysis; CI, confidence interval; A:G, albumin:globulin; NS, not significant; ALP, alkaline phosphatase; ALT, alanine aminotransferase.

not referred to the University Veterinary Teaching Hospital by other veterinarians, but are more often euthanized because of the poor prognosis. Prevalence in this study may also be lower because cats for which there was not a definitive diagnosis were excluded from analysis. For many of the excluded cats, the diagnosis of FIP was considered likely, but owners chose not to pursue definitive diagnostic testing. In addition, owners increasingly decline necropsy examinations, which is another reason definitive diagnoses are lacking. These considerations could have resulted in bias in the population in this study, and the exclusion of approximately one-third of all cats with effusions may have affected the calculated sensitivity and specificity of the test and the predictive values.<sup>11,12</sup> On the other hand, excluded cats could have had diseases other than FIP, and if definitive diagnoses had been obtained, pre-

dictive values would have been affected. Another reason for the lower prevalence of FIP in the present study is that some feline diseases, such as various neoplasms, pancreatitis, or heart disease, are diagnosed more frequently today than in the past owing to improved imaging and cytologic techniques; increased diagnosis of diseases other than FIP in the present study would have decreased the prevalence of FIP. The higher prevalence of FIP in cats  $\leq 2$  years of age was expected because the disease affects young cats,<sup>1,3,27–29</sup> and other causes of effusions are rare in such young animals.<sup>30</sup>

The PPV of the Rivalta test for FIP was lower than the PPV of 84 or 85% reported in earlier studies<sup>3,9</sup>; in those studies, cats in which the Rivalta test was positive rarely had diseases other than FIP.<sup>3</sup> In the present study, which included a larger number

of cats, a higher proportion of effusions with positive Rivalta test results were caused by other diseases. It is possible that some of these effusions were present in body cavities for long time periods, resulting in inflammation and serositis secondary to the effusion; subsequent production of inflammatory mediators and cytokines and their presence in the effusion could have resulted in a positive Rivalta test result, which may have been negative during the early stages of the primary disease. Alternatively, cats with a positive Rivalta test and a definitive diagnosis of a disease other than FIP could potentially have had FIP in addition to the other disease, as further diagnostic testing was not performed once a disease associated with development of effusion was diagnosed. However, this is unlikely, because many of the cats with other diseases lived longer than 14 days, the median survival time of cats with FIP has been reported to be 8 days, although some cats live longer.<sup>31</sup> Predictive values depend on disease prevalence, which may vary considerably from one clinical situation to another.<sup>11,12</sup> Thus, the lower prevalence of FIP in this study explains the lower PPV of the Rivalta test, but predictive values reported in this study must be interpreted in light of the population evaluated and may not be assigned to other clinical situations. In cats  $\leq 2$  years, the PPV of a positive Rivalta test for FIP reaches approximately 90% owing to the higher prevalence of FIP in this population.<sup>1,3,27-29</sup> Although the sensitivity of the Rivalta test in the diagnosis FIP was also lower than previously reported (98 and 100%),<sup>9,32</sup> it was still high. In addition, the NPV was also high, indicating that if the Rivalta test is negative, the likelihood of FIP as a cause for the effusion is low.

The many significant differences among variables between Rivalta-positive and Rivalta-negative effusions reflected the large number of cats in the study. It was long assumed that a positive Rivalta test would depend on the TP concentration in the effusion<sup>6,33-35</sup>; however, in the present study, neither TP nor globulin concentrations of Rivalta-positive and Rivalta-negative effusions were significantly different, and high and low TP concentrations were found in both Rivalta-negative and Rivalta-positive effusions. Thus, TP and globulin concentrations appear not to be the major factors correlated with the Rivalta test result, and other components might result in the formation of a precipitate. In a study of human body cavity effusions, several acute phase proteins in Rivalta-positive precipitates could be identified.<sup>36</sup> Therefore, it is possible that some protein fractions, eg, acute phase pro-

teins, cause the positive reaction in feline effusions. In this retrospective study, neither electrophoretic analysis nor measurement of acute phase proteins was performed, but these analyses are warranted in a future prospective study. Cholesterol concentration was significantly higher in Rivalta-positive effusions and the OR was highest for this analyte. Cholesterol concentration has been found to be useful in differentiating between transudates and exudates in human effusions,<sup>37-39</sup> possibly because of degeneration of cellular components, eg, WBCs and RBCs, in the fluid or because cholesterol can exit the vasculature due to increased capillary permeability in vasculitis.<sup>38,40</sup> In the present study, more Rivalta-positive effusions were classified as exudates, and cholesterol concentrations are increased in exudates; thus, cholesterol concentrations may have been higher in these Rivalta-positive effusions. However, it remains unknown whether cholesterol causes or contributes to the precipitate formed in a positive Rivalta test. Interestingly, triglyceride concentrations were lower in Rivalta-positive effusions than in Rivalta-negative effusions. Triglycerides may not follow the same pattern as cholesterol, as it has been shown in mice fed cholesterol that high cholesterol concentrations actually decreased the production of triglycerides.<sup>41</sup> Formation of "pseudochyle", which typically contains cholesterol but not triglycerides,<sup>42</sup> can occur in long-standing effusions. SG depends on concentration of TP and other analytes<sup>43</sup> in effusions, but this variable did not differ between Rivalta-positive and Rivalta-negative effusions in the multivariate analysis.

Cats with Rivalta-positive effusions had significantly lower serum A:G ratios associated with high globulin and low albumin concentrations. Hypoalbuminemia may be caused by reduced hepatic production, but most likely was caused by vasculitis. A decrease in albumin also may reflect compensation for increased globulin production.<sup>1</sup> Albumin also acts as a negative acute phase protein in cats with FIP and could therefore be downregulated by the immune system. Serum bilirubin concentration, which had the highest OR of all blood variables, was markedly higher in cats with Rivalta-positive effusions, possibly due to the high percentage of cats with FIP in which hyperbilirubinemia in the absence of increased hepatic enzyme activity often occurs.<sup>2</sup> It has been suggested that cytokines released in FIP affect bilirubin metabolism resulting in altered excretion into the biliary system<sup>1</sup>; cytokines can affect degradation of bilirubin by reducing transporter gene expression or directly inhibiting transport protein function in hepatocytes, with consequent hyperbilirubinemia.<sup>44</sup> Serum



bilirubin concentrations may also increase in FIP from secondary immune-mediated hemolysis or hemolysis caused by disseminated intravascular coagulation, from hepatic involvement, or from cholestasis.<sup>1,4,5</sup>

This study had several limitations. As a retrospective analysis, not all variables were measured in every cat. The Rivalta test is a subjective assay with no available reference method to assess performance. In addition, during the long investigation period, there was turnover of laboratory personnel performing the test, which may be a source of bias. For cats with diagnoses other than FIP, FIP as a possible concurrent disease was often not investigated. On the other hand, although unlikely, cats with FIP may have had a second disease that might have resulted in effusion. The measurement of blood analytes, including proteins, was performed on serum or plasma, depending on the available specimen. This could have masked some differences between results, as fibrinogen and other proteins involved in coagulation are not present in serum, but remain present in plasma.

In conclusion, the Rivalta test can be a valuable diagnostic aid in evaluating feline effusions; for FIP, the PPV is good and the NPV is excellent. The components in effusions that lead to a positive Rivalta test result have not been elucidated in cats, and, along with electrophoresis of effusions and analysis of globulin fractions, analysis of the precipitate formed in a positive test is an important next step.

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## References

- Hartmann K. Feline infectious peritonitis. *Vet Clin North Am Small Anim Pract.* 2005;35:39–79.
- Norris JM, Bosward KL, White JD, Baral RM, Catt MJ, Malik R. Clinicopathological findings associated with feline infectious peritonitis in Sydney, Australia: 42 cases (1990–2002). *Aust Vet J.* 2005;83:666–673.
- Hirschberger J, Hartmann K, Wilhelm N, Frost J, Lutz H, Kraft W. Clinical symptoms and diagnosis of feline infectious peritonitis. *Tierarztl Prax.* 1995;23:92–99.
- Pedersen NC. Serologic studies of naturally occurring feline infectious peritonitis. *Am J Vet Res.* 1976;37:1449–1453.
- Rivalta F. Su di una nuova reazione per la diagnosi chimica differenziale fra gli essudati sierosi e i semplici trasudati [About a new reaction for chemical differentiation of serous exudates and simple transudates]. *La Riforma Medica.* 1895;242.
- Berti-Bock G, Vial F, Premuda L, Rulliere R. Exudates, transudates and the Rivalta reaction (1895). Current status and historical premises. *Minerva Med.* 1979;70:3573–3580.
- Eisenack W. Untersuchungen über Exsudat und Transudat [Examinations of exudates and transudates]. Doctoral dissertation, Gießen, Germany: Justus-Liebig-Universität Gießen; 1951.
- Virchow R. *Jahresbericht über die Leistungen und Fortschritte in der gesammten Medizin.* Annual report on performance and progress in medicine; 1895.
- Hartmann K, Binder C, Hirschberger J, et al. Comparison of different tests to diagnose feline infectious peritonitis. *J Vet Intern Med.* 2003;17:781–790.
- Kasbohm C. Effusions of body cavities in the dog (2). Clinical and diagnostic study with special reference to punctate cytology. *Tierarztl Prax.* 1976;4:85–94.
- Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clin Chem.* 2003;49:7–18.
- Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for reporting of diagnostic accuracy. *Clin Chem.* 2003;49:1–6.
- Addie DD, Paltrinieri S, Pedersen NC. Recommendations from workshops of the second international feline coronavirus/feline infectious peritonitis symposium. *J Feline Med Surg.* 2004;6:125–130.
- Sparkes AH, Gruffydd-Jones TJ, Harbour DA. Feline infectious peritonitis: a review of clinicopathological changes in 65 cases, and a critical assessment of their diagnostic value. *Vet Rec.* 1991;129:209–212.
- Tammer R, Evensen O, Lutz H, Reinacher M. Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Vet Immunol Immunopathol.* 1995;49:177–182.
- Ferasin L. Feline myocardial disease 2: diagnosis, prognosis and clinical management. *J Feline Med Surg.* 2009;11:183–194.
- Bonagura JD. Feline echocardiography. *J Feline Med Surg.* 2000;2:147–151.
- Hirschberger J, DeNicola DB, Hermanns W, Kraft W. Sensitivity and specificity of cytologic evaluation in the diagnosis of neoplasia in body fluids from dogs and cats. *Vet Clin Pathol.* 1999;28:142–146.
- Ottenjann M, Lubke-Becker A, Linzmann H, Brunnberg L, Kohn B. Pyothorax in 26 cats: clinical signs, laboratory results and therapy (2000–

- 2007). *Berl Munch Tierarztl Wochenschr.* 2008;121:365–373.
20. Barrs VR, Beatty JA. Feline pyothorax – new insights into an old problem: part 1. Aetiopathogenesis and diagnostic investigation. *Vet J.* 2009;179:163–170.
  21. Newell SM, Selcer BA, Girard E, Roberts GD, Thompson JP, Harrison JM. Correlations between ultrasonographic findings and specific hepatic diseases in cats: 72 cases (1985–1997). *J Am Vet Med Assoc.* 1998;213:94–98.
  22. Patsikas MN, Papazoglou LG, Papaioannou NG, Savvas I, Kazakos GM, Dessiris AK. Ultrasonographic findings of intestinal intussusception in seven cats. *J Feline Med Surg.* 2003;5:335–343.
  23. Forman MA, Marks SL, De Cock HE, et al. Evaluation of serum feline pancreatic lipase immunoreactivity and helical computed tomography versus conventional testing for the diagnosis of feline pancreatitis. *J Vet Intern Med.* 2004;18:807–815.
  24. Gerhardt A, Steiner JM, Williams DA, et al. Comparison of the sensitivity of different diagnostic tests for pancreatitis in cats. *J Vet Intern Med.* 2001;15:329–333.
  25. Noone KE. Pleural effusions and diseases of the pleura. *Vet Clin North Am Small Anim Pract.* 1985;15:1069–1084.
  26. Stepien RL. Feline systemic hypertension: diagnosis and management. *J Feline Med Surg.* 2011;13:35–43.
  27. Marioni-Henry K, Vite CH, Newton AL, Van Winkle TJ. Prevalence of diseases of the spinal cord of cats. *J Vet Intern Med.* 2004;18:851–858.
  28. Rohrbach BW, Legendre AM, Baldwin CA, Lein DH, Reed WM, Wilson RB. Epidemiology of feline infectious peritonitis among cats examined at veterinary medical teaching hospitals. *J Am Vet Med Assoc.* 2001;218:1111–1115.
  29. Foley JE, Poland A, Carlson J, Pedersen NC. Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. *J Am Vet Med Assoc.* 1997;210:1313–1318.
  30. Beatty J, Barrs V. Pleural effusion in the cat: a practical approach to determining aetiology. *J Feline Med Surg.* 2010;12:693–707.
  31. Ritz S, Egberink H, Hartmann K. Effect of feline interferon-omega on the survival time and quality of life of cats with feline infectious peritonitis. *J Vet Intern Med.* 2007;21:1193–1197.
  32. Hirschberger J. Cytology of body cavity effusions. *Tierarztl Prax.* 1995;23:192–199.
  33. Matraccia S, Campo G, Titone C. Interaction between globin and Rivalta-positive colloids. *Boll Soc Ital Biol Sper.* 1963;39:148–149.
  34. Moritz F. Beiträge zur Lehre von den Exsudaten und Transsudaten [Contribution to the knowledge of exudates and transudates]. Doctoral dissertation, München, Germany: Ludwig-Maximillan-Universität München; 1986.
  35. Staehelin R. Ueber den durch Essigsäure fällbaren Eiweisskörper der Exsudate und des Urins [On the protein content precipitated by adding acetic acid to exudates and urine]. *Munch Med Wochenschr.* 1902;34:1413–1415.
  36. Sakai N, Iijima S, Shiba K. Reinvestigation of clinical value of Rivalta reaction of puncture fluid. *Rinsho Byori.* 2004;52:877–882.
  37. Jenss U, Towe D, Diwok K. Determination of individual lipid parameters in non-chylous pleural effusions. *Z Erkr Atmungsorgane.* 1989;173:161–165.
  38. Valdes L, San-Jose E, Estevez JC, et al. Cholesterol in pleural exudates depends mainly on increased capillary permeability. *Transl Res.* 2010;155:178–184.
  39. Guleria R, Agarwal SR, Sinha S, Pande JN, Misra A. Role of pleural fluid cholesterol in differentiating transudative from exudative pleural effusion. *Natl Med J India.* 2003;16:64–69.
  40. Valdes L, Pose A, Suarez J, et al. Cholesterol: a useful parameter for distinguishing between pleural exudates and transudates. *Chest.* 1991;99:1097–1102.
  41. Van der Veen JN, Havinga R, Bloks VW, Groen AK, Kuipers F. Cholesterol feeding strongly reduces hepatic VLDL-triglyceride production in mice lacking the liver X receptor alpha. *J Lipid Res.* 2007;48:337–347.
  42. Hillerdal G. Chylothorax and pseudochylothorax. *Eur Respir J.* 1997;10:1157–1162.
  43. Meyers DG, Meyers RE, Prendergast TW. The usefulness of diagnostic tests on pericardial fluid. *Chest.* 1997;111:1213–1221.
  44. Zollner G, Trauner M. Molecular mechanisms of cholestasis. *Wien Med Wochenschr.* 2006;156:380–385.
  45. Pedersen NC. A review of feline infectious peritonitis virus infection: 1963–2008. *J Feline Med Surg.* 2009;11:225–258.