Concentrations of C-reactive protein in effusions in dogs

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The concentration of C-reactive protein (CRP) was measured in effusions from 50 dogs to assess the potential for measuring this protein to differentiate body cavity fluids. The effusions were classified as either transudates, modified transudates or exudates according to their total protein concentration, total nucleated cell count, cytological findings and aetiology, and the concentration of CRP was determined by a time-resolved immunofluorometric assay. There were significant differences between the concentrations of CRP in the three types of effusion; the highest concentrations were observed in the exudates (4·47 to 54·59 µg/ml), the lowest were in the transudates (0·0094 to 7·87 µg/ml), and the modified transudates contained intermediate concentrations of CRP (0·045 to 10·78 µg/ml). A cut-off value of 4 µg/ml had a sensitivity of 100 per cent and a specificity of 94·4 per cent for differentiating transudates from exudates, and a cut-off value of 11 µg/ml had a sensitivity of 88·2 per cent and a specificity of 100 per cent for distinguishing modified transudates from exudates. However, a cut-off value of 1 µg/ml had a lower sensitivity (80 per cent) and an unacceptably low specificity (66·7 per cent) for differentiating transudates from modified transudates.

EFFUSIONS form when there is an increase in hydrostatic pressure and/or a decrease in oncotic pressure in capillaries and changes in vascular permeability. Effusions in the body cavity are generally classified as transudates, modified transudates or exudates. Transudates are formed as a result of systemic factors such as hypoalbuminaemia or liver insufficiency that result in the development of a diluted clear fluid, whereas exudates are formed as a result of inflammation or neoplasia, resulting in a fluid that may macroscopically resemble plasma (Cowell and others 1999). Modified transudates are caused by right-sided heart failure and less commonly by lesions that constrict the caudal vena cava (Meyer and others 1998). Most effusions caused by tumours, especially those that do not exfoliate, are classified as modified transudates (Dunn and Villiers 1998).

In veterinary medicine, the most frequently used criteria for differentiating body cavity effusions are the concentration of total protein, which can be determined by the use of urine test strips, refractometry or the Bradford and biuret techniques (Braun and others 2001), the nucleated cell count (NCC) and cell differentiation; however, there are small differences in how these should be interpreted depending on the reference consulted. For example, according to Duncan and others (1994), canine transudates are characterised by a total protein concentration less than 30 g/l and a low NCC (<1·5 x 10^9/l), modified transudates have higher cell counts or total protein concentrations than transudates, whereas exudates have a total protein concentration greater than 30 g/l and a NCC above 1·5 x 10^9/l. However, Meyer and others (1998) stated that transudates have a total protein concentration less than 25 g/l and a NCC less than 1 x 10^9/l, modified transudates have a total protein concentration greater than 25 g/l and a NCC less than 5 x 10^9/l, and exudates have a total protein concentration greater than 25 g/l and a NCC greater than 5 x 10^9/l.

In human medicine the most common criteria for differentiating transudates from exudates are the total protein concentration and lactate dehydrogenase activity (Eid and others 2002, Gouteux-Frezzotti and others 2002, Segura 2003), but alternative criteria involving the use of acute phase proteins have been proposed. C-reactive protein (CRP) has been used in the classification of pleural effusions (Castano Vidriales and Amores Antequera 1992, Yilmaz Turay and others 2000, García-Pachon and Llorca 2002) and α_2-macroglobulin, α_1-acid glycoprotein and α_1-antitrypsin have been reported to be useful parameters for distinguishing exudates from transudates in pleural and peritoneal effusions (Alexandrakis and others 2000, 2001).

The acute phase response is induced by protein hormones called cytokines, which act as messengers between the local site of injury and the hepatocytes that synthesise the acute phase proteins (Petersen and others 2004). In dogs, CRP is one of the major acute phase proteins, and increases in its concentration indicate the presence and extent or severity of inflammation (Hayashi and others 2001, Martínez-Subiela and others 2002, Jergens and others 2003).

The aims of the present study were to measure the concentration of CRP in body cavity effusions from dogs by using a highly sensitive immunoassay based on time-resolved fluorometry (Parra and others 2005), to compare the concentrations of CRP in effusions that were classified as transudates, modified transudates and exudates, and to determine the cut-off concentrations that would provide the best sensitivity and specificity for distinguishing between the different types of effusion.

MATERIALS AND METHODS

Samples

Fifty effusions (36 abdominal, 12 pleural and two pericardial) were collected from adult dogs of various breeds and ages that had presented with various diseases to the veterinary hospitals of Murcia university or Bristol university, into EDTA and plain tubes. Samples from the plain tubes were transferred into Eppendorf tubes and frozen at –20°C until CRP analysis.

Total protein, total NCC and determination of CRP

The total protein concentration in each sample was measured by the biuret technique in an automated chemistry analyser (Cobas Mira Plus; ABX Diagnostics).

The total NCC was determined within 30 minutes of sample collection with an automated microcellcounter (Sysmex F-800; TOA Instruments) at Murcia university and a cell counter (CellDyn 3700; Abbott Diagnostics Division) at Bristol university. Direct smears and sediment smears from centrifuged samples were also examined cytologically.

The concentration of CRP was determined by using the time-resolved immunofluorometric assay (TR-IFMA) described by Parra and others (2005). Biotinylated anti-CRP antibodies (200 µl) were pipetted into the streptavidin-coated microtitration wells and incubated for 45 minutes. Next, strips were washed and then 200 µl of diluted samples (1:10,000 or 1:100) and standards were added to each well. The samples and standards were incubated for 45 minutes,

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TABLE 1: Concentrations of C-reactive protein (CRP), total protein (TP) and nucleated cell counts (NCCs) in effusions from 50 dogs

Type of effusion	Diagnosis	TP (g/l)	NCC (x 10^9/l)	CRP (µg/ml)
Transudate				
Abdominal	Liver insufficiency	2·9	0·5	0·79
Abdominal	Liver insufficiency	1·0	0·2	0·009
Abdominal	Liver insufficiency	15·3	0·4	0·33
Abdominal	Liver insufficiency	2·7	0·5	0·12
Abdominal	Liver insufficiency	1·2	0·2	0·02
Abdominal	Liver insufficiency	2·4	0·7	2·37
Abdominal	Lymphangectasia	8·8	0·7	1·19
Abdominal	Protein-losing nephropathy and hypoalbuminaemia	16·4	0·8	7·87
Abdominal	Liver and splenic lymphoma	3·7	1·1	0·71
Abdominal	Liver insufficiency	0·7	1·3	0·14
Abdominal	Liver insufficiency	4·6	0·6	0·72
Abdominal	Liver insufficiency	0·2	0·4	0·36
Abdominal	Intestinal disease	0·8	5·8	0·24
Abdominal	Intestinal disease	0·7	0·2	0·04
Pleural	Cardiac disease	24·8	0·6	2·09
Pleural	Cardiac disease	11·2	0·2	2·69
Pleural	Liver insufficiency	4·1	2·6	3·66
Pleural	Cardiac disease	22·8	0·6	0·86
Modified transudate				
Abdominal	Mass in caudal thorax pressing on caudal vena cava	53·9	0·8	5·98
Abdominal	Neoplastic process	31·3	2·0	10·78
Abdominal	Heart neoplasia	46·7	4·4	5·39
Abdominal	Cardiac disease	34·5	0·3	0·86
Abdominal	Liver disease causing portal hypertension	25·2	1·5	3·20
Abdominal	Intestinal lymphoma	31·2	1·4	5·89
Abdominal	Cardiac disease	29·5	0·9	3·98
Abdominal	Cardiac disease	30·3	0·5	1·16
Abdominal	Cardiac disease	36·9	8·8	0·16
Pleural	Chemodectoma causing cardiac disease	32·8	5·2	0·04
Pleural	Lung adenocarcinoma	34·6	5·0	5·59
Pleural	Lung mast cell tumour	31·9	3·5	2·94
Pleural	Mesothelioma	39·2	2·5	1·09
Pericardial	Neoplastic process	29·2	1·8	7·39
Pericardial	Heart neoplasia	50·0	2·4	2·25
Exudate				
Abdominal	Peritonitis postenterectomy	34·0	18·0	51·60
Abdominal	Abdominal mass	34·0	7·2	7·98
Abdominal	Intussusception	36·0	800·0	13·07
Abdominal	Prostatic carcinoma with abdominal metastasis	28·0	18·0	13·34
Abdominal	Peritonitis postgastropexia	40·0	19·6	50·98
Abdominal	Pyometra, peritonitis	60·0	6·7	13·92
Abdominal	Aseptic peritonitis	42·0	42·0	45·02
Abdominal	Haemangioma	42·0	1700·0	16·09
Abdominal	Spleen carcinoma	26·0	550·0	21·92
Abdominal	Prostatic adenocarcinoma	37·8	12·4	40·12
Abdominal	Intestinal and liver lymphoma	26·7	56·4	54·59
Abdominal	Osteosarcoma involving thoracic wall and ribs	29·6	87·1	19·55
Abdominal	Cholescystatitis and mild pancreatic hyperplasia	29·3	35·0	11·67
Pleural	Neoplastic process	40·9	8·4	4·47
Pleural	Lymphoma	42·1	16·5	13·30
Pleural	Pleural metastasis of mammary adenocarcinoma	36·0	43·0	11·75
Pleural	Pyothorax	25·6	450·0	50·35

and after a second wash, 200 µl of europium-labelled anti-CRP antibodies were added to each well. The strips were incubated for 45 minutes and then washed again. Finally, 200 µl of enhancement solution was added to each well and the strips were incubated for 20 minutes. The enhanced fluorescence was measured in a time-resolved fluorometer (Victor 1420 multilabel counter; PerkinElmer Life Sciences). This assay was validated by the following procedures: first, its intra-assay variation, expressed as the coefficient of variation (CV), was determined by measuring eight effusions, four with low (0·32 µg/ml) and four with high (20·7 µg/ml) concentrations of CRP concentrations, six times in the same analytical series, and its interassay variation was assessed by measuring the same samples on three different days. Secondly, its accuracy was evaluated by determining the linearity of the results obtained when measuring the concentration of CRP in serial dilutions (1:2, 1:4, 1:8 and 1:16) in assay buffer of two effusions with high concentrations. Thirdly, its limit of detection was calculated by analysing the zero standard (assay buffer) 12 times, and expressed as three standard deviations above the mean response for this standard.

Classification of effusions

The effusions were classified as either transudates, modified transudates or exudates, on the basis of their total protein concentration and NCC, following the criteria of Meyer and others (1998) and the aetiology involved in their production. In each case, the cause of the effusion was established by performing a detailed clinical examination and by using additional laboratory tests and diagnostic methods, including haematology, blood biochemistry, radiography, ultrasonography and histopathology, as necessary.

Statistical analysis

The data were analysed by using the statistical program SPSS version 11.0. The statistically significant level was set at $P<0·05$. A simple regression analysis was used to compare the CRP concentrations expected and observed in the linearity study. A Kruskal-Wallis test was used to compare the CRP concentrations in the three types of effusions. Mann-Whitney U tests were used to compare paired groups. The sensitivity, specificity, positive predictive value and negative predictive value of CRP at different cut-off points were calculated as

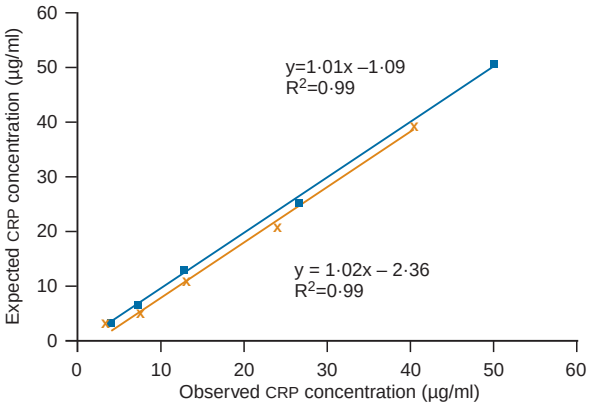

FIG 1: Comparison of the measured concentrations of C-reactive protein (CRP) in serial dilutions of two effusions with high CRP concentrations with the expected concentrations

follows: sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative + false positive); positive predictive value = true positive/(true positive + false positive); negative predictive value = true negative/(true negative + false negative), and the values were expressed as percentages. The cut-off point with the highest sensitivity and specificity pair was fixed as the cut-off point for clinical decisions.

In addition, receiver operating characteristic (ROC) curves were used to evaluate the sensitivity and specificity for different cut-off points. A ROC curve is constructed by plotting the sensitivity against 1 – specificity for every cut-off point. The area under the ROC curve (AUC) and its se were calculated by the trapezoidal rule (DeLong and others 1988). The size of the AUC can be interpreted as the probability that a randomly chosen animal from the positive reference population will have a CRP concentration significantly higher than a randomly chosen animal from the negative reference population. Highly discriminatory tests have ROC curves that tend toward the upper left corner with AUCs approaching 1. For the ROC analysis, GraphROC for Windows, version 2.0 (Maxiwatti) was used.

RESULTS

The mean intra- and interassay CVs of the assay were 5·81 per cent and 13·03 per cent, respectively, for samples with a low CRP concentration, and 6·92 per cent and 13·30 per cent, respectively, for samples with a high CRP concentration. The concentrations of CRP measured in the two diluted effusions were linear and proportional (Fig 1); the limit of detection of the assay was 0·066 ng/ml.

TABLE 2: Median and 25th and 75th percentile concentrations of C-reactive protein (µg/ml) in 18 canine transudates, 15 modified transudates and 17 exudates

	25th percentile	Median	75th percentile
Transudates	0·13	0·72	2·17
Modified transudates	1·09	3·20[a]	5·89
Exudates	12·41	16·09[b,c]	47·68

[a] P<0·01 between transudates and modified transudates
[b] P<0·001 between modified transudates and exudates
[c] P<0·001 between transudates and exudates

The total protein concentration, NCC and CRP concentration of each effusion are shown in Table 1. In the transudates the CRP concentrations ranged from 0·0094 to 7·87 µg/ml, in the modified transudates from 0·045 to 10·78 µg/ml, and in the exudates from 4·47 to 54·59 µg/ml. The Kruskal-Wallis test revealed significant differences among groups (P<0·001). The concentrations of CRP in the transudates were significantly lower than those in the modified transudates and exudates, the concentrations of CRP in the modified transudates were significantly lower than in the exudates but significantly higher than those in the transudates, and the concentrations of CRP in the exudates were significantly higher than in the transudates and modified transudates (Table 2). However, the concentrations of CRP in the different groups did overlap (Fig 2). The overlap between the transudates and the exudates was minimal, with only one of the exudates having a CRP concentration (4·47 µg/ml) that could be included within the range for the transudates. Values of sensitivity, specificity and positive and negative predictive values for distinguishing between the different types of effusion were calculated at different cut-off points. The ROC curves are shown in Fig 3. Thus, with a cut-off value of 1 µg/ml, the sensitivity of CRP for distinguishing transudates from modified transudates was 80 per cent, the specificity was 66·7 per cent, the positive predictive value was 66·7 per cent and the negative predictive value was

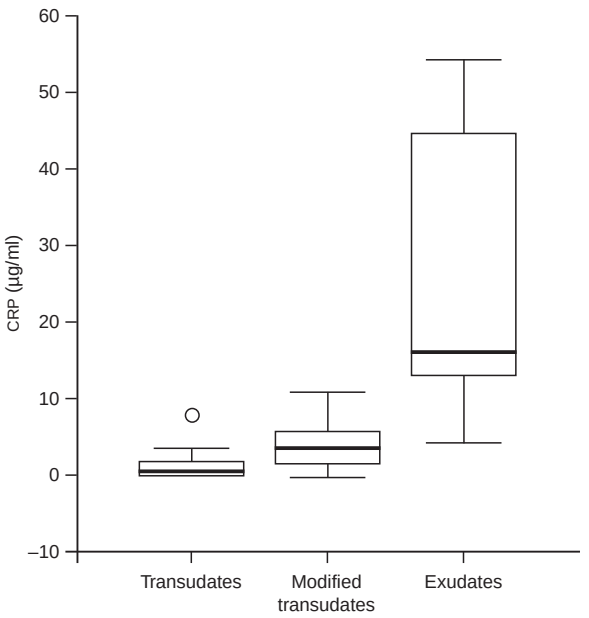

FIG 2: Box and whisker plots of the concentrations of C-reactive protein (CRP) in the 18 canine transudates, 15 modified transudates and 17 exudates. The circle indicates an outlier

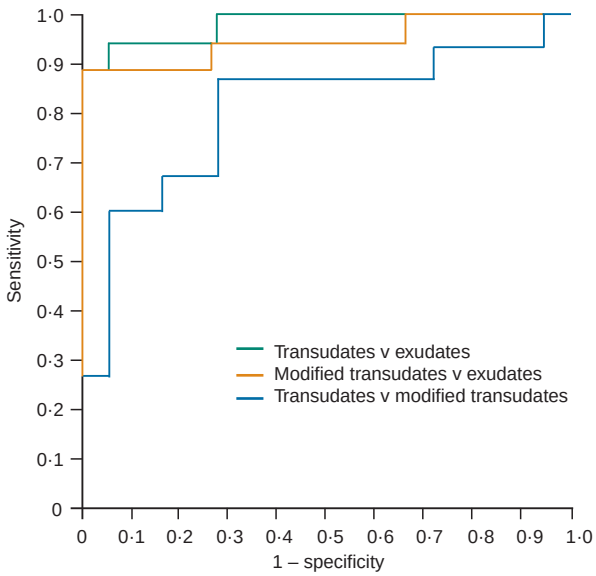

FIG 3: Receiver operating characteristic curves of C-reactive protein used to differentiate transudates from exudates (area under the curve [AUC] [se] 0·98 [0·039]); modified transudates from exudates (AUC 0·94 [0·049]); and transudates from modified transudates (AUC 0·80 [0·085])

TABLE 3: Sensitivity, specificity and positive and negative predictive values of the concentrations of C-reactive protein (CRP) for differentiating transudates from modified transudates at different cut-off points

	CRP (µg/ml)		
	<3	<2	<1
Sensitivity	53·3	66·7	80·0
Specificity	88·8	72·2	66·7
PV+	80·0	66·7	66·7
PV–	69·6	72·2	80·0

PV+ Positive predictive value, PV– Negative predictive value

TABLE 4: Sensitivity, specificity and positive and negative predictive values of the concentration of C-reactive protein (CRP) for differentiating transudates from exudates at different cut-off points

	CRP (µg/ml)		
	<8	<7	<4
Sensitivity	88·2	94·1	100
Specificity	100	94·4	94·4
PV+	100	94·1	94·4
PV–	90·0	94·4	100

PV+ Positive predictive value, PV– Negative predictive value

TABLE 5: Sensitivity, specificity and positive and negative predictive values of the concentration of C-reactive protein (CRP) for differentiating modified transudates from exudates at different cut-off points

	CRP (µg/ml)				
	<11	<10	<7	<5	<4
Sensitivity	88·2	88·2	94·1	94·1	100
Specificity	100	93·3	86·7	60·0	60·0
PV+	100	93·8	88·9	72·7	73·9
PV–	88·2	87·5	92·9	90·0	100

PV+ Positive predictive value, PV– Negative predictive value

80 per cent (Table 3). A CRP concentration below 4 µg/ml had a sensitivity of 100 per cent, a specificity of 94·4 per cent, a positive predictive value of 94·4 per cent and a negative predictive value of 100 per cent for differentiating transudates from exudates (Table 4). A CRP concentration less than 11 µg/ml had a sensitivity of 88·2 per cent, a specificity of 100 per cent, a positive predictive value of 100 per cent and a negative predictive value of 88·2 per cent for differentiating modified transudates from exudates (Table 5).

DISCUSSION

To the authors' knowledge, this is the first time that measurements of CRP have been used to investigate effusions in veterinary medicine. Duthie and others (1997) observed that the acute phase protein α_1-acid glycoprotein was useful for distinguishing cats with infectious peritonitis from cats with diseases with similar clinical signs. The present results show that CRP measurements by TR-IFMA had a sensitivity of 100 per cent and a specificity of 94·4 per cent for differentiating exudates from transudates at a cut-off point of 4 µg/ml, and a sensitivity of 88·2 per cent and a specificity of 100 per cent for distinguishing exudates from modified transudates at a cut-off point of 11 µg/ml.

Although there were significantly higher CRP concentrations in the modified transudates than in the transudates, from a clinical point of view, the transudates could not be differentiated accurately from the modified transudates because of the significant overlap of CRP concentrations between the two groups; this overlap resulted in a lower sensitivity (80 per cent) and an unacceptably low specificity (66·7 per cent). A possible explanation for this overlap might be that transudates are usually associated with hypoalbuminaemia, which results in a decreased oncotic pressure, allowing fluid to accumulate in the body cavity. The serum albumin concentration must usually be below 10 g/l for a transudate to develop as a result of hypoalbuminaemia alone (Meyer and others 1998). On the other hand, most modified transudates develop as a result of increased vascular permeability or increased hydrostatic pressure of hepatic blood and/or lymph (Tyler and Cowell 1989). However, in some circumstances, for example, if there is hypertension, a transudate can develop with a serum albumin concentration above 10 g/l, whereas early congestive heart failure and diaphragmatic hernia, the two conditions that most commonly lead to a modified transudate, can be included in the differential diagnosis of transudates (Dunn and Villiers 1998). In the present study, three cases of early cardiac disease were included as transudates.

Four of the effusions had total protein concentrations below 25 g/l but a NCC above 1×10^9/l, as a result of which they should have been classified as modified transudates. However, they were classified as transudates on the basis of their aetiology: one case of hepatic lymphoma with a total protein concentration of 3·7 g/l and NCC of $1{\cdot}1 \times 10^9$/l; one case of intestinal disease that was causing severe hypoalbuminaemia with a total protein concentration of 0·8 g/l and NCC of $5{\cdot}8 \times 10^9$/l; and two cases of liver insufficiency with total protein concentrations and NCCs of 0·7 g/l and $1{\cdot}3 \times 10^9$/l, and 4·1 g/l and $2{\cdot}6 \times 10^9$/l, respectively. The same occurred with two modified transudates that might have been classified as exudates on the basis of their total protein concentrations (36·9 and 32·8 g/l) and NCCs ($8{\cdot}8 \times 10^9$ and $5{\cdot}2 \times 10^9$/l) but were classified as modified transudates because they were caused by cardiac disease. In these doubtful effusions, the CRP concentrations agreed with the aetiological classification; the CRP levels in three of the four transudates were within the 25th to 75th percentiles of the transudates, and in the two modified transudates were below the 25th percentile of the modified transudates.

The use of the very sensitive technology of time-resolved fluorometry (Lövgren and Pettersson 2000) made it possible to measure the very low concentrations of CRP that are present in some transudates and modified transudates. A commercially available canine CRP ELISA kit for dogs (Tridelta Development) did not detect CRP in three of five of the transudates tested. However, a good correlation was found between the TR-IFMA and the ELISA kit when CRP was measured by both methods in the exudates (R^2=0·97; y=1·01x – 13·35, where y=TR-IFMA results and x=ELISA results). The ELISA method could therefore be used to measure the high concentrations of CRP that appear in exudates.

The results of this study show that CRP can be measured in canine body cavity effusions using the quantitative immunofluorometric assay described, and that measurement of CRP can be a useful additional diagnostic test for differentiating transudates from exudates and modified transudates from exudates. Further studies should be carried out to evaluate whether measurements of other acute phase proteins are useful in the investigation of canine body cavity effusions.

References

ALEXANDRAKIS, M. G., COULOCHERI, S. A., BOUROS, D., VLACHONIKOLIS, I. G. & ELIOPOULOS, G. D. (2000) Significance of alpha-2-macroglobulin, alpha-1-acid glycoprotein, and C-reactive protein in pleural effusion differentiation. *Respiration* **67,** 30-35

ALEXANDRAKIS, M. G., KYRIAKOU, D. S., BOUROS, D., XYLOURI, I., ANTONAKIS, N. & SIAFAKAS, N. M. (2001) Interleukin-6 and its relationships to acute phase proteins in serous effusion differentiation. *Oncology Reports* **8,** 415-420

BRAUN, J. P., GUELFI, J. F. & PAGES, J. P. (2001) Comparison of four methods for determination of total protein concentrations in pleural and peritoneal fluid from dogs. *American Journal of Veterinary Research* **62,** 294-296

CASTANO VIDRIALES, J. L. & AMORES ANTEQUERA, C. (1992) Use of pleural fluid C-reactive protein in laboratory diagnosis of pleural effusions. *European Journal of Medicine* **1,** 201-207

COWELL, R. L., TYLER, R. D. & MEINKOTH, J. H. (1999) Abdominal and thoracic fluid. In Diagnostic Cytology and Hematology of the Dog and Cat. 2nd edn. Eds R. L. Cowell, R. D. Tyler, J. H. Meinkoth. St Louis, Mosby-Year Book. pp 142-158

DELONG, E. R., DELONG, D. M. & CLARKE-PEARSON, D. L. (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44,** 837-845

DUNCAN, J. R., PRASSE, K. W. & MAHAFFEY, E. A. (1994) Cytology. In Veterinary Laboratory Medicine. Clinical Pathology. 3rd edn. Eds J. R. Duncan, K. W. Prasse, E. A. Mahaffey. Ames, Iowa State University Press. pp 204-211

DUNN, J. & VILLIERS, E. (1998) Cytological and biochemical assessment of pleural and peritoneal effusions. *In Practice* **20,** 501-505

DUTHIE, S., ECKERSALL, P. D., ADDIE, D. D., LAWRENCE, C. E. & JARRETT, O. (1997) Value of α1-acid glycoprotein in the diagnosis of feline infectious peritonitis. *Veterinary Record* **141,** 299-303

EID, A. A., KEDDISSI, J. I., SAMAHA, M., TAWK, M. M., KIMMELL, K. & KINASEWITZ, G. T. (2002) Exudative effusions in congestive heart failure. *Chest* **122,** 1518-1523

GARCÍA-PACHON, E. & LLORCA, I. (2002) Diagnostic value of C-reactive protein in exudative pleural effusions. *European Journal of Internal Medicine* **13,** 246-249

GOUTEUX-FREZZOTTI, D., BOLARD, F., RAMON, P. M. & STEENHOUWER, F. (2002) Biological criteria for distinguishing exudative and transudative pleural effusions. Usefulness in a general hospital. *Revue de Pneumologie Clinique* **58,** 11-16

HAYASHI, S., JINBO, T., IGUCHI, K., SHIMIZU, M., SHIMADA, T., NOMURA, M., ISHIDA, Y. & YAMAMOTO, S. (2001) A comparison of the concentrations of C-reactive protein and α1-acid glycoprotein in the serum of young and adult dogs with acute inflammation. *Veterinary Research Communications* **25,** 117-120

JERGENS, A. E., SCHREINER, C. A., FRANK, D. E., NIYO, Y., AHRENS, F. E., ECKERSALL, P. D., BENSON, T. J. & EVANS, R. (2003) A scoring index for disease activity in canine inflammatory bowel disease. *Journal of Veterinary Internal Medicine* **17,** 291-297

LÖVGREN, T. & PETTERSSON, P. (2000) Time-resolved fluoroimmunoassay, advantages and limitations. In Luminescence Immunoassay and Molecular Applications. Eds. K. Van Dyke, R. Van Dyke. Boca Raton, CRC Press. pp 233-253

MARTÍNEZ-SUBIELA, S., TECLES, F., ECKERSALL, P. D. & CERÓN, J. J. (2002) Serum concentrations of acute phase proteins in dogs with leishmaniasis. *Veterinary Record* **150,** 241-244

MEYER, D. J., COLES, E. H. & RICH, L. J. (1998) Evaluation of effusions. In Veterinary Laboratory Medicine. Interpretation and Diagnosis. Philadelphia, W. B. Saunders. pp 125-130

PARRA, M. D., TUOMOLA, M., CABEZAS-HERRERA, J. & CERÓN, J. J. (2005) Use of a time-resolved immunofluorometric assay for determination of canine C-reactive protein concentrations in whole blood. *American Journal of Veterinary Research* **66,** 62-66

PETERSEN, H. H., NIELSEN J. P. & HEEGAARD, P. M. H. (2004) Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research* **35,** 163-187

SEGURA, R. M. (2003) Useful clinical biological markers in diagnosis of pleural effusions in children. *Paediatric Respiratory Reviews* **83,** 219-226

TYLER, R. D. & COWELL, R. L. (1989) Evaluation of pleural and peritoneal effusions. *Veterinary Clinics of North America: Small Animal Practice* **19,** 743-768

YILMAZ TURAY, U., YILDIRIM, Z., TURKOZ, Y., BIBER, C., ERDOGAN, Y., KEYF, A., UGURMAN, F., AYAZ, A., ERGUN, P. & HARPUTLUOGLU, M. (2000) Use of pleural fluid C-reactive protein in diagnosis of pleural effusions. *Respiratory Medicine* **94,** 423-435

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