

Peritoneal Fluid Analysis in the Diagnosis of Abdominal Disorders In Cattle: A Retrospective Study

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SUMMARY

A retrospective study of bovine peritoneal fluids collected over a two year period was conducted. Of a total of 66 cattle studied, 31 had a nonseptic peritonitis, 11 acute bacterial peritonitis, eight ascites and 16 miscellaneous disorders such as abomasal impaction, enteritis and lymphosarcoma. Peritoneal fluid analysis was a useful aid in the diagnosis of abdominal disorders of cattle, especially as hematological changes were absent in many cases. Due to relatively low nucleated cell counts in bovine peritonitis, all parameters (i.e. nucleated cell count, total protein and differential cell counts) must be evaluated before interpretation. A nucleated cell count of greater than 6000 cells/ μ L and total protein content of greater than 3 g/dL was consistent with the diagnosis of peritoneal inflammation in 80% of the cases studied. An atlas of cell types common to bovine peritoneal fluid is presented.

RÉSUMÉ

Une étude rétrospective de l'analyse des épanchements péritonéaux, dans le diagnostic des affections péritonéales bovines

Cet article rapporte les résultats d'une étude rétrospective des échantillons d'épanchements péritonéaux prélevés chez des bovins, au cours d'une période de deux ans. Des 66 bovins impliqués dans cette étude, 31 étaient atteints d'une péritonite aseptique, 11 souffraient d'une péritonite septique et huit ne présentaient que de l'ascite, tandis que les 16 autres se partageaient les conditions suivantes: impaction de la caillette, entérite et lymphosarcome. L'analyse de ces épanchements se révéla utile pour le diagnostic des affections abdominales des bovins

inclus dans l'étude, puisque les changements hématologiques faisaient défaut dans plusieurs cas. À cause du nombre relativement peu élevé de cellules nucléées dans les cas de péritonite bovine, il convient d'en déterminer le nombre, ainsi que la quantité de protéines et les numérations différentielles, avant de procéder à l'interprétation de la cytologie des épanchements péritonéaux. Une numération des cellules nucléées, supérieure à 6000 cellules/ μ L, et une quantité de protéines, plus élevée que 3 g/dL, accompagnèrent 80% des cas d'inflammation péritonéale. Les auteurs présentent un atlas des différentes cellules qu'on retrouve communément dans le liquide péritonéal des bovins.

INTRODUCTION

Peritoneal fluid analysis has considerable potential as an aid to the hematological and clinical examination in the diagnosis of bovine abdominal disorders since it can indirectly assess the volume of peritoneal fluid, cellularity and protein characteristics thereby giving an indication of inflammatory changes in the peritoneal cavity (9,10). This technique could therefore aid in the diagnosis of traumatic reticuloperitonitis (TRP) and acute abdominal crises in cattle. It may be particularly valuable in the diagnosis of chronic peritonitis in cattle, a condition that is rarely accompanied by the hematological changes typical of peritoneal inflammation in other species (3,5,11).

Although peritoneal fluid analysis in cattle was investigated as early as 1964, it is still not a widely accepted diagnostic procedure in this species (9,10). Previous reports are incomplete, stressing differential cell counts with little information on total cell counts and total protein content

(9,10). In addition, reference values for these parameters in peritoneal fluid from normal cattle are not available. More complete data is available on equine peritoneal fluid (1,2,4,7,8,12) but it is unwise to extrapolate between these two species due to major differences in the types of abdominal disorders in cattle as opposed to horses (3,6).

This paper reports the findings of a retrospective study of peritoneal fluid samples from bovine clinical cases. The purpose of the study was to document the cellular and protein characteristics of peritoneal fluid in cattle with peritonitis, ascites or other abdominal disorders and to establish guidelines for differentiating peritonitis from noninflammatory peritoneal fluid. An additional objective was to present a cytological atlas of cell types common to peritoneal fluid in cattle in order to assist the large animal practitioner in the interpretation of peritoneal fluid cytology.

MATERIALS AND METHODS

All bovine peritoneal fluid samples collected from clinical cases admitted to the Large Animal Clinic of the Western College of Veterinary Medicine during the years 1978 to 1981 were retrospectively analyzed. Only samples collected from cases with a subsequent exploratory laparotomy or necropsy examination were included in the study. All samples of less than 0.5 mL were excluded from the study.

Techniques for Collection of Fluid

The technique for collection of peritoneal fluid has been described (9,10). Fluid was collected from the cattle in the standing position. The recommended site for collection is about 4 cm medial and 5 to 7 cm cranial to

the point where the milk vein enters the abdomen. The site was clipped, surgically prepared and locally anesthetized. A small stab incision was made through the skin and external fascia and a 9 cm blunt ended bovine teat cannula passed through the incision. The cannula was then gently pushed through the *rectus abdominis* muscle and with a quick, short thrust popped through the peritoneum. To prevent blood contamination, the shaft of the cannula was wrapped with a sterile gauze sponge. If no fluid was obtained from this site, the tap was repeated a few centimetres caudal to the site at the most dependent aspect of the abdomen.

The fluid obtained was collected in a small tube containing dipotassium ethylene diamine tetraacetic acid (EDTA) and analyzed as soon as possible after collection. If this was not possible, smears of the fluid were made immediately and the remainder of the sample stored at 4°C for total cell counts and total protein analysis.

Laboratory Evaluation

Three parameters of the peritoneal fluid were examined: (a) the nucleated cell count (NCC), (b) the total protein content (TP) and (c) cytological characteristics including a differential cell count. The nucleated cell count was determined using an automated cell counter.¹ The total protein content was determined with a refractometer² on the supernatant of a centrifuged sample. Smears of both the original fluid sample and cellular sediment from the centrifuged sample were stained with Wright's-Giemsa for cytological examination.

White blood cell counts were done on all cattle using an automated cell counter and differential leukocyte counts were done on Wright's-Giemsa smears. Plasma fibrinogen levels were determined using a heat precipitation technique (11). The leukon was interpreted as either normal, a stress leukon (i.e. neutrophilia and lymphopenia or neutrophil to lymphocyte ratio of $\geq 2:1$), mild left shift, or degenerative left shift by the criteria cited by Schalm (11).

Classification of Fluids

The fluids from each case were first grouped based on the presence or absence of peritonitis at necropsy or laparotomy into (a) noninflammatory and (b) inflammatory fluids. The inflammatory fluids were further subcategorized on the microscopic presence or absence of bacteria, to determine whether there were distinct characteristics for the two categories, (i) nonseptic peritonitis and (ii) septic peritonitis. Bacteriological examination was not done consistently on all the fluids, therefore fluids were considered to be nonseptic on the basis of absence of bacteria by microscopic examination.

The changes in the leukon and plasma fibrinogen levels were correlated with the classification of the fluid in order to determine how reliable these parameters were as an indicator of peritoneal inflammation in cattle.

RESULTS

Sixty-six cattle with a variety of disorders were evaluated. The majority of cattle in this study were mature animals. A total of ten calves were also included in the study, but the conclusions should probably only be applied to mature cattle. The mean nucleated cell counts and total proteins and ranges for each of these categories are summarized in Table I.

Ascites

There were eight mature cattle in this category with a diagnosis of congestive heart failure. Five of the eight animals had a stress or normal leukon and the remaining three had a mild left

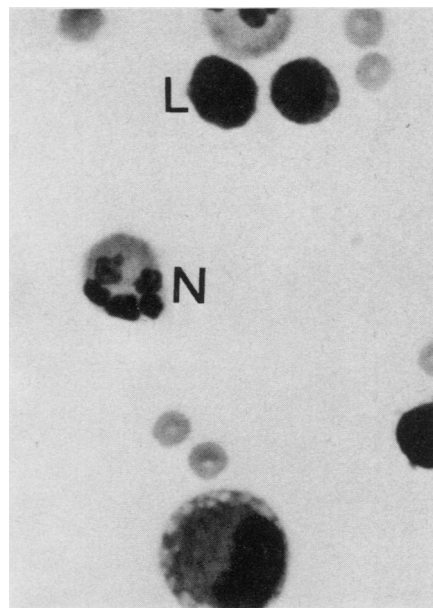


FIGURE 1. Wright's-Giemsa stained smear of peritoneal fluid from a cow with abomasal impaction. Nondegenerative neutrophils (N), lymphocytes (L) and a mesothelial cell (arrow) are present. X880.

shift. The plasma fibrinogen in all eight cases was in the normal range (Table II).

Cytological studies of these fluids revealed a mixture of cell types with a ratio of approximately 1:1 mononuclear cells to neutrophils (Figure 1). The neutrophils were nondegenerate and the mononuclear cells consisted of small lymphocytes and mesothelial cells. A few active macrophages and reactive mesothelial cells were present but did not predominate. Occasionally, sheets of mesothelial cells were present. Some of these fluids contained up to 20% eosinophils (Figure 2).

TABLE I
CHARACTERISTICS OF BOVINE PERITONEAL FLUID

Classification of fluid	Nucleated Cell Count ($\times 10^3/\mu\text{L}$)		Total Protein (g/dL)	
	Mean	Range	Mean	Range
Nonseptic Peritonitis (n = 31)	14.7	3.2-47.4	5.3	2.8-9.8
Septic Peritonitis (n = 11)	7.0	1.0-31.1	3.9	2.5-5.8
Ascites (n = 8)	2.4	0.8- 6.4	1.6	0.4-2.8
Miscellaneous Noninflammatory (n = 16)	4.3	1.2-16.3 ^a	1.9	0.1-3.3

^aRange not including neoplastic effusions was $1.2-5.3 \times 10^3/\mu\text{L}$.

¹Coulter Model S, Coulter Electronics Inc., Hialeah, Florida.

²TS Meter, American Optical Co., Buffalo, New York.

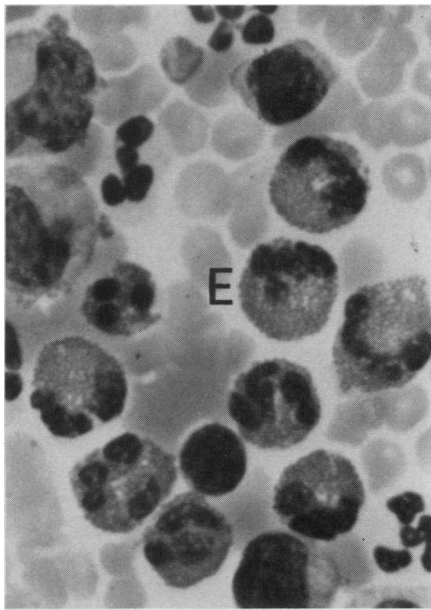


FIGURE 2. Peritoneal fluid cytology from a cow with congestive heart failure showing a large proportion of eosinophils (E), some neutrophils and macrophages (Wright's-Giemsa). X880.

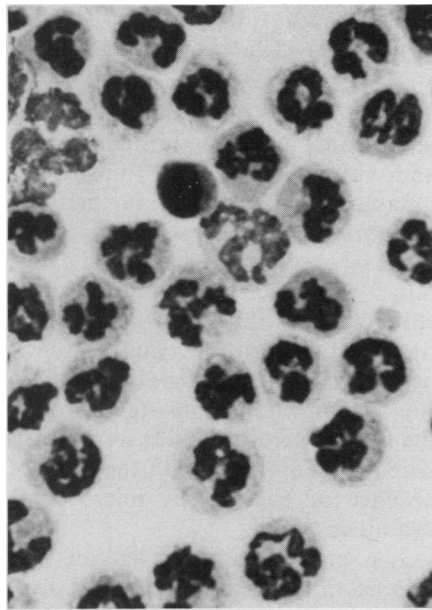


FIGURE 3. Peritoneal fluid cytology from a cow with nonseptic peritonitis due to traumatic reticuloperitonitis showing nondegenerate neutrophils. Bacteria cannot be seen (Wright's-Giemsa stain). X880.

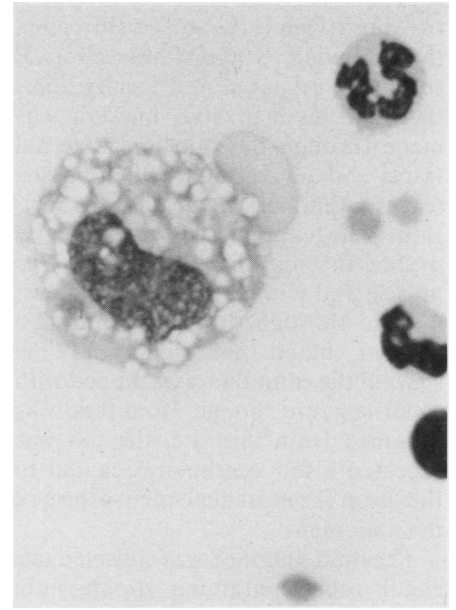


FIGURE 4. An actively phagocytic macrophage in a cow with traumatic reticuloperitonitis (Wright's-Giemsa stain). X880.

Nonseptic Peritonitis

These fluids were collected from 31 cattle, including 22 cases of traumatic reticuloperitonitis (TRP), five abdominal accidents, three postsurgical samples and from one cow with severe enteritis.

Nondegenerate neutrophils predominated in these samples (Figure 3). The mononuclear to neutrophil ratio ranged from 1:3 to 1:9. Mononuclear cells were usually actively phagocytic macrophages (Figure 4) and reactive mesothelial cells (Figures 5 and 6). Occasionally an exudate contained mostly macrophages. Eosinophils were not commonly seen. No bacteria were found on cytological preparations.

The leukons of cattle in this cate-

gory were normal or consistent with stress in 65% of the cases. In only 35% a left shift was present (Table II). In contrast, 68% of these cases had an elevated plasma fibrinogen of greater than 600 mg/dL, and an average fibrinogen of 700 mg/dL.

Septic Peritonitis

Eleven cattle had a bacterial peritonitis. Three were due to a ruptured abomasal ulcer, four to acute traumatic reticuloperitonitis and the remainder due to miscellaneous causes (i.e. surgery, urinary bladder rupture, ruptured abdominal abscess). Considerable overlap with cell counts of noninflammatory fluids occurred (Figure 10), therefore the cytological

characteristics were important differentiating features.

These fluids contained degenerate neutrophils and bacteria, both within neutrophils and extracellularly (Figure 7). In the cases with a perforation of the gastrointestinal tract, the fluid contained plant fibres, squamous cells and a mixed bacterial population (Figure 7). These fluids could be differentiated from gut contents by the elevated pro-

TABLE II
HEMATOLOGICAL FINDINGS AND PLASMA FIBRINOGEN AS CORRELATED
WITH CLASSIFICATION OF PERITONEAL FLUID

Classification of Fluid	Normal Leukon	Stress Leukon	Mild Left Shift in Leukon	Degenerative Left Shift	Plasma Fibrinogen of > 600 mg/dL
Nonseptic Peritonitis	45(14) ^a	20(6)	22(7)	13(4)	68(21)
Septic Peritonitis	0(0)	0(0)	40(4)	60(7)	55(6)
Noninflammatory fluid	40(8)	20(4)	30(6)	10(2)	40(8)

^aNumbers in parentheses indicate number of cases.

Numbers without parenthesis indicate percentage of cases.

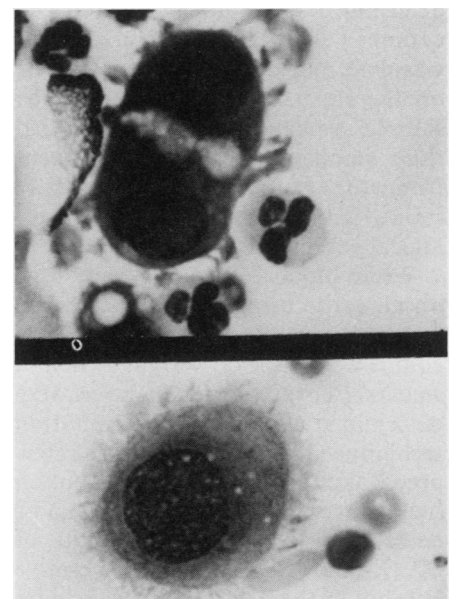


FIGURE 5. Reactive mesothelial cells. Both have a ruffled border and one cell has an eosinophilic brush border. Note the large size in comparison to neutrophils (Wright's-Giemsa stain). X880.

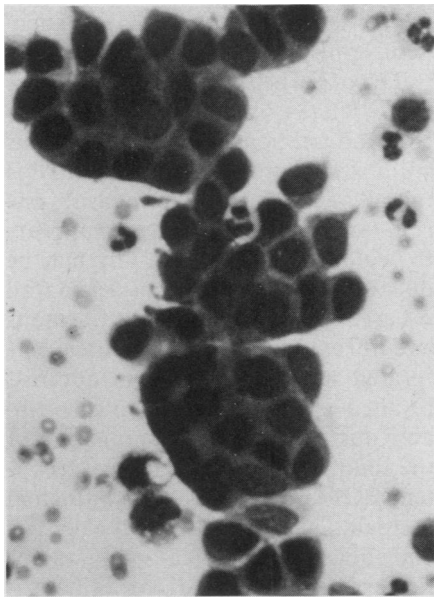


FIGURE 6. A large clump of mesothelial cells with uniform nuclear size (Wright's-Giemsa stain). X350.

tein content and the presence of degenerate neutrophils.

A shift in the leukon varying from mild to degenerative was present in all cases. The plasma fibrinogen was elevated in six of the eleven cases (Table II)



FIGURE 7. Peritoneal fluid from a cow with a septic peritonitis due to a perforated abomasal ulcer. The top shows severely degenerate neutrophils with swollen pink nuclei and indistinct cytoplasm which contain bacteria. Some free bacteria also present. X880. Bottom shows low power view of same fluid with plant fibres evident (Wright's-Giemsa stain). X90.

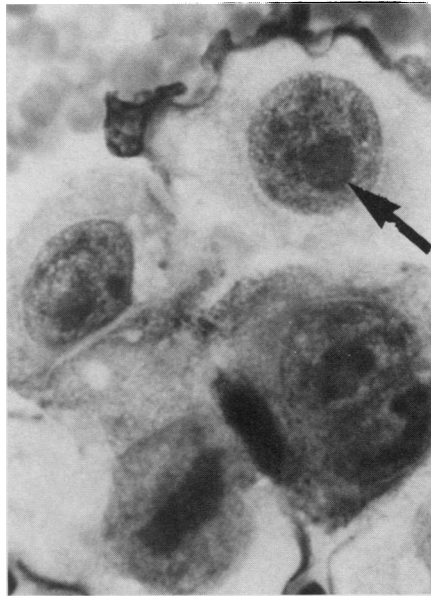


FIGURE 8. Large bizarre epithelial cells with nucleoli from peritoneal fluid in a case of disseminated squamous cell carcinoma. Arrow indicates a prominent nucleolus (Wright's-Giemsa stain). X880.

Miscellaneous Conditions

All of the cattle included in this category had fluids which were considered to be noninflammatory in nature.

The 16 cases in this category were represented by vagus indigestion (n = 2), bloat (n = 2), abomasal impaction (n = 2), enteritis (n = 3), metritis (n = 2), fat necrosis (n = 2) and neoplasia (n = 2).

The cytological features of these peritoneal fluids varied with the etiology. Those cattle with functional disorders such as abomasal impaction had a mononuclear to neutrophil ratio of approximately 1:1, consisting of non-degenerate neutrophils, lymphocytes and mesothelial cells, similar to the cattle with ascites. In fat necrosis, non-degenerate neutrophils predominated. Cytological preparations of fluid from the cow with lymphosarcoma revealed many bizarre lymphocytes and mitotic figures. A population of large, pleomorphic, epithelial cells in clusters predominated in peritoneal fluid from a cow with disseminated squamous cell carcinoma (Figure 8).

Comparison of Inflammatory and Noninflammatory Fluids

Statistical analysis was not done on this data due to the nonparametric distribution of results. The nucleated cell



FIGURE 9. Appearance of peritoneal fluid from a cow with traumatic reticuloperitonitis. The fluid is yellow, cloudy and contains fibrin clumps.

counts (Figure 10), total protein content (Figure 11) and plasma fibrinogen levels (Figure 12) were compared graphically between the four different categories. Elevations of both the nucleated cell count (NCC) and total protein (TP) content in peritoneal fluid from cases with peritoneal inflammation were observed.

DISCUSSION

This study demonstrates that peritoneal fluid analysis is a useful aid in the diagnosis of peritonitis in cattle. Adequate normal reference values for bovine peritoneal fluid have not been reported and therefore an arbitrary division was made between inflammatory and noninflammatory fluids based on the results as presented in Figures 10 and 11. A fluid with a NCC of greater than 6000 cells/ μ L in addition to a TP content of greater than 3 g/dL was always associated with peritonitis. Eighty percent of confirmed cases of nonseptic peritonitis had results consistent with these guidelines (Table III). In the remaining cattle only one parameter of the peritoneal fluid, either NCC or TP was significantly increased. In contrast, 85% of peritoneal fluids obtained from cattle with no peritoneal inflammation had NCC and TP values lower than these arbitrary values and both values never increased concurrently (Table

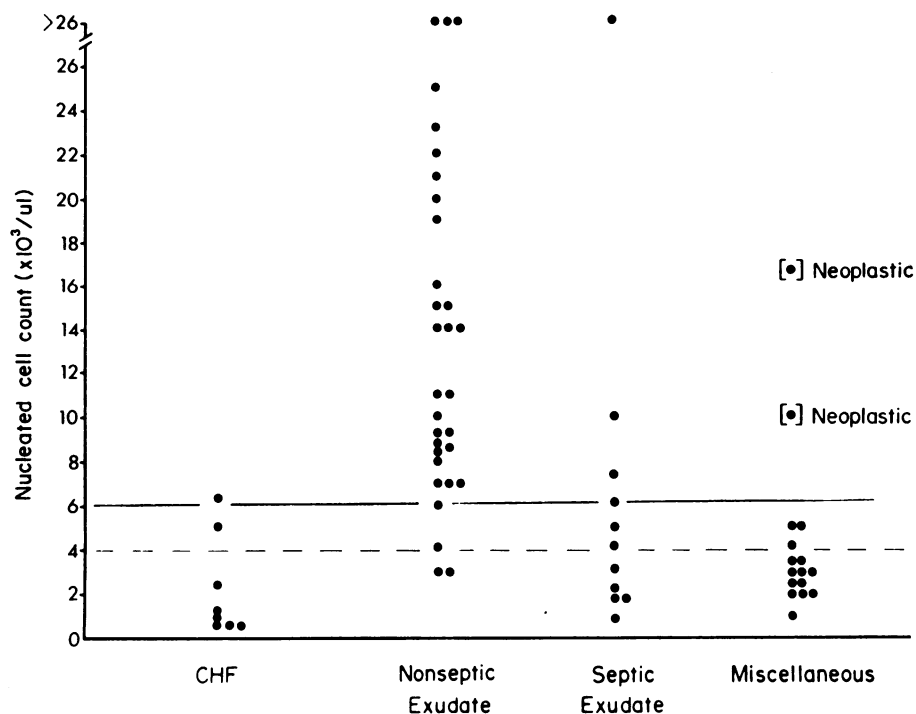


FIGURE 10. Graphical representation of nucleated cell counts of peritoneal fluid from cattle with congestive heart failure, nonseptic and septic peritonitis and other miscellaneous noninflammatory conditions. Division between inflammatory and noninflammatory fluids (—). A gray area exists between 4000 cells (.....) and 6000 (—) where interpretation is difficult.

III). Therefore, in 80 to 85% of cases in this study, a definitive diagnosis could be made on the basis of peritoneal fluid analysis. In contrast to nonseptic peritonitis, fluid from cases with sepsis had more variable characteristics, the

most notable being a low NCC (Figure 10). However, the presence of bacteria and degenerate neutrophils on cytological preparations were a clear differentiating feature. These lower NCC values probably reflect the acute

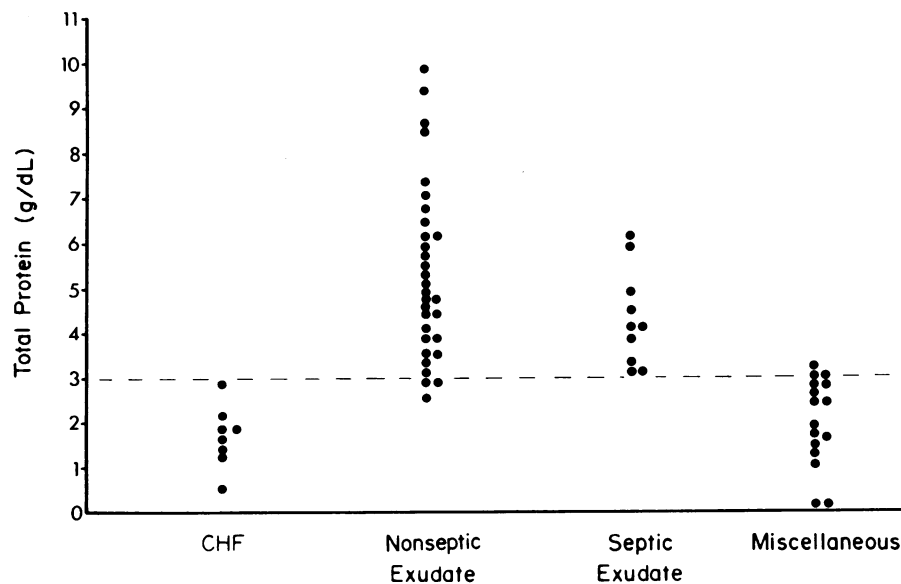


FIGURE 11. Graphical representation of total protein content of peritoneal fluids from cattle with congestive heart failure (CHF), nonseptic peritonitis, septic peritonitis and miscellaneous noninflammatory conditions. Possible division between inflammatory and noninflammatory fluids (---).

course of the disease and also lysis of neutrophils by bacterial toxins (5,11).

The overwhelming majority of cases with TRP (84%) were classified as having a nonseptic peritonitis. From this study it appears that although TRP is initially caused by a septic insult, the resultant peritonitis frequently appears to be nonseptic in nature. This may be due to the ability of this species to wall off areas of inflammation by fibrinous and fibrous adhesions (6). Acutely perforated abomasal ulcers in contrast resulted in a septic peritonitis, probably due to massive contamination of the abdominal cavity by abomasal contents at the time of rupture. Abdominal accidents such as intestinal volvulus and abomasal torsion caused a nonseptic peritonitis but the degree of peritoneal inflammation may reflect the duration of the disease process. Peritoneal fluid analysis was also helpful in the diagnosis of generalized abdominal neoplasia. In this study cytological preparations revealed neoplastic cells in one case of squamous cell carcinoma and one cow with lymphosarcoma. The incidence of these neoplasms in cattle is low in contrast to other abdominal disorders.

Peritoneal fluid analysis has become established in the routine laboratory screening of equine colic cases (1,2,4,5,7,8,12). In this species, changes in the integrity of the bowel wall are rapidly reflected in the peritoneal fluid by increased volume, cellularity and the appearance of bacteria in later stages of gut devitalization (1,7,8). It is obvious when comparing nucleated cell counts of peritoneal fluid from cases of peritonitis in cattle and horses that distinct differences exist. Peritonitis in the horse is characterized by high cell counts in the range of 50 000 to 100 000 cells/ μ L (1,7,8) whereas the average nucleated cell count of fluids in cases of bovine peritonitis was only 14 700 cells/ μ L. This may reflect the chronic course of peritonitis in cattle, as caused by traumatic reticuloperitonitis, but might also be the result of a lesser ability of this species to mobilize neutrophils from the bone marrow in response to inflammation (3,5,11).

The evaluation of hematological findings of the different categories in this study shows that cases with septic peritonitis consistently reflected the severe inflammatory process by the

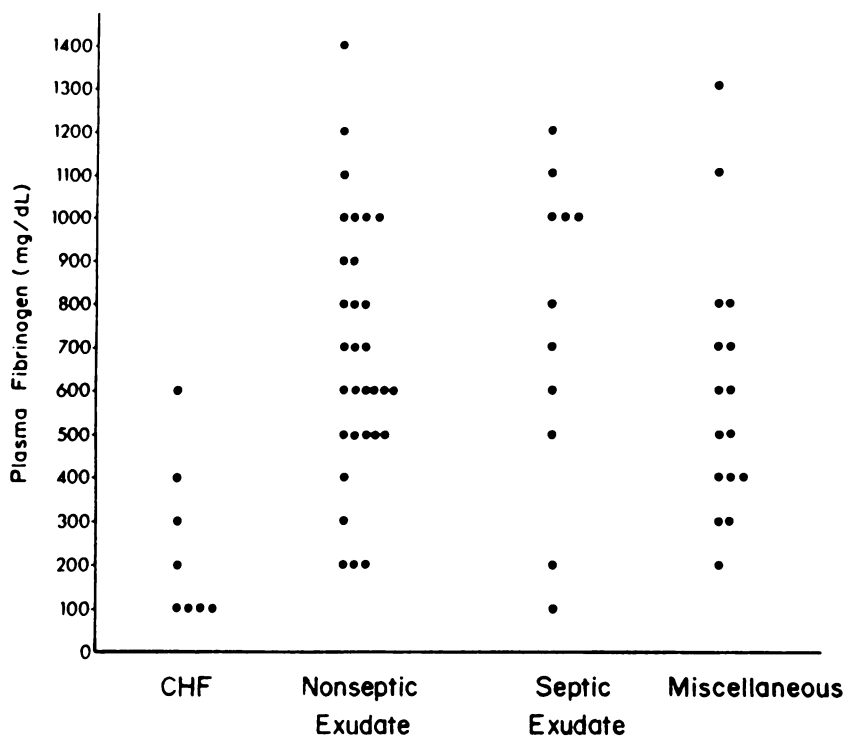


FIGURE 12. Graphical representation of plasma fibrinogen in congestive heart failure, nonseptic peritonitis and septic peritonitis and miscellaneous noninflammatory conditions. Notice the wide range of values obtained in all categories.

development of a degenerative left shift, whereas the hematological findings in nonseptic peritonitis were often unrevealing. In fact, 65% of the latter cases showed no hematological evidence of inflammation and only 13% of cases had hematological findings consistent with a severe inflammatory process. The plasma fibrinogen was more frequently elevated, indicating an ongoing inflammatory reaction; however, the plasma fibrinogen was not elevated in all cases of peritonitis and was increased in some cases from which noninflammatory peritoneal fluid was obtained. This only confirms that

plasma fibrinogen is a nonspecific indicator of inflammation in cattle, increasing in peritonitis as well as metritis, mastitis, pyelonephritis and other generalized inflammatory conditions (11).

Values for total cell counts, total protein content and cytological characteristics were not established for cattle without peritoneal disease since we were unable to obtain samples by paracentesis from normal cattle. The fluid obtained from cattle with such noninflammatory conditions as abomasal impaction is probably comparable to the peritoneal fluid in normal cattle. It appears to be difficult to obtain perito-

neal fluid from cattle if excess fluid is not present. Therefore, although peritonitis is not ruled out by a negative paracentesis, a positive free-flowing paracentesis indicates excess abdominal fluid.

Previous reports suggest that only one or two drops of peritoneal fluid are necessary for analysis (9,10). The findings of this study, indicate a fine line of division between characteristics of inflammatory and noninflammatory peritoneal fluid. As only moderately elevated nucleated cell counts occurred even in severe peritonitis, small samples should not be interpreted unless complete analysis is possible (i.e. NCC, TP content and cytological examination). These problems are compounded in small samples, if the anticoagulant used is EDTA; this anticoagulant, having a high refractive index can markedly elevate the apparent total protein content of small samples especially if these have a low protein content initially.

In summary, peritoneal fluid analysis is very helpful in the diagnosis of bovine chronic, nonseptic peritonitis. Increases in the cellularity, total protein content, and a predominant cytological cell population of neutrophils in peritoneal fluid may be the only laboratory indications of peritoneal inflammation. Analysis of peritoneal fluid from cattle must be complete to prevent misinterpretation due to moderate elevations of cell counts in peritonitis. More data on expected values in peritoneal fluid from normal or pregnant cattle and cattle with localized peritonitis would still be valuable.

ACKNOWLEDGMENTS

The authors appreciate the assistance of Dr. D. Wilson in attempting to obtain peritoneal fluid from normal cattle.

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TABLE III
CONSISTENCY OF NUCLEATED CELL COUNT (NCC) AND TOTAL PROTEIN (TP) CONTENT WITH THE PRESENCE OR ABSENCE OF PERITONITIS USING A NCC OF > 6000 CELLS/ μ L AND TP OF > 3 g/dL AS INDICATIVE OF PERITONITIS

Classification of Fluid	Increased NCC and TP	Increased TP only	Increased NCC only	Neither Increased
Nonseptic peritonitis	80(25) ^a	10(3)	10(3)	0(0)
Septic peritonitis	36(4)	54(6)	0(0)	10(1)
Noninflammatory fluids	(0)	5(1)	10(2)	85(19)

^aNumbers in parentheses indicate number of cases.

Numbers without parenthesis indicate percentage of cases.

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BOOK REVIEW

Diseases of Cage and Aviary Birds. Second Edition. Edited by M.L. Petrak. Published by Lea & Febiger, Philadelphia. 1982. 680 pages. Price US\$89.50 C\$107.50.

Margaret Petrak's long awaited second edition of "Diseases of Cage and Aviary Birds" is finally available. This volume proves to be a worthy successor to the original, which has become accepted as the standard of avian medical texts.

The book is extremely broad in scope with sections pertaining to virtually all aspects of avian care. The early chapters are concerned with the environment, nutrition, anatomy, physiology, etc. of cage birds. These sections are very well written but the practitioner may find that some portions go into greater depth than he is likely to require. The chapter on avian genetics is an example of this. One must bear in mind, however, that the book is aimed

at a much wider readership than strictly veterinary practitioners.

Part two of the text contains an outstanding amount of information pertinent to the practitioner. The chapters are exceptionally well written. It is enjoyable as well as profitable to sit down and read through them rather than using the text solely as reference material. Once the practitioner has absorbed the more clinical information, he will likely find himself returning to the sections on behavior and environment which were initially skimmed over.

It seems that a much larger portion of the avian practitioner's consultation time is taken up with discussions of environment and behavior than is usual in general small animal practice. Armed with the knowledge presented in this book, a veterinarian should be able to offer practical advice to avian owners who are often faced with a variety of conflicting opinions in the

owner oriented manuals available in pet shops.

Since these hard economic times affect veterinarians as well as everyone else, the question of whether it is worthwhile to spend in excess of a hundred dollars for volume two when you possess volume one is bound to arise. In this reviewer's opinion the investment is certainly justified. Thirteen years is a long time span between editions, particularly in a field where information is multiplying as rapidly as in avian medicine. Two of the original contributing authors are now deceased, and several of the chapters in the new edition are either new or have been completely rewritten.

Considering the wide variety of subject material, this volume could be considered a mini library of books on birds in health and disease. If a veterinarian planned to purchase only one book on avian medicine this year, this should be the one. *D.A. McKiel*

PRIX VÉTÉRIINAIRE GAINES

Dans le but d'encourager le progrès en médecine et en chirurgie des petits animaux, la compagnie General Foods Limitée, par l'entremise du Centre de Service professionnel Gaines, a institué le "Prix vétérinaire Gaines".

Ce prix sera décerné à un vétérinaire dont on aura jugé que le travail a contribué à l'avancement de la médecine et de la chirurgie des petits animaux, soit en recherches cliniques ou en recherches fondamentales, ou s'est distingué dans la gérance d'une pratique pour petits animaux contribuant à aider le public à prendre connaissance de leurs responsabilités en tant que propriétaires d'animaux.

On considérera en premier lieu les travaux exécutés au cours de cinq dernières années ainsi que les travaux des membres qui demeurent toujours actifs dans la profession.

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