

Abdominocentesis in Cattle: Technique and Criteria for Diagnosis of Peritonitis

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ABSTRACT

A reliable method for the collection of peritoneal fluid from cattle using a trocar and cannula is described. Peritoneal fluid was collected from three groups of cattle: periparturient, normal and with peritonitis. The fluid was examined by white cell count, differential cell count, total protein concentration and bacteriology. The results were analysed to determine the best criteria for peritonitis. Greater than 10% eosinophils were typical of normal peritoneal fluid. Peritoneal fluid with a relative neutrophil count greater than 40% and a relative eosinophil count of less than 10% was frequently associated with the diagnosis of peritonitis. Parturient cattle had large volumes of peritoneal fluid with low total protein and white cell counts. Growth of Gram-negative or anaerobic organisms was associated with mortality.

Key words: Peritoneal fluid, peritonitis, eosinophils.

RÉSUMÉ

Technique d'abdominocentèse, chez les bovins, et critères de diagnostic de la péritonite

Cet article décrit une méthode fiable d'effectuer une abdominocentèse, chez des bovins, à l'aide d'un trocart et d'une canule. Cette méthode permet de récolter du liquide péritonéal, chez des vaches normales, ou atteintes de péritonite, ou à la veille de vêler. On y recherche ensuite le nombre total de leucocytes et leur proportion respec-

tive, la quantité totale de protéines, ainsi que des bactéries. On analysa les résultats obtenus, afin de déterminer les meilleurs critères de diagnostic de la péritonite. Une proportion d'éosinophiles supérieure à 10% se révéla caractéristique du liquide péritonéal normal; par ailleurs, une proportion relative de neutrophiles supérieure à 40%, jointe à une proportion d'éosinophiles inférieure à 10%, accompagnait souvent une péritonite. Les vaches sur le point de vêler affichaient une quantité abondante de liquide péritonéal, pauvre en protéines et en leucocytes. L'isolement de bactéries gram-négatives signifiait une péritonite fatale.

Mots clés: liquide péritonéal, péritonite, éosinophiles.

INTRODUCTION

There are several accounts of the analysis of peritoneal fluid in pathological conditions of the bovine abdomen (1,2,3). There are also descriptions of normal bovine peritoneal fluid (1,2) although no details of numbers of cows examined were published. In preliminary studies at this college we experienced difficulty in obtaining samples from healthy cows. From approximately 70% of cattle either no fluid, or fluid severely contaminated with blood was obtained. It is possible that this could lead to a biased assessment of normal peritoneal fluid if only cows with high amounts of fluid yield a

sample. In our experience and that of others (1) cows in late pregnancy have increased amounts of peritoneal fluid but detailed descriptions of its cytology have not been published. In this paper we describe a reliable technique for collection of bovine peritoneal fluid. Using this technique we were able to collect and characterize samples of peritoneal fluid from normal cows and compare these to samples from parturient cows and cattle with peritonitis.

MATERIALS AND METHODS

Three groups of cattle were sampled.

Group 1 consisted of 19 cows presented to the Western College of Veterinary Medicine, University of Saskatchewan for alleviation of dystocia by hysterotomy. These cows were selected for the presence of a live calf, the absence of other clinical disease, and no history of extensive fetal manipulation. Peritoneal fluid was collected during surgery, the skin and muscle layers were incised, and a 5 cm 14 gauge test cannula pushed through the peritoneum prior to opening the abdomen. The resulting free-flowing peritoneal fluid was collected into 2 ml sterile tubes for bacteriology and 2 ml tubes containing 4.5 mg potassium EDTA for cytology and total protein assay. When possible, samples were examined immediately and if not, direct and spun smears were made and the sample stored at 5°C until it could be examined. All samples were examined within 18 hours.

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Group 2 contained 21 apparently healthy nonpregnant Holstein cows. Each cow was examined and a 5 ml blood sample collected for a complete blood count. Abdominocentesis was performed at a site 10 cm cranial and 10 cm to the right-hand side of the umbilicus (Figure 1). The area was clipped, washed with a surgical scrub containing 5% povidone iodine (Betadine Scrub — Purdue Frederick Company (Canada) Ltd., Toronto, Ontario) and swabbed with 70% alcohol. Five mL of 2% Lidocaine HCl containing 1:100,000 epinephrine (Armitage Carroll Ltd., London, Ontario) was injected into the subcutaneous tissue and tissues of the abdominal wall. A bleb of local anesthetic was left under the skin so that the anesthetized site could be readily identified. A stab incision was made through the skin using a No. 15 scalpel blade (Figure 1), taking care to avoid cutting large cutaneous blood vessels. If excessive bleeding occurred it was controlled by applying pressure to the site with a sterile gauze sponge. A sterile 80 mm logn Nelson trocar and cannula of 4 mm internal diameter (Aesculop-Werke, A.G. Normals Jetter and Scheerer, D-71, Tuttlingen, Germany) was pushed vertically through the abdominal wall to its full length (Figure 2). If the skin was incised completely only a modest pressure was required to penetrate the remaining structures of the abdominal floor. Once the cannula was in position the trocar was removed and a sterile 80 cm long 10 French gauge infant feeding tube (Argyle Division of Sherwood Medical, St. Louis, Missouri) inserted into the abdomen via the cannula (Figure 3) leaving 10-20 cm outside.

Fluid was collected by allowing it to flow from the tube into containers as in group 1. Also, the tube acted as a wick, fluid being drawn through the cannula and running down the outside of the tubing to be collected into a container. The time taken to collect sufficient (0.5 mL each for bacteriology and cytology) varied from five seconds to ten minutes. All samples were analysed immediately following collection.

Subsequently all cows in this group were subjected to laparotomy.

Group 3, 22 cattle with clinical peritonitis, were sampled using a teat cannula pushed through a skin incision in

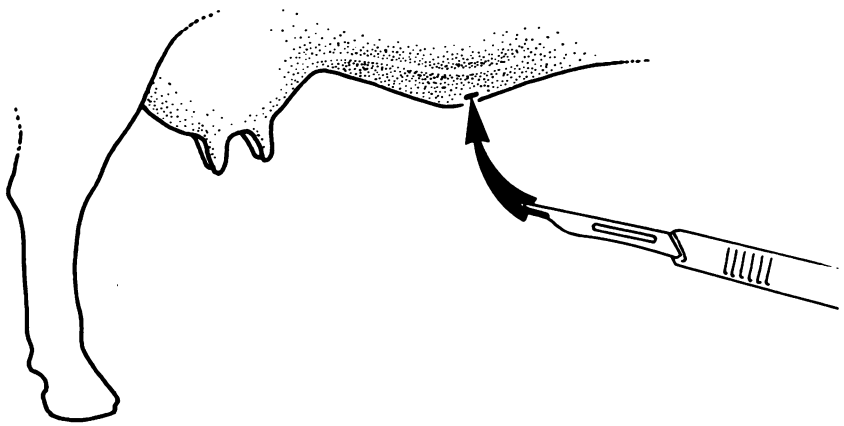


FIGURE 1. Site of abdominocentesis in cattle using the trocar and cannula method.

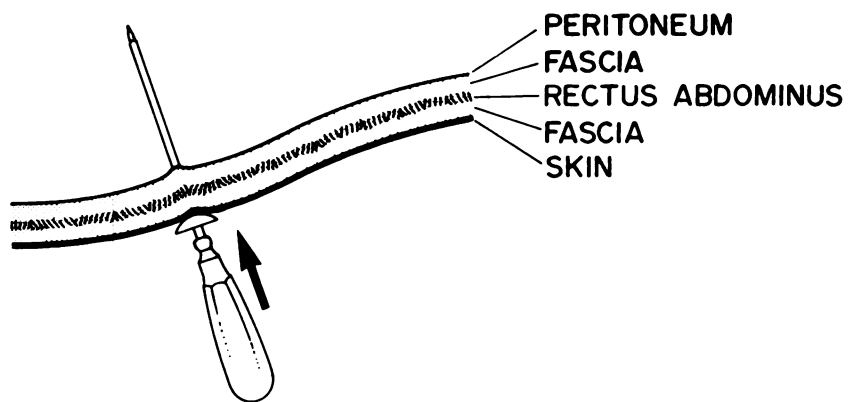


FIGURE 2. Collection of bovine peritoneal fluid showing the tissues penetrated by trocar and cannula.

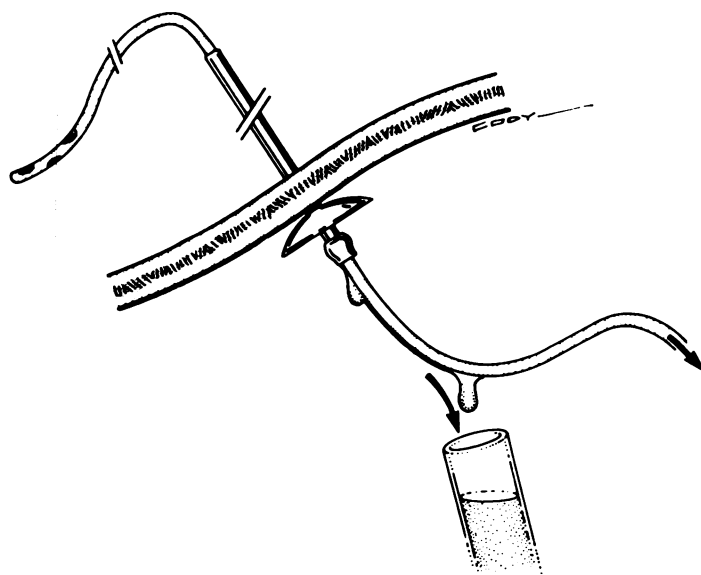


FIGURE 3. Collection of peritoneal fluid using trocar and cannula and fenestrated tube showing sites of collection (arrows), through and around the tube.

the abdomen 5 cm cranial and 5 cm medial to the point where the left milk vein disappears. On some occasions the method using the trocar and cannula described above was used when no fluid could be obtained by teat cannula. Traumatic reticuloperitonitis, ruptured abdominal viscus and post-operative sepsis were the most common causes of peritonitis in this series. In all cases diagnosis was confirmed by exploratory laparotomy or postmortem. The cows were further classified as acute or chronic cases, based on the nature of the pathological changes. Any cow in which organized fibrous adhesions were observed was classified as chronic irrespective of whether other changes, such as fibrin deposition, indicative of a more acute reaction, were present. Those in which no fibrous adhesions were detected were classified as acute, regardless of the apparent time course of the disease.

Analysis of Peritoneal Fluid

The white blood cell count (WBC) was determined using an automated cell counter (Coulter Model S, Coulter Electronics Inc., Hialeah, Florida). Total protein was determined on the supernatant using a refractometer (T.S. Meter, American Optical Co., Buffalo, New York). Fibrinogen was determined by the method described by Kaneko and Smith (4) on all 19 samples obtained in group 2 and ten samples in group 3. Direct and spun smears were stained with Wright's-Giemsa for cytological examination. One hundred cells were counted from a representative area of each sample to yield a differential cell count. Large mononuclear cells, including macrophages, activated macrophages and mesothelial cells were all classified as monocytes. Samples for bacteriology were cultured aerobically and anaerobically on blood agar at 37°C.

Statistical Analysis

Due to the asymmetrical distribution of many of the sample variables and the small sample sizes, medians and observed ranges were used to describe the location and distribution of the samples. Comparisons between two samples were performed using the Mann-Whitney U test (5). Also, multiple comparisons between groups were performed by the Kruskal-Wallis

one-way analysis of variance (6) and Dunn's Multiple Range Test (7). Two-by-two tables were constructed by standard methods (8).

RESULTS

Group 1

Peritoneal fluid was collected from every cow in the parturient group. Large volumes were present in every

case. The fluid was yellow in color, slightly opaque and clotted on standing. No lesions were detected during surgery in any of these cows.

Group 2 (normal nonpregnant)

Twenty-one attempts were made to collect peritoneal fluid. In one case (5%) no fluid was collected and in another (5%) the fluid was severely

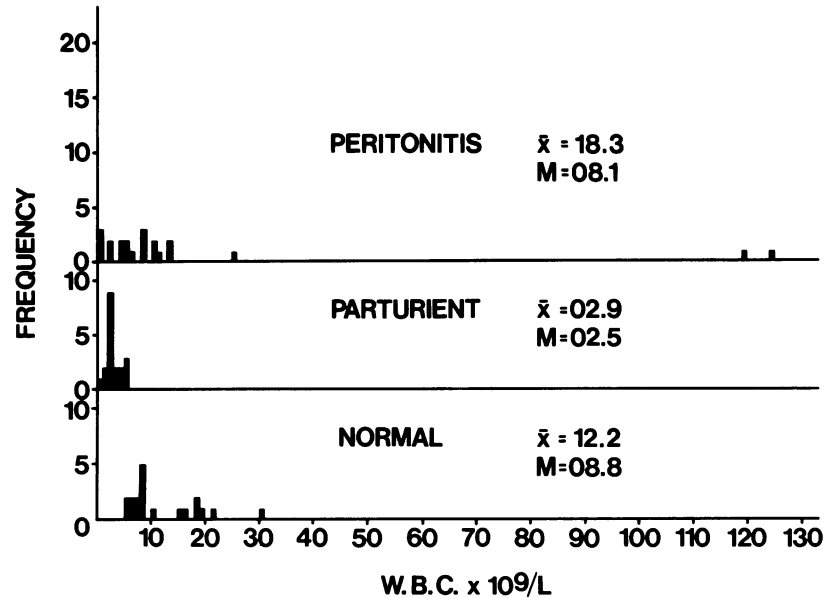


FIGURE 4. Histograms of white blood cell count in peritoneal fluid of normal, parturient cattle and cattle with peritonitis.

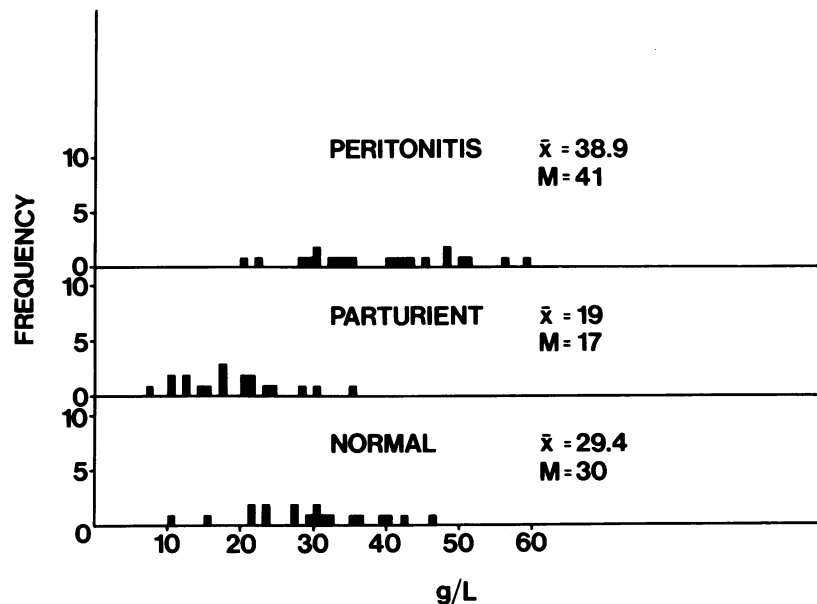


FIGURE 5. Histograms of total protein in peritoneal fluid of normal and parturient cattle with peritonitis.

contaminated with blood and could not be analyzed. In the remaining 19 (90%) sufficient (> 0.5 mL) fluid for each of bacteriology and cytology was collected. The fluid clotted on standing. The color varied from clear to yellow and the turbidity also varied. Hematological abnormalities were not demonstrated in this group. No lesions were detected at laparotomy.

Group 3

The volume of fluid obtained from cattle with peritonitis varied from less than 1 mL to more than 10 L. Color and turbidity were highly variable — some samples contained pieces of fibrin. Not all samples clotted in this group.

Frequency histograms of the total white cell counts, the differential cytology and total protein assays performed on peritoneal samples from all three groups are shown in Figures 4-9. From these and Table I, it can be seen that the observed ranges overlap extensively. Relative neutrophil and eosinophil numbers had the clearest separation.

Fibrinogen levels in peritoneal fluid were $\leq 1 \text{ g/L}^{-1}$ in 15 out of 19 samples taken from normal cows. The mean value was 1.42 g/L^{-1} , median 1 g/L^{-1} and range $1-4 \text{ g/L}^{-1}$. Fibrinogen values in peritoneal fluid of cattle with clinical peritonitis had a mean value of 3.5 g/L^{-1} , a median value of 3 g/L^{-1} and a range from $1-8 \text{ g/L}^{-1}$. The differences between the group medians was significant ($P \leq 0.05$).

Plasma fibrinogen data was also available for the normal cows and 20 out of 21 of the cattle with peritonitis. A cut-off point of 6 g/L , the upper normal reference value for the clinical laboratory, was used and a 2×2 table constructed (Table II). For comparison, Table III shows a 2×2 table in which peritoneal fluids were classified as suggestive of peritonitis if both relative eosinophil counts were $\leq 10\%$ and relative neutrophil counts were $\geq 40\%$. The relative neutrophil and eosinophil counts were also useful in distinguishing parturient (Group 1) cows from cattle with peritonitis. Using the same criteria as above true +ve rate = 100%, false -ve rate = 0%, true -ve rate = 90% and false +ve rate = 10%.

The fluid from parturient (Group 1) cows exhibited interesting features.

TABLE I
RESULTS OF KRUSKAL-WALLIS 1-WAY ANALYSIS OF VARIANCE ON SIX PARAMETERS OF BOVINE PERITONEAL FLUID IN NORMAL AND PARTURIENT CATTLE AND CATTLE WITH PERITONITIS

	Mean Ranks ^a			Chi-Square	Significance
	Group 1 (Parturient)	Group 2 (Normal)	Group 3 (Peritonitis)		
White Blood Cells	14.55	<u>42.50</u>	<u>32.70</u>	25.94	< 0.001
Total Protein	14.95	<u>30.68</u>	<u>43.00</u>	26.65	< 0.001
Monocytes	46.05	<u>19.11</u>	<u>25.33</u>	25.83	< 0.001
Neutrophils	<u>20.11</u>	<u>19.97</u>	<u>48.02</u>	35.91	< 0.001
Eosinophils	31.13	47.50	13.14	40.69	< 0.001
Lymphocytes	<u>32.53</u>	<u>37.58</u>	<u>20.86</u>	11.14	< 0.001

^aValues underlined indicate no significant differences was demonstrated between mean ranks of groups at the $\alpha = 0.5$ level using Dunn's Method of Comparisons.

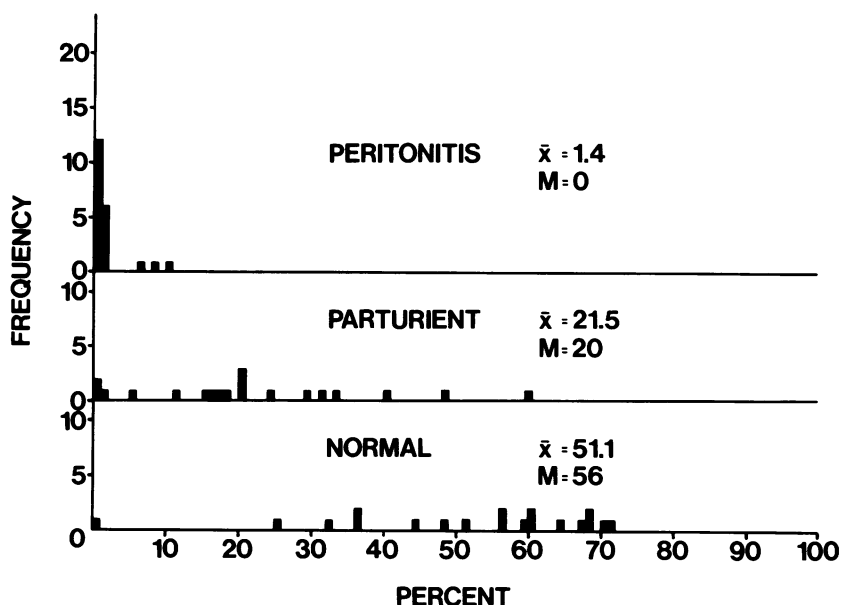


FIGURE 6. Histograms of relative eosinophil count in peritoneal fluid of normal and parturient cattle and cattle with peritonitis.

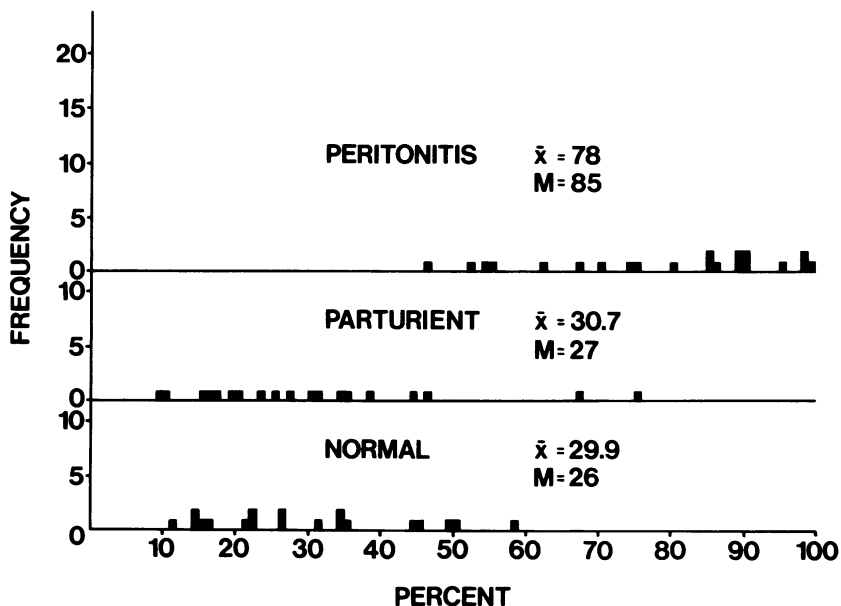


FIGURE 7. Histograms of relative neutrophil count in peritoneal fluid of normal and parturient cattle and cattle with peritonitis.

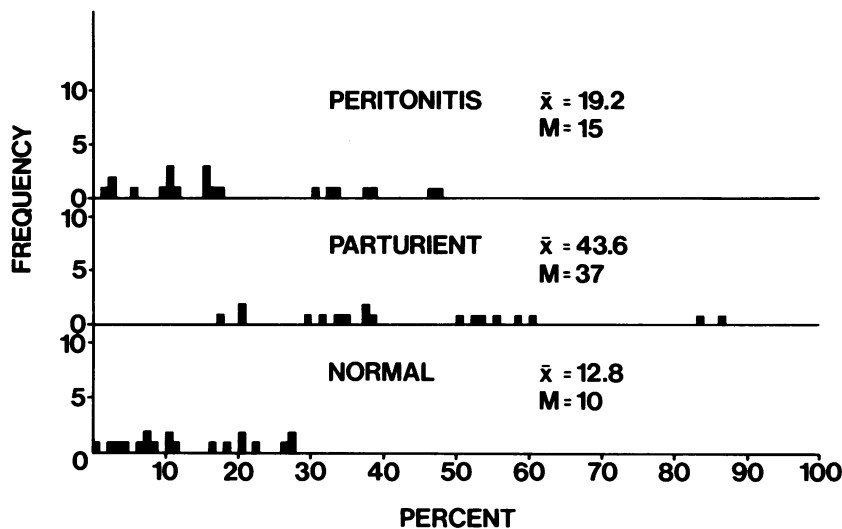


FIGURE 8. Histograms of relative monocyte count in peritoneal fluid of normal and parturient cattle and cattle with peritonitis.

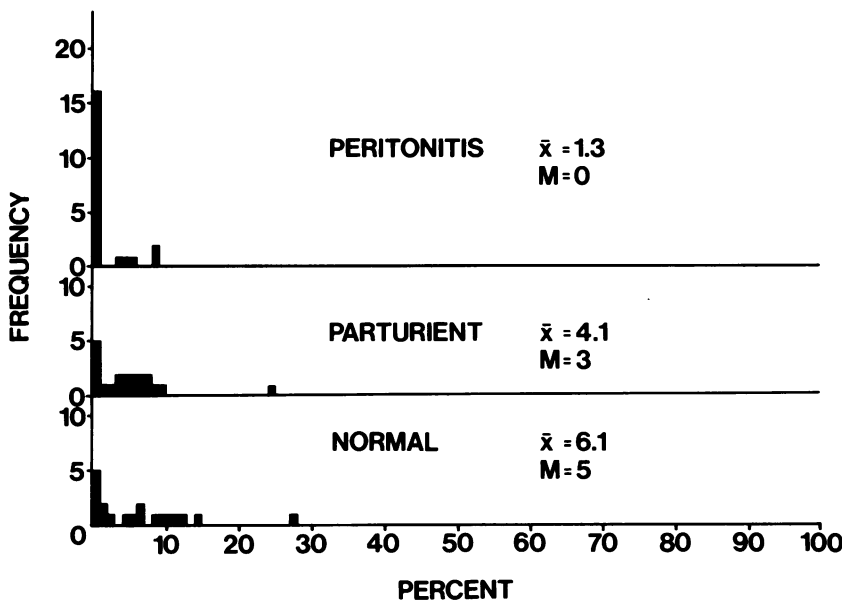


FIGURE 9. Histograms of relative lymphocyte count in peritoneal fluid of normal and parturient cattle and cattle with peritonitis.

TABLE II
2 x 2 TABLE OF PLASMA FIBRINOGEN AS A TEST FOR PERITONITIS

	Peritonitis ^a	Normal ^b	Total
Test Positive ^c	16	1	17
Test Negative ^d	4	18	22
Total	20	19	39
True Positive Rate	80%		
False Negative Rate	20%		
False Positive Rate	5%		
True Negative Rate	95%		

^a20 out of 21 cattle in peritonitis group (3).

^bAll cows in normal group (2).

^cFibrinogen $\geq 6 \text{ g/L}^{-1}$

^dFibrinogen $< 6 \text{ g/L}^{-1}$

From Table I it can be seen that WBC and TP were significantly lower than in either normal (Group 2) or peritonitis (Group 3) samples and relative monocyte counts were higher.

The median plasma fibrinogen levels between the acute and chronic cases of peritonitis were not significantly different; however, in cows which had peritonitis the median relative monocyte count in peritoneal fluid was significantly higher in the chronic group. Using a cut-off point of $\geq 15\%$ monocytes, 18 out of 21 samples (86%) were correctly classified as acute or chronic peritonitis.

The frequency with which different organisms were isolated from peritoneal fluid in each of the three groups of cows is shown (Table IV). The samples from group 1 taken aseptically during surgery were all sterile. The growth of organisms in group 2 samples most likely represents contamination during collection. The same organisms are found in samples from the peritonitis group with similar frequency. In addition, *Corynebacterium pyogenes*, *Escherichia coli*, *Actinomyces* and anaerobic Gram-negative rods were cultured. Growth of Gram-negative organisms was always associated with death or the necessity for euthanasia of cases.

DISCUSSION

In our experience, the trocar and cannula method of collecting peritoneal fluid has proved reliable. The high success rate (90%) in clinically normal cows should prevent bias as a result of selecting only cows in which there is a large volume of peritoneal fluid. It also proved useful in collecting peritoneal fluid from some cases where prior attempts with a teat cannula had failed. No complications were encountered during the study. However, subsequently, penetration of a viscus has occurred on two occasions. In one the rumen was penetrated; exploratory laparotomy showed this was due to the rumen having been totally adhered to the floor of the abdomen. In a second case the abomasum was penetrated. A subsequent diagnosis of impacted abomasum was made and surgery revealed a grossly distended abomasum laying on the floor of the abdomen. Both cases were able to return home after treatment and in neither

TABLE III
2 x 2 TABLE OF PERITONEAL TAP DATA AS A TEST FOR PERITONITIS

	Peritonitis ^a	Normal ^b	Total
Test Positive ^c	21	0	21
Test Negative ^d	0	19	19
Total	21	19	40
True Positive Rate	100%		
False Negative Rate	0%		
False Positive Rate	0%		
True Negative Rate	100%		

^aAll cows in peritonitis group (3).

^bAll cows in normal group (2).

^cPositive test $\leq 10\%$ eosinophils and $\geq 40\%$ neutrophils.

^dNegative test $> 10\%$ eosinophils and $< 40\%$ neutrophils.

TABLE IV
FREQUENCY OF ISOLATION OF BACTERIA SPECIES IN PERITONEAL FLUID SAMPLES

Organism	Group 1 (Parturient)	Group 2 (Normal)	Group 3 (Peritonitis)
<i>Bacillus</i> sp.	0	5	3
<i>Staphylococcus epidermidis</i>	0	4	6
<i>Streptococcus</i> sp.	0	2	2
<i>Corynebacterium</i> sp.	0	0	1
<i>Corynebacterium pyogenes</i>	0	0	1
<i>Escherichia coli</i>	0	0	4
<i>Enterobacter</i> sp.	0	1	1
<i>Clostridium</i> sp.	0	2	2
<i>Actinomyces</i> sp.	0	0	1
Gram-negative anaerobes	0	0	3
No growth	19	8	5

case did we detect side effects directly attributable to the penetration. The small amount of available information suggests that this procedure is not associated with undue complications. However, it is the authors' opinion that this method of collecting abdominal fluid should only be used if more conservative methods have failed to yield an adequate sample, and gross distension of abdominal organs cannot be detected by rectal palpation.

There were few gross differences between samples of peritoneal fluid from healthy and diseased cows. All normal and most diseased samples clotted readily. This could not be related to the total protein content. All the cows in the parturient group had a high volume of peritoneal fluid which also clotted readily, despite having a significantly lower total protein. A previous report (1) indicated that normal peritoneal fluid is clear and watery with only a small amount present; pathological fluids were described as increased in volume, cloudy and yellow to bloody in color. The findings of this study do not support these obser-

ations. It has been stated that a peritoneal fluid sample with greater than 6×10^9 WBC/L¹ and total protein greater than 3 g/dL¹ was consistent with the diagnosis of peritoneal inflammation in 86% of cases (2). Although this statement is supported by the findings in this paper, it would also be true of 50% of the normal group in this series, making it a poor diagnostic criterion. The WBC counts in this series have mean values of 18.3×10^9 /L¹ in the peritonitis group and 12.2×10^9 /L¹ in the normal group. However, median values were very similar (8.8 and 8.1 respectively, Figure 4). Therefore, only in extreme cases is WBC count of peritoneal fluid a clear diagnostic sign of peritonitis. Similarly, total protein in peritoneal fluid is not a useful indicator of peritonitis in the cow. Fibrinogen levels measured on peritoneal fluid are higher in cows with peritonitis but the complete overlap of the ranges prevents its use as a good diagnostic test for peritonitis.

The most useful criteria for classifying peritoneal fluid samples in this

study were the relative numbers of eosinophils and neutrophils. By considering two variables, i.e. both eosinophils $\leq 10\%$ and neutrophils $\geq 40\%$, simultaneously, a better separation of peritonitis cases and normal cows was achieved than by the use of either variable on its own. This same classification was also useful in differentiating between parturient cows and those with peritonitis.

From Tables II and III it can be seen that peritoneal fluid analysis is slightly better than the measurement of plasma fibrinogen as a diagnostic test for peritonitis. In addition, high plasma fibrinogen may be associated with many other diseases, including fat necrosis, hepatitis, mastitis, pericarditis, pneumonia, metritis, displaced abomasum and abscesses (9). This seems to indicate that peritoneal fluid analysis would be a better diagnostic test of peritonitis than plasma fibrinogen determination.

The classification procedure used in this study relies heavily on the absence of eosinophils; previous reports have stated that eosinophils were uncommon in bovine peritoneal fluid (1,2,3). The almost ubiquitous occurrence of eosinophils in this study is contrary to these observations. None of the blood samples examined had eosinophil counts outside the normal range. It is possible that the eosinophils may be present as a result of some pathological process. For example, nematodes of the genus *Setaria* are commonly encountered in the bovine abdomen. *Setaria* have been reported in cattle from Colorado, Connecticut, Florida, Georgia, Illinois, Maryland, Minnesota, Montana, New York, North Carolina, Pennsylvania, South Dakota, and Texas in the United States (12) and are also encountered in Saskatchewan.

Alternatively, eosinophils may be present in the peritoneal fluid of normal, nonparasitized cattle. Studies in rats show that eosinophils are primarily distributed in subepithelial tissues of the digestive, urinary and respiratory tracts (13). However, repeated washings of the peritoneum with normal saline yielded an increasingly large percentage of eosinophils in the fluid (14). Similar observations have been made in guinea pigs and, in addition, eosinophils were noted to be present in some cases before stimula-

tion with saline (15). Also, the study in guinea pigs showed increased neutrophil numbers and decreased eosinophil numbers with injection of many antigens, including an *Ascaris* extract.

A third possibility is that the eosinophils are a result of the sampling procedure. The presence of a foreign body in the peritoneal cavity for periods up to ten minutes could induce changes of cell type in the sample. This theory is not supported by the observation of large numbers of eosinophils in samples collected in less than one minute, and by a different method from parturient cows.

Generally used criteria, such as WBC count and total protein, proved unreliable for diagnosis of peritonitis. A larger body of information will be required before definite statements on diagnosis and prognosis based on peritoneal fluid analysis can be made.

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BOOK REVIEW

Atlas of Skin Diseases of the Horse: Diagnosis and Treatment in Equine Dermatology. L.F. Montes and J.T. Vaughan. Published by W.B. Saunders Company, Toronto. 1983. 202 pages. Price \$85.80.

The Atlas of Skin Diseases of the Horse is an attempt to present some common equine dermatoses in a clear and concise manner, according to the authors experience, and represents a collaborative effort between Leopold Montes, a human dermatologist, and T. Thomas Vaughan, a recognized veterinary clinician. In their introduction the authors state clearly that it was not their intention to produce either a textbook or a treatise on equine dermatology. The text on the whole conforms well to the loosely accepted definition of an "Atlas", being replete with illustrations and containing limited text, however the comprehensive though superficial, treatment which one may expect of a publication in this style is not fully achieved.

The presentation of the book is of an

extremely high quality, being well bound and printed on a high quality, glossy bond. The layout is very tidy and the liberal use of space makes the book very easy to scan. The organization follows mainly an etiologic categorization of skin diseases with some arrangement by topography/anatomical structure. A brief review of structure and function of the skin as an organ, and glossary of terms lead off, and the text concludes with a brief discussion of treatment. Within each etiologic category, diseases and their management are described by presentation of one or more case examples, each lavishly illustrated by full-colour gloss and histopathologic photographs. With the exception of some of the high-power photomicrographs which lack definition, the photography is of a high quality.

The principal strengths of the book lie in the quality of presentation, the quality of the photographic reproductions and the succinctness of the text. Detracting from these strengths are shortcomings in content and often some limitations in the use of English.

In reviewing structure and function of the skin the text uses some excellent and fascinating scanning electron micrographs. Since most veterinarians view the structure of skin histologically however, more emphasis in this area would have been valuable, especially with regard to the detailed structure of the epidermis, which reflects so many of the changes occurring in the skin disease. The glossary is very useful for the uninformed, though it contains mistakes. Before proceeding to case presentation, a brief review of the limited responses which skin can make to insult and of their significance would have helped greatly in promoting understanding of the specific dermatoses.

In the case presentations some conditions, especially those for which a surgical correction is frequently applied appear to be over-emphasized, while little material is presented on parasitic skin diseases, and immune-mediated conditions are not covered at all. While references are provided in each section, they primarily support

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