THE DIFFERENTIAL LEUKOCYTE COUNT OF PERITONEAL FLUID AS ASSOCIATED WITH TRAUMATIC RETICULOPERITORITIS IN THE BOVINE

by

MUHAMMAD ASLAM PASHA

B.V.Sc., University of the Punjab (W. Pakistan), 1954

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Madicine

KANSAS STATE UNIVERSITY Manhattan, Kansas

1964

Approved by:

Major Professor

my Odler

LD 2668 T4 1964 P27 2.2 Docume -

TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	3
Blood Examination	3
Metal Detectors	7
Correlation of Pain	8
Urine Test	8
Exfoliative Cytology	8
Peritoneal Fluid Leukocytes	9
MATERIALS AND METHODS	9
Experimental Animals	9
Collection of Peritoneal Specimens	
Contrifugation of the Poritonnal Specimens	
Preparation of Peritonsal Fluid Films on Microslides	
Peritoneal Fluid Differential Loukocyte Count	
Collection of Circulating Blood Samples	
Blood Leukooyto Total and Differential Count	
Exploratory Operations	
RESULTS AND DISCUSSION	
Acute Transatic Reticuloperitonitie	
Chronic Traumatic Reticuloperitonitis	
Traumatic Pericarditis	
Clinical Conditions other than Transatic Reticuloperitonitis	23
SUPMARY	29
ACKNOWLEDGMENTS	32
LITERATURE CITED	33

INTRODUCTION

Transatic reticuloperitoritis is a disease of ruminants resulting from the perforation of the reticulum by a sharp object which may introduce infection of varying severity. The disease is also termed traumatic gastritis, traumatic reticulitis, traumatic peritoritis and most commonly as 'hardware disease'.

With the advancement of animal husbandry there is an increased tendency to maintain cattle under stable conditions and dry feed for longer periods during the year. This fact, coupled with the mechanization of agriculture and consequent increase in metallic debris, seems to be predisposing factors for the increasing number of transactic reticuloperitonitis each year. Kingery (1955) observed that this disease is common where silage and hay are made from fields containing old rusting fences and baling wires, or from areas where buildings have recently been constructed, burned or term down.

Trausatic reticuloperitoritis is one of the most important problems confronting many modern day dairy veterinary practitioners. Its importance stems from untold economic less (milk production, weight gain and reproductive ability) as well as the individual fatalities. Blood and Hutchins (1955) stated that approximately 8 percent of all cases of traumatic reticuloperitonitis will develop into traumatic pericarditis and many animals die or suffer from chronic peritonitis and do not return to completely normal health.

The condition is under almost constant consideration in the differential diagnosis of diseases of cattle because of the similarity of symptoms to these of indigestion and other diseases. Udall (1954) stated that so many

symptoms of traumatic gastritiz are common to those of primary indigestion that an indefinite diagnosis is frequent, especially at the first examination. Fincher and Fox (1956) stated that with careful consideration of the history and syndrome following a complete examination, the diagnosis of traumatic gastritis is not difficult. Blood and Hutchins (1955) and Frank (1961) suggested that the diagnosis of an early case of traumatic gastritis is frequently very difficult as the symptoms will vary with different animals.

An early diagnosis in traumatic lesions of the bovine stomach is important so that the foreign body may be removed before extensive peritonitis with abscess fermation or pericarditis complicates the condition. Diagnostic aids such as blood examination and metal detectors are most commonly recognized and employed by several workers to reach a tentative diagnosis of traumatic reticuloperitonitis, whereas other workers are of the opinion that these techniques have very limited value and therefore, cannot be relied upon in establishing a tentative diagnosis of this disease.

Since the diagnostic aids, presently recognized and employed have limited application for the diagnosis of traumatic reticuloperitonitis, it was felt necessary to work out an effective technique to help the veterinary clinicians to detect this disease at an early stage so that further economic loss as well as fatalities could be minimized. Therefore, the present study was undertaken with a view to observe (1) the differential leukocyte count of the peritoneal fluid associated with traumatic reticuloperitonitis in cows, (2) to correlate the peritoneal and blood differential leukocyte counts associated with traumatic reticuloperitonitis in cows, and (3) to correlate peritoneal leukocyte count with surgical exploratory operations associated with traumatic reticuloperitonitis.

REVIEW OF LITERATURE

Blood Exemination. Dougherty (1939) was impressed by the inadequacy of the methods available for the diagnosis of traumatic reticulitis and stressed the need for some supplementary laboratory tests. He, therefore, studied the symptoms and blood changes in three cattle in which traumatic reticulitis was induced by inserting a piece of wire, via rumenal fistula, through the anterior reticular wall. Changes in the blood were present four to six hours after insertion of the foreign body. These changes comprised an increase in neutrophiles, particularly in immature forms, and a relative decrease in lymphocytes. In addition he made blood examination in 30 suspected cases and the blood changes corrobrated the clinical findings. The conclusion reached was that a study of the blood picture is a definite aid in the diagnosis of traumatic reticulities and pericardities when taken in conjunction with clinical observations.

Arthur (1946) investigated the blood leukocyte picture in cases of traumatic reticulitis and a number of other infections having some clinical resemblence to them. He stated that although not pathognomenic, for similar blood pictures can arise from several other causes including infections with corynebacterium pyogenes, it is contended that a hemogram can help to differentiate between traumatic reticulitis and other diseases showing similar symptoms. Also, the total leukocyte appears of value in distinguishing between reticuloperitoritis and traumatic pericarditis and thus can be used as a guide to the urgency of the condition with regard to surgical interference. He concluded that a study of the blood picture is of diagnostic and prognostic value in traumatic reticulitis and pericarditis.

Matteeon et al. (1953) observed that the total red blood cell count and homoglobin percentage apparently are of no diagnostic value as compared to the use of the white cell total and differential count in the traumatic gastritis. They further stated that although it is apparent that total blood cell count and differential count could be relied upon alons for the diagnosis of "Hardware Disease"; analysis of 18 cases of traumatic gastritis, peritonitis and pleurocarditis treated at the University of Missouri Veterinary Clinic, revealed periodic, often daily shift of the total white cells from higher to lower counts, or vice veres, plus definite neutrophils shift to the left. High cosinophilia were observed in some cases, and near normal ratio in other cases, which were interpreted as being the result of stress in varying degree.

Blood and Hutchins (1955) observed that the total and differential leukocyte counts at least indicate the presence or absence of an inflammatory process and elimination of the other entities by careful examination confirms the diagnosis of traumatic gastritis. They further stated that hematology is often inconclusive for although there may be a mederate neutrophilia it is often insufficiently marked to be diagnostic and the total leukocyte count is unchanged.

In ten eases of experimently induced traumatic gastritis, Kingrey (1955) observed an increase in the total leukocyte count with the enset of symptoms. The percentage of non-segmented neutrophils increased in general in direct proportion to the total white cell count and to the percentage of the segmented neutrophils. He concluded that the changes in the blood due to reaction were consistent and should be considered significant in suspected cases of traumatic gastritis.

William (1956) reported that he observed leukocytosis with a 'shift to the left' in the 15 cases he examined, and considered it to be significant for the diagnosis of traumatic reticulitis.

in confirming the diagnosis of traumatic gastritis. The total leukocyte count was found to be of little value in indicating the presence of infection in such cases. In all instances of the traumatic gastritis studied, the total white blood cell count was within the normal range of 4,000 to 12,000 cells per c.mm. They were of the opinion that a monocyte percentage of 5 or more, with a leveling of the lymphocyte:neutrophil ratio, was indicative of a poor prognosis for surgical interference.

Brown et al. (1959) reported that reticuloperitoritis remains one of the diagnostic problems in the bovine practice. They attempted to determine if the hemogram in chronic traumatic reticulitis followed a pattern consistant enough to be of a value in diagnosis and prognosis. It was observed that since the clinical syndrome of chronic cases is less definite than that of acute cases, the detection of the former is often more difficult and early discovery is indefinite except in cows which are milked and observed daily. They concluded that a blood examination, including the leukocyte and differential count is of value in the diagnosis and prognosis of the disease. Even though one factor may be within the normal limits at a particular time, it is probable that a differential count will disclose a significant disturbance.

Frank (1961) stated that if the patient has been treated with antibiotics or sulfonamides the differential leukocyte count will not be as accurate. He further stated that there will be cases in which the blood count is almost normal but the symptoms are typical of traumatic gastritis. Schalm (1961) reported 16 cases of traumatic reticulities which were proven by surgical procedure. He suggested that a marked shift to the left does not necessarily mean traumatic reticulities in the digestive problems of bovine. He also reported a case in which hemogram could have been misleading.

Fraser (1961) recorded total and differential white cell counts in cases of traumatic reticulitis and observed that the changes in the blood picture were evident in certain cases but were not constant. With the blood picture and typical clinical symptoms these animals were diagnosed as traumatic reticulitis.

According to the Editorial Board (1961) the blood examination is an aid in making diagnosis of traumatic gastritis, and especially following the initial perforation of the reticulum by the foreign body.

Hjerpe (1961) observed elevation of the total leukecytes, increase in the incidence of unsegmented neutrophils in the differential count and reversal of the normal lymphocytes neutrophil ratio. He was of the opinion that although the hemogram is of considerable value as a diagnostic aid in acute traumatic reticuleperatoritis, the above mentioned changes are non-specific and are seen in association with other bacterial infections, severe stress as occurs in parturition, and following the administration of corticosteroids. Moreover, occasional returns to near normal hematologic values do occur during the course of the traumatic reticuloperatoritis. Hemograms performed during such a period may be misleading. He suggested that hemogram should be used as an aid to diagnosis with primary attention assigned to the physical examination. He also referred to the report of Carrol et al. (1958) that a monocyte percentage of 5 or more is indicative

of poor prognosis for surgical interventions. In his study only one count of this type was observed and the cow recovered without complications.

According to Hjerpe's observations, hemograms were not of prognostic value for cows treated with the platform and antibiotics.

Hjerpe (1963) undertook another study to determine what hemotologic changes occur in cattle with field cases of acute indigestion, in order to determine the value of the hemogram in differentiating acute indigestion from acute traumatic reticuloperitonitis. He suggested that the hemogram has limited value for differentiating acute indigestion from traumatic reticuloperitonitis and that physical examination of the patient should not be neglected in making a diagnosis.

Coles (1963) stated that the leukocyte picture in various diseases is not static. There may be significant variations in the total count, in the ratio of cell types and in the morphological characteristic within the space of a few hours and also there will be approximately 10 percent technical error involved in the leukocyte counts.

Motal Detectors. Notal detectors are used as a diagnostic aid in recognizing this disease. Jackson (1951) recommended the use of electromagnetic metal detector in the diagnosis of traumatic gastritis. Fincher and Fox (1956) stated that the metal detectors were originally developed to detect mines in the World War II. They suggested that the metal detectors must be used with reservation and with the clinical symptoms constantly borne in mind. They concluded that the disadvantages lie in the fact that so many cows have metallic objects in the reticulum without deterimental effect, and therefore, a positive reaction does not in any way guarantee that the presence of this material is producing the symptoms.

Milson (1956) reported successful use of metal detector routinely in suspected cases of 'Hardware Disease'. Animals suffering from traumatic gastritis were diagnosed on the basis of history, symptoms, and findings with the metal detector.

Correlation of Fain. William (1956) claimed a new method for diagnosing transmits reticulities in the form of a grunt associated with reticular movements. He suggested that a grunt should be heard 2 to 3 seconds before, or when, the primary runimal nevement resched its maximum contractions.

This diagnostic method was found to be completely reliable in the 64 affected cattle he exemined. Mingrey (1955), Blood and Mutchins (1955), Fincher and Fox (1956) and Blood and Mutchins (1960) have also reported that the pain in the area of migheid cartilage is an important manifestation for differential diagnosis.

Prine Test. Silver (1951) reported that he made 'on the spot' wrine test with Sulkewitch reagent and found an absence of calcium in urine in a positive case of traumatic reticulities. Hall (1956) utilized this test on six cases that were positive for a perforating foreign body, confirmed on rumenotemy, and which were positive to the Sulkewitch test. William (1956) applied this test to 10 positive cases of traumatic gastritis and on each occasion a negative reaction was obtained. Normal cows in the herd were also examined with this test and most of those gave similar results. He suggested that tests were applied during winter season which might account for the negative calcium wrine results in the normal results.

<u>Marfoliative Cytology</u>. Wayne (1959), Omender and Christensen (1959) and Bernhard et al. (1960) reported the importance of exfoliative cytology

as an aid in diagnosis of some severe infections in human, but so far no literature on this subject is available in the field of veterinary medicine.

<u>Peritoneal Fluid Leukocytes</u>. Review of the literature did not reveal information on the presence of leukocytes in normal peritoneal fluid.

MATERIALS AND METHODS

Experimental Animals. Twenty dairy type cows and one Hereford bull which were admitted to the Kansas State University Veterinary Hospital with a history of digestive disturbances were used as subjects for this experiment.

The management background of these cows varied with each individual and in many cases inadequate history was available. Several of the cows had been treated previously with antibiotics or other medicines either by veterinarians or by the owners. The exact time the subjects first exhibited symptoms was uncertain in many cases.

The cases used were those admitted routinely in the hospital and no attempt was made to pick the so-called ideal subject.

Collection of Peritoneal Specimens. Peritoneal paracentesis was accomplished just prior to surgery. Sterile 10cc glass syringes and 2½ inch 18 ga. needles were used in the puncture. The hair was clipped from a 2 inch square area left of the xiphoid cartilage. Specimens were collected from the peritoneal cavities by puncturing into the space dorsal to the xiphoid cartilage, below the reticulum and in front of the anterior blind sac of the rumen. Specimens were collected by aspiration. Occasionally a second or third insertion was used to obtain sufficient amount of the fluid.

The peritoneal fluid was mixed with 2 to 3 drops of ethylenediaminetetraacetate solution to prevent coagulation.

Centrifugation of the Peritoneal Specimens. All of the peritoneal specimens, with the exception of those consisting of a few drops, were immediately transferred to sterile 10cc blood tubes and were centrifuged for 10 minutes at 4,000 RPM.

Premaration of Peritemeal Fluid Miles on Microslides. Approximately 75% of the expernatant fluids of the centrifuged specimens were discarded. Peritoneal fluid films on microslides were prepared by spreading 4 to 5 drops from the remaining pertian of the centrifuged specimens. In cases where the specimen was inadequate for centrifugation, films were prepared by direct spreading of the collected specimen on the microslides.

The peritoneal films on microslides were air dried and stained by Wright's Method as described below:

- 1. Eight to ten drops of the Wright's stain were added. The stain was allowed to act for 2 minutes.
- 2. An equal number of drops of buffer solution (3.80 grams Na_2HPO_b and 5.47 grams KH_2PO_b in 1000 ml. distilled water) were added and mixed until a metallic film formed on the surface of stain-buffer mixture.
 - 3. Mluted stain was allowed to act for 4 minutes.
- 4. The soun and precipitated stain was floated off by quickly flooding water over the slides.

Giomsa's stain was also tried on four slides but the results were unsatisfactory as compared to the Wright's stain.

^{*}Sequester-Sol., Product of Cambridge Chemical Products, Inc., Dearborn, Michigan.

Peritoneal Fluid Leukocyte Differential Count. The differential leukocyte counts were made, with the microscope using the oil immersion lens, by counting 100 cells.

Collection of Circulating Blood Samples. The blood samples were obtained from the left jugular vein prior to surgery. Approximately 5cc of whole blood was mixed with 2 to 3 drops of ethylenediaminetetrascetate solution to prevent coagulation.

Blood Loukocyte Total and Differential Counts. The total and differential loukocyte counts were made on the blood samples. Standard laboratory techniques were used. The hemocytemeter was used for the total leukocyte counts and the Wright's stain was used for differential leukocyte counts.

Exploratory Operations. The surgical operations were accomplished by standard surgical procedures for abdominal exploratory and rumenotomy as described by Frank (1961). The exploratory operations were performed to confirm the diagnosis.

RESULTS AND DISCUSSION

Acute Traumatic Reticuloperitoritis. The blood leukocyte total and differential counts of 4 scute traumatic reticuloperitoritis cases are shown in Table 1.

Table 1. Blood leukocyte total and differential counts in acute cases of trammatic reticuloperitoritis.

Case	A	Total WBC	Bands	60-	46	mtial co	unt per	cent base.
No.	Age	TO PRIT MIDO	Danis	Seg.	lym.	2003204	9034	nespi
5577*	6 yr.	22,118		71	23	5	23	
7417*	4 yr.	10,700		27	50			
9188*	6 yr.	14,600	1	66	28	2	3	
9776*	6 yr.	6,700		50	40	2	8	
Average	5.5	13,379	.25	53.5	35	2	8.5	

Unable to obtain enough peritoneal fluid for differential cell count in all four soute reticuloperitonitis cases.

The total leukocyte count was within the normal (Table 2) range in ease No. 7417 and 9776, whereas marked leukocytosis was observed in case No. 5577 and slight increase in case No. 9188.

Table 2. Total and differential leukocyte counts of blood in normal bovine according to Schalm.

	Range	Average
Total Leukocyte count	4-12,000 c.mm.	(9.25)(5.48)(8.9)(5.6) ¹⁰³
Bands %	0-12	0.5
Segmenters %	15-45	28
Lymphocytes %	45-75	53
Monocytes %	2-7	4
Eosiniphils \$	2-20	9
Basophils %	0-2	0.5
Neutrophil: Lymphocyte ratio	28/58%	

The differential leukocyte count in case No. 5577 and 9776 revealed neutrophilia and lymphopenia. A slight 'shift to the left' and lymphopenia was observed in case No. 9188. A normal differential leukocyte count was observed in case No. 7417 (Table 2).

Adequate peritoneal specimens for differential leukocyte count could not be obtained from the 4 cases of acute traumatic reticuloperitoritis. It may be due to the fact that the perforations of the reticulum and formation of the adhesions in acute traumatic reticuloperitoritis cases resulted so rapidly that it did not produce adequate peritoritis for peritoneal paracentesis and cellular study. It is also possible that in acute traumatic reticuloperitoritis cases, the leukocytes concentrated at the site of perforation and did not migrate sufficiently to be demonstrated in peritoneal fluid obtained by paracentesis.

The average blood leukocyte total and differential count in 4 cases of acute traumatic reticuloperitonitis exhibited leukocytosis with a slight 'shift to the left' and reversal of normal (Table 2) neutrophililymphocyte ratio. The monocyte and ecsinophils were in the normal (Table 2) percentage. The blood picture in 4 acute traumatic reticular perferation. Schalm (1960) stated that neutrophilia above 50 percent or band neutrophils in excess of 2 percent, irrespective of total count, should be regarded as evidence of stress reaction in bovine. The results in this study agree with the work done by Dougherty (1939), Blood and Hutchins (1955) and Schalm (1960).

Chronic Traumatic Reticuloperitoritis. The blood leukweyte total and differential counts of seven cases of chronic traumatic reticuloperitoritis are shown in Table 3(a).

Table 3(a). Blood leukocyte total and differential counts in chronic cases of traumatic reticuloperitonitis.

Case No.	Age	Total WBC	Bands	Seg.	Differ lym.	mono.	count per	cent baso.
			2,763,842,9			230340	2	1
5488	4 yr.	9,631		51	45		4	
6368	3 yr.	14,537	2	67	25	3	2	
6374	3 yr.	12,918		78	17	3	2	
6867	2 yr.	No CBC.						
7662*	2 yr.	11,931		77	13	14	1	
9204*	5 yr.	7,000	6	68	20	4		
10231	4 yr.	7.274		62	29	2	7	
Average	3.5	12,928	2.5	67	25.5	3	2	

^{*}Previous antibiotic therapy.

The blood examination was not accomplished in sase No. 6867. The total leukocyte count was within the normal (Table 2) range in case No. 5488. 9204 and 7662. Leukocytosis was observed in case No. 6368 and 6374.

The differential lenkocyte count in seven cases of chronic traumatic reticuloperitonitis exhibited a 'shift to the left' with lymphopenia and normal (Table 2) percentage of monocyte and essinophils in case No. 6368 and 9204. Neutrophilia and lymphopenia, reversal of normal (Table 2) neutrophil: lymphocyte ratio, was an outstanding feature in cases No. 5483, 6374, 6367, 7662 and 10231.

The average blood isubmoyte total and differential counts showed leukscytoms with a 'shift to the left' and lymphopenia. The monocytes and econophils were in their normal (Table 2) percentage. This indicated stress reaction which was quite marked in chronic traumatic reticuloperitonitis cases and agrees with the results of Brown et al. (1959).

The peritoneal fluid leukwayte differential counts in seven chronic traumatic reticuloperitoritis cases are shown in Table 3(b).

Table 3(b). Peritoneal fluid differential cell count in chronic traumatic reticuleperitorities cases.

					count per	
Case No.	Bands	Seg.	lym.	mono.	905.	baso.
5483*						
5483* 6363*						
6374		82	15	3		
6867		95	5			
6867 7662*						
9204						
0231	2	67	21	10		
lverage	.5	85.5	17	4		

Well organized adhesions—apparently of long duration. Inadequate fluid available for differential cell count.

The peritoneal differential leukecyte count could be accomplished only in case No. 6374, (Plate I. II); 6867, (Plate III); 9204 and 10231. Three types of leukocytes viz. segmenters, lymphocytes and memocytes ware differentiated. Marked neutrophilia and lymphopenia was observed in all 4 chronic traumatic reticuloperitoritis cases. Monocytes were present in the percentage of 3, 4, and 10 in case No. 6374, 9204 and 10231 respectively.

EXPLANATION OF PLATE I

Photograph of peritoneal fluid film in chronic traumatic reticuloperitonitis case No. 6374.

a. Segmenter b. Lymphocyte (X 500)

EXPLANATION OF PLATE II

Photograph of peritoneal fluid film in chronic traumatic reticuloperitonitis case No. 6374.

a. Segmenter b. Lymphocyte (X 1250)

PLATE I

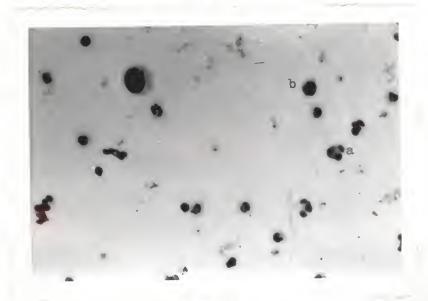
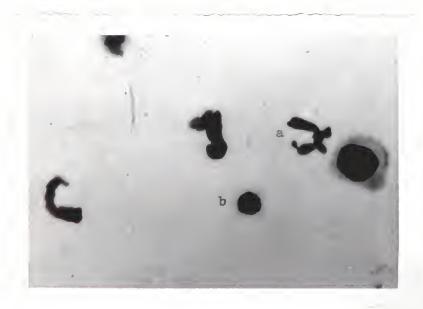


PLATE II



EXPLANATION OF PLATE III

Photograph of peritoneal fluid film in chronic traumatic reticuleperitomitis case No. 6867.

a. Segmenter b. Lymphocyte (X 500)

PLATE III



Monocytes were not observed in case No. 6857. It is of interest that neither eccinophils nor basephils were seen in the peritoneal fluid of these cases by counting 100 cells.

The peritoneal fluid differential laukedyte count could not be accomplished in three chronic traumatic reticuleperitonitis cases No. 5003, 6368 and 7662 due to the inadequate peritoneal fluid available in these cases. Well organized adhesions, apparently of long duration, were observed on exploratory operations in these cases. It is considered that well organized adhesions were associated with inadequate peritoneal fluid for aspiration and collular study.

The average peritoneal differential leukeeyte count in 4 cases of chronic traumatic reticuloperitoritis revealed neutrophilia, marked lymphopenia and 4 percent monocytes.

While comparing the average differential leukedyte counts of the peritoneal fluid and direculating blood in 4 chronic cases of traumatic reticuloperitonitis, it was observed that neutrophil: lymphocyte ratio was similar in both cases. The percentage of neutrophils was high whereas low in lymphocytes in the peritoneal fluid when compared to the percentage of these cells in the circulating blood. There was correlation in the differential leukecyte counts of the peritoneal fluid and blood as both were indicative for a stress reaction in chronic traumatic reticuloperitonitis cases.

<u>Providing Periperditie</u>. The blood leukecyte tetal and differential counts of 3 cases of transatic periperditis are shown in Table 4(a).

Table 4(a). Blood leukocyte total and differential counts in traumatic pericarditis cases.

Caso No.	Age	Total WBC	Bands	Seg.	Diffe:	rential mono.	count	percent baso.
5513	2 yr.	10,326	2	70	24	4		
6808	5 yr.	6,600		79	16	4		
9103	2 yr.	25,000		65	31	4		
Average	3 yr.	13,975	1	71	24	Zş.		

Marked leukocytosis was observed in case No. 9108. Normal (Table 2) total leukocyte count was seen in case No. 5513 and 6308.

The differential leukwayte count in the blood samples of case No. 5513 exhibited a 'shift to the left' with lymphopenia and normal (Table 2) monocyte percentage. Neutrophilia, lymphopenia, eosinophilia and normal percentage of (Table 2) monocytes were observed in case No. 6608 and 9108. A marked reversal of neutrophil:lymphocyte ratio (Table 2) was observed in all the 3 cases of traumatic pericarditis. This indicated a stress condition of severe nature.

The average total and differential leukocyte counts in blood samples of 3 cases of traumatic pericarditis revealed leukocytesis with a 'shift to the left' and lymphopenia. The average total leukocyte count in these cases was much lower than was expected and did not agree with the results of Arthur (1946) and Blood and Henderson (1960). The average differential leukocyte count indicated presence of infection of severe nature which was in close conformity with the observations made by the above mentioned research workers.

The peritoneal fluid leukocyte differential count in 3 cases of traumatic pericarditis are shown in Table 4(b).

Table 4(b). Peritoneal fluid differential cell count in traumatic pericarditis cases.

Case No.		Ban	ds	Seg.	Different lym. m	dal count	percent
5513 [*]	Clear	fluid-inadequate	cells	for	differential	counts.	
6808				85	10	3	
9108**	Clear	fluid-inadequate	cells	for	differential	count.	

whire penetrated through anterior pertion of reticulum—slight peritoneal adhesions between reticulum and diaphragm.

The peritoneal leukocyte differential count could be accomplished in only one case (No. 6808). Marked neutrophilia and lymphopenia was observed. This indicated presence of a stress reaction of severe nature in traumatic pericarditis case.

The peritoneal fluid leukecyte count could not be accomplished in the other two cases of traumatic pericarditis (No. 5513 and 9108). The peritoneal fluid in both cases was clear and had inadequate leukecytes for cell study. In case No. 5513, on exploratory operation, it was observed that the wire penetrated directly through the anterior wall of the reticulum into the pericardium. Only slight peritoneal adhesions were noted between the reticulum and the disphragm. It is postulated that the differential leukecyte count could not be accomplished in this case, due to inadequacy of the leukecytes in the peritoneal fluid. It is probable that due to the slight peritoneal reaction, less numbers of leukecytes were consentrated at the cite of adhesions and migration into the peritoneal fluid did not occur. In case No. 9108 the differential leukecyte count was not accomplished, and this can possibly be due to the fact the case was of very long duration which might have been associated with an inactive peritonitis.

By comparing the average differential leukocyte counts of the blood and the peritoneal fluid in traumatic pericarditis case No. 6308, it was observed

Long term condition.

that several of the neutrophil: lymphocyte ratios were quite outstanding and similar in both instances. But the percentage of neutrophils was high whereas low in lymphocytes in the peritoneal fluid as compared to the percentage of these cells in the circulating blood. There was definite correlation in the differential leukocyte counts of the blood and peritoneal fluid; both indicated the presence of a severe infection due to stress reaction, as was observed in the case of traumatic pericarditis.

Clinical Conditions other than Reticuloperitoritis. The differential leukecyte counts in the blood and peritorial fluids of seven cases, which produced clinical syndromes somewhat similar to traumatic peritoritis but were negative for this disease are shown in Table 5 as A and B respectively. The blood total leukecyte count was in normal (Table 2) range in case No. 11030, 7642 and 10666. Leukocytosis was observed in case No. 6635, 3144 and 6645. Case No. 9111 exhibited a leukemoid blood picture. It is of interest to note that the total leukecyte count in case No. 6645 was similar to a typical case of traumatic pericarditis as stated by Arthur (1946) and Blood and Henderson (1960), but was diagnosed and confirmed as malignant lymphoma of the abomasum.

The differential leukocyte count of blood samples revealed a slight 'shift to the left' and lymphopemia in case No. 6635 and 9111. These cases were suspected for reticular perforation. Normal (Table 2) leukocyte distribution was seen in case No. 11031 and 6645. Neutrophilia and lymphopemia was observed in cases No. 3144, 7664, and 10666. Schalm (1960) stated that a 'shift to the left' should not always be taken for transatic reticuloperitemitis. In this study it was observed that the differential and total leukocyte counts were quite misleading and exhibited similar blood picture as was observed in cases of transatic reticuloperitonitis.

Table 5. (A) Blood leukesyte total and differential counts, and
(B) Peritoneel fluid leukesyte differential counts, in clinical conditions other than traumatic reticuloperitoritis.

Case	Age	Clinical diagnosis		Total WBC	Bands	Seg.	Diffe lym.	mono.	eos.	percent base.
6635	2 yr.	Displaced abovesure	(A) (B)*	14,300	2	74	16	8		
11030	5 yr.	Displaced abonasum	(A) (B)	4,921		25 60	60 40	6	8	
3144	5 yr.	Ketosis	(A) (B)*	12,700	2	62.	22	2þ	12	
6645	6 yr.	Malignant Lymphora	(A)	27,841	2	20	46	8		
7664**	5 gr.	Indiges- tion rumen impaction	(A) (B)*	7:427		51	his	l.	1	
10666	4 yr.	Indiges-	(A) (B)*	9,000		69	26	l÷	1	1
9111	6 yr.	Enteritis	(A)	40,000	12	78	7	4		

^{*}Inadequate peritoneal fluid or total cells for differential count.

The differential leukecyte count of the periteneal specimens of suspected cases for traumatic reticuloperitenitis were accomplished in case No. 11030 (Plate IV) and 6645 (Plate V, VI). Neutrophilia and lymphopenia was observed in the case of displaced abonases (No. 11030). The picture was guite similar to that of a traumatic reticuloperitenitis case and did indicate a stress reaction. The blood differential leukecyte sount of the case did not show any change in its cell distribution.

The differential leukocyte count of the peritoneal specimen of case No. 6645 exhibited lymphocytosis and neutropenia which was quite indicative for a lymphoma case. The blood differential leukocyte count of the case was not indicative for the presence of lymphoma. The total blood leukocyte count

Male Hereford.

EXPLANATION OF PLATE IV

Photograph of peritoneal fluid film in displaced abonasum case No. 11030.

a. Segmenter b. Lymphocyte (X 500)

PLATE IV



EXPLANATION OF PLATE V

Photograph of peritoneal fluid film malignant lymphoms case No. 6645.

a. Segmenter b. Lymphocyte (X 500)

EXPLANATION OF PLATE VI

Photograph of peritoneal fluid film in malignant lymphoma case No. 6645.

a. Lymphocyte b. Lymphoblast (X 1250)

PLATE V

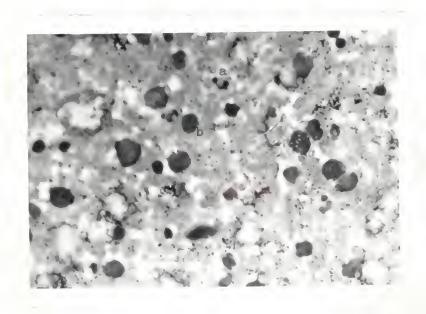
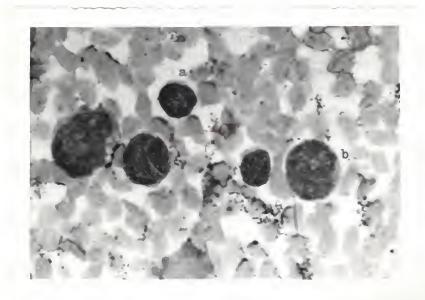


PLATE VI



did indicate an absolute lymphocytosis. On the exploratory operation of the animal it was observed that the animal had a malignant lymphoma in the abomasal area.

The peritoneal fluid leukocyte differential could not be accomplised in cases No. 6635, 3144, 10666 and 9111. These cases were negative for reticular perforation but produced clinical syndromes and blood pictures somewhat similar to a typical case of traumattle reticuloperationitis. The peritoneal specimens from these cases were inadequate for differential count. It is considered that these cases did not produce peritonitis of consequence and the peritoneal fluid did not increase sufficiently for successful paracentesis.

In this study it was observed that digestive disturbances, other than these which produced physical stress in the peritencel carity such as displaced abonasum and malignant lymphoma of abonasum and simple localized penetrating cases of reticuloperitonitis, apparently will not cause sufficient peritoncal fluid for differential count.

It is suggested that the cellular contents of the peritoneal fluid apparently will depend upon the point of penetration, degree of local reaction, and the duration of the disease syndrome. The results would suggest that the cellular study of the peritoneal fluid is most useful in determining late acute diffuse cases of traumatic reticuloperitomitis.

SUMMARY

A technique for the diagnosis of traumatic reticuleparitonitis (TRF) cases at an early stage was studied. The technique consists of paracentesis of peritoneal fluid and its differential leukocyte counts. Peritoneal paracentesis was made in twenty dairy type cows and one Hereford bull

which were admitted to the Kansas State University Veterinary Hospital. with a history of digestive disturbances.

Differential leukocyte counts on peritoneal fluid were accomplished in seven out of the 21 cases. Four cases were confirmed as chronic traumatic reticuloperitonitis. The other 3 cases were confirmed as traumatic pericarditis, displaced abomasum and malignant lymphoma of abomasum.

The differential leukocyte counts of the peritoneal specimens in the 7 diffuse traumatic reticuloperitonitis cases exhibited a picture which indicated the presence of stress reaction and infection of varying nature.

While comparing the peritoneal fluid and blood differential leukocyte count in 4 cases of chronic traumatic reticuloperitoritis and a case of traumatic pericarditis, it was observed that neutrophilia and lymphopenia was distinct in both cases. There was definite correlation in the differential leukocyte count of the blood and peritoneal fluids in these cases as both counts were indicative for a stress reaction and infection.

The differential leukocyte count in the peritoneal fluid of the displaced abonasum case indicated the presence of a stress reaction, whereas the blood leukocyte differential count was within the normal range.

The differential leukocyte count in the peritoneal fluid in the case of malignant lymphoma of the abomasum was quite indicative for this entity.

The 4 acute traumatic reticuloperitoritis cases which produced a slight peritoritis, and 3 chronic cases of traumatic reticuloperitoritis with circumscribed and well organized peritoritis did not produce adequate peritoneal fluid for cellular study.

From the results of this study it was concluded that the cellular contents of the peritoneal fluid apparently will depend upon the point of

penetration, degree of local reaction, and the duration of the disease syndrome.

The cellular study of the peritoneal fluid is most useful in determining late acute diffuse cases of traumatic reticuloperitonitis.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and indebtedness to his major professor, Dr. John L. Noordsy, for his encouragement, patient supervision, guidance, help in collecting samples and surgical procedures throughout the course of study and preparation of this manuscript.

The author is grateful to Dr. Jacob E. Mosier, Head, Department of Surgery and Medicine, for providing facilities in carrying out this investigation.

Special thanks are tendered to Dr. Harry C. Mussman, for his valuable suggestions and assistance in carrying out the laboratory work.

The author wishes to express his deep sense of appreciation to Mrs. Virginia Herren for her timely help in the laboratory work.

LITERATURE CITED

- Arthur, G. H. The Diagnostic Value of the Blood Leukocyte Picture in Traumatic Gastritis and Pericarditis in Bovine. Vet. Rec. 58, (1946):356.
- Blood, D. C. and Hutchins, D. R. Traumatic Reticular Perforation of Cattle. Aust. Vet. J. 31. (1955):113.
- Brown, J. M., Kingrey, B. W. and Rosenquist, B. D. The Hematology of Chronic Bovine Reticuloperitonitis. Am. J. Vet. Res. 20, (1959):225.
- Blood, D. C. and Henderson, J. A. Veterinary Medicine. The Williams and Wilkins Company, Baltimore. 1960:112.
- Bernhardt, H., Young, J. M. and Gourley, R. D. A Simplified Millipore Filter Technique for Isolation of Cancer Cells in Body Fluids. Cancer, 13. No. 3. (1960).
- Carrol, R. E. and Robinson, R. R. The Differential Leukocyte Count in the Diagnosis and Prognosis of Bovine Traumatic Gastritis. J.A.V.M.A. 132, (1958):248.
- Coles, E. H. Lecture Notes and Personal Communication. Professor Clinical Pathology, School of Veterinary Medicine, Kansas State University. Fall Semester 1963-64.
- Dougherty, R. W. Induced Cases of Traumatic Gastritis and Pericarditis. J.A.V.M.A. 94. (1939):357.
- Fincher, M. G. and Fox, F. H. Diseases of Cattle. American Veterinary Publication. Inc., Evanston. Ill. 1956:168.
- Frank, E. R. Veterinary Surgery. 6th Ed., Burges Publishing Company, Minneapolis 15, Minnesota, 1961.
- Fraser, C. M. Conservative Treatment of Traumatic Reticulitis. Can. Vet. Jour. 2, (1961):65.
- Hall, S. A. Diagnosis of Reticulitis. Vet. Rec. 68, (1956):82.
- Hjerpe, C. A. Studies on Acute Bovine Traumatic Reticuloperitoritis i. Platform Method of Treatment, ii. Signs of Traumatic Reticuloperitoritis. iii. Hematology. J.A.V.M.A. 139, (1961):227.
- in Dairy Cattle. J.A.V.M.A. 143. (1963):1324.
- Jackson, S. Electromagnetic Metal Detector. J.A.V.H.A. 130, (1957):477.

- Kingrey, B. W. Experimental Bovine Traumatic Gastritis. J.A.V.H.A. 127, (1955):477.
- Matteson, A. M., Ebert, E. F. and Case, A. A. The Value of Blood Counts in Traumatic Gastritis. Vet. Med. 48, (1953): 345.
- Milson, L. S. Magnets: A Therapoutic in the Treatment of Traumatic Gastritis. Vet. Med. 51, (1956):56.
- Omender, D. L. and Christenson, H. N. Transcellular Concentration as a Consequence of Intracellular Accumulation. J. of Biological Chemistry, 234, No. 9 (1959):2321.
- Ryan, H. E. The Diagnosis and Treatment of Traumatic Gastritis. North Am. Vet. 28, (1947):294.
- Silver, J. The Sulkowntch Test in Traumatic Gastritis. North Am. Vet. 32. (1951):829.
- Schalm, O. W. Veterinary Hamatology. Les & Febiger. Philadelphia. 1961: 326.
- Shaw, J. C. Miscellaneous Publication 139, University of Maryland, Asri. Ebr. St. 1952.
- The Editorial Board. The Merck Veterinary Manual. 2nd Ed., Merck and Co. Inc., Rahway, N.J. 1961:128.
- Udall, D. H. The Practice of Veterinary Medicine. 6th Ed., Ithaca, N.Y. Udall Publishing Co., 1954.
- William, E. Further Observations on Traumatic Reticulities and Bloat in Cattle. Vet. Rec. 68, (1956):835.
- Wayne, L. G. Quantitative Aspects of Neutral Red Reactions of Typical and Atypical Mycobacteria. Am. Rev. Tuberculosis and Pulmonary Diseases. 79:4, (1959):526.

THE DIFFERENTIAL LEUKOCYTE COUNT OF PERITONEAL FLUID AS ASSOCIATED WITH TRAUMATIC RETICULOPERITORITIS IN THE BOVINE

by

MUHAMMAD ASLAM PASHA

B.V.Sc., University of the Punjab (W. Pakistan), 1954

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY Hanhattan, Kansas A technique was studied to assist the veterinary clinicians to detect traumatic reticuleperitonitis (TRP) at an early stage.

Twenty dairy type cows and one Hereford bull which were admitted to the Kansas State University Veterinary Hospital with a history of digestive disturbances were used as subjects for this experiment.

Attempts for peritoneal paracentesis were made in all cases just prior to the surgery. Peritoneal specimens were collected by aspiration from the peritoneal cavities by paneturing into the space dersal and lateral to siphoid cartilage, below the reticulum and in front of the anterior blind sac of the rumen. Peritoneal specimens were contribuged and after discarding the supermatant fluid, microslide films were prepared by spreading a portion of the remaining specimen. Wright's method was used for staining. A differential leukesyte count was made with the microscope using the cil immersion laws.

The blood samples were obtained from the left jugular vein prior to the surgery. Standard laboratory techniques were used for total and differential loukocyte counts.

The exploratory operations were accomplished by standard surgical procedures for abdominal exploratory and rumenetomy.

Peritoneal fluid leukesyte differential counts were accomplished in seven out of twenty-one cases. Four cases were confirmed as chronic transatic reticuloperitoritis. The other three cases were confirmed as transatic pericarditis, displaced abonasum and malignant lymphoma of the abonasum. The differential leukesyte counts of the peritoneal specimens in the seven cases exhibited pictures which indicated the presence of stress reaction and infection of varying degree.

While comparing the differential leukocyte counts of the peritoneal fluid and the blood of the above mentioned 5 cases, marked neutrophilia and lymphoponia was observed in all instances. The differential leukocyte count in the peritoneal fluid in the case of displaced abomasum indicated the presence of stress reaction, whereas the blood leukocyte differential did not reveal important changes. The differential leukocyte count of the peritoneal fluid in the case of malignant lymphoma of abomasum indicated the presence of this entity, whereas the differential leukocyte count of the blood did not exhibit anything specific for malignant lymphoma. But the total blood leukocyte count exhibited absolute lymphositosis.

The four scute traumatic reticuloperitonitis cases which produced slight peritonitis and three chronic traumatic reticuloperitonitis cases with circumscribed and well organized adhesions did not produce adequate peritoneal fluid for cell study. Similarly two cases of traumatic pericarditis did not exhibit adequate leukocytes in the peritoneal fluid for differential study.

From the results of this study it was concluded that the cellular contents of the peritoneal fluid apparently will depend upon the point of penetration, degree of local reaction and the duration of the disease syndrome.

It was further concluded that the collular study of the peritoneal fluid is most useful in determining late acute diffuse cases of traumatic reticuloperitomitis.