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

by

MUHAMMAD ASLAM PASHA
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Approved by:

[Signature]
Major Professor
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INTRODUCTION

Traumatic reticuloperitonitis 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 peritonitis 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 pre-disposing factors for the increasing number of traumatic reticuloperitonitis each year. Kingerly (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 torn down.

Traumatic reticuloperitonitis is one of the most important problems confronting many modern day dairy veterinary practitioners. Its importance stems from untold economic losses (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 those of indigestion and other diseases. Udall (1954) stated that so many
symptoms of traumatic gastritis 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 formation 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 Examination.** 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 corroborated the clinical findings. The conclusion reached was that a study of the blood picture is a definite aid in the diagnosis of traumatic reticulitis and pericarditis 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 resemblance to them. He stated that although not pathognomonic, 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 reticuloperitonitis 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.
Matteson et al. (1953) observed that the total red blood cell count and hemoglobin 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 alone 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 versa, plus definite neutrophils shift to the left. High eosinophilia 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 moderate neutrophilia it is often insufficiently marked to be diagnostic and the total leukocyte count is unchanged.

In ten cases of experimentally induced traumatic gastritis, Kingrey (1955) observed an increase in the total leukocyte count with the onset 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.

Carrol et al. (1958) found differential leukocyte count of great value 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 reticulopectonitis remains one of the diagnostic problems in the bovine practice. They attempted to determine if the hemogram in chronic traumatic reticulitis followed a pattern consistent 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.
Sohalm (1961) reported 16 cases of traumatic reticulitis which were proven by surgical procedure. He suggested that a marked shift to the left does not necessarily mean traumatic reticulitis 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 leukocytes, increase in the incidence of unsegmented neutrophils in the differential count and reversal of the normal lymphocyte:neutrophil ratio. He was of the opinion that although the hemogram is of considerable value as a diagnostic aid in acute traumatic reticuloenteritis, 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 reticuloenteritis. 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.

Metal Detectors. Metal 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 detrimental effect, and therefore, a positive reaction does not in any way guarantee that the presence of this material is producing the symptoms.
Nilson (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 Pain. William (1956) claimed a new method for diagnosing traumatic reticulitis 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 ruminal movement reached its maximum contractions. This diagnostic method was found to be completely reliable in the 64 affected cattle he examined. Kingrey (1955), Blood and Hutchins (1955), Fincher and Fox (1956) and Blood and Hutchins (1960) have also reported that the pain in the area of xiphoid cartilage is an important manifestation for differential diagnosis.

Urine Test. Silver (1951) reported that he made 'on the spot' urine test with Sulkowitch reagent and found an absence of calcium in urine in a positive case of traumatic reticulitis. Hall (1956) utilized this test on six cases that were positive for a perforating foreign body, confirmed on rumenotomy, and which were positive to the Sulkowitch 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 urine results in the normal results.

Exfoliative Cytology. Wayne (1959), Oxender 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.

**Peritoneal Fluid Leukocytes.** 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.

**Preparation of Peritoneal Fluid Films on Microslides.** Approximately 75% of the supernatant fluids of the centrifuged specimens were discarded. Peritoneal fluid films on microslides were prepared by spreading 4 to 5 drops from the remaining portion 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 (3.30 grams Na$_2$HPO$_4$ and 5.47 grams KH$_2$PO$_4$ in 1000 ml. distilled water) were added and mixed until a metallic film formed on the surface of stain-buffer mixture.

3. Diluted stain was allowed to act for 4 minutes.

4. The scum and precipitated stain was floated off by quickly flooding water over the slides.

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

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 ethylenediaminetetraacetate solution to prevent coagulation.

Blood Leukocyte Total and Differential Counts. The total and differential leukocyte counts were made on the blood samples. Standard laboratory techniques were used. The hemocytometer 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 Reticuloperitonitis. The blood leukocyte total and differential counts of 4 acute traumatic reticuloperitonitis cases are shown in Table 1.
Table 1. Blood leukocyte total and differential counts in acute cases of traumatic reticuloperitonitis.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Total WBC</th>
<th>Bands</th>
<th>Seg.</th>
<th>Differential count percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>5577*</td>
<td>6 yr.</td>
<td>22,118</td>
<td></td>
<td>71</td>
<td>23 5 23</td>
</tr>
<tr>
<td>7417*</td>
<td>4 yr.</td>
<td>10,700</td>
<td></td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td>9188*</td>
<td>6 yr.</td>
<td>14,600</td>
<td>1</td>
<td>66</td>
<td>28 2 3</td>
</tr>
<tr>
<td>9776*</td>
<td>6 yr.</td>
<td>6,700</td>
<td></td>
<td>50</td>
<td>40 2 8</td>
</tr>
<tr>
<td>Average</td>
<td>5.5</td>
<td>13,379</td>
<td>25</td>
<td>53.5</td>
<td>35 2 8.5</td>
</tr>
</tbody>
</table>

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

The total leukocyte count was within the normal (Table 2) range in case 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 bovines according to Schalm.

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leukocyte count</td>
<td>4-12,000 c.mm.</td>
<td>(9.25)(5.48)(8.9)(5.6) x 10^3</td>
</tr>
<tr>
<td>Bands %</td>
<td>0-12</td>
<td>0.5</td>
</tr>
<tr>
<td>Segments %</td>
<td>15-45</td>
<td>28</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>45-75</td>
<td>58</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>2-7</td>
<td>4</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>2-20</td>
<td>9</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0-2</td>
<td>0.5</td>
</tr>
<tr>
<td>Neutrophil:Lymphocyte ratio</td>
<td>28/58%</td>
<td></td>
</tr>
</tbody>
</table>
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. 9183. 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 reticuloperitonitis. It may be due to the fact that the perforations of the reticulum and formation of the adhesions in acute traumatic reticuloperitonitis cases resulted so rapidly that it did not produce adequate peritonitis for peritoneal paracentesis and cellular study. It is also possible that in acute traumatic reticuloperitonitis 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) neutrophil:lymphocyte ratio. The monocyte and eosinophils were in the normal (Table 2) percentage. The blood picture in 4 acute traumatic reticuloperitonitis cases discussed above was indicative for stress reaction in reticular perforation. 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 Reticuloperitonitis. The blood leukocyte total and differential counts of seven cases of chronic traumatic reticuloperitonitis are shown in Table 3(a).

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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Total WBC</th>
<th>Bands</th>
<th>Seg.</th>
<th>Differential count percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lym.</td>
</tr>
<tr>
<td>5483</td>
<td>4 yr.</td>
<td>9,631</td>
<td>51</td>
<td>45</td>
<td>2</td>
</tr>
<tr>
<td>6363</td>
<td>3 yr.</td>
<td>14,537</td>
<td>67</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>6374</td>
<td>3 yr.</td>
<td>12,918</td>
<td>78</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>6867</td>
<td>2 yr.</td>
<td>No CBC.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7662*</td>
<td>2 yr.</td>
<td>11,931</td>
<td>77</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>9204*</td>
<td>5 yr.</td>
<td>7,000</td>
<td>6</td>
<td>68</td>
<td>20</td>
</tr>
<tr>
<td>10231</td>
<td>4 yr.</td>
<td>7,274</td>
<td>62</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Average</td>
<td>3.5</td>
<td>12,920</td>
<td>67</td>
<td>25.5</td>
<td>3</td>
</tr>
</tbody>
</table>

*Previous antibiotic therapy.

The blood examination was not accomplished in case No. 6867. The total leukocyte count was within the normal (Table 2) range in case No. 5483, 9204 and 7662. Leukocytosis was observed in case No. 6363 and 6374.

The differential leukocyte count in seven cases of chronic traumatic reticuloperitonitis exhibited a 'shift to the left' with lymphopenia and normal (Table 2) percentage of monocyte and eosinophils in case No. 6363 and 9204. Neutrophilia and lymphopenia, reversal of normal (Table 2) neutrophil:lymphocyte ratio, was an outstanding feature in cases No. 5483, 6374, 6867, 7662 and 10231.
The average blood leukocyte total and differential counts showed leukocytosis with a 'shift to the left' and lymphopenia. The monocytes and eosinophils 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 leukocyte differential counts in seven chronic traumatic reticuloperitonitis cases are shown in Table 3(b).

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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Bands</th>
<th>Seg.</th>
<th>lym.</th>
<th>mono.</th>
<th>eos.</th>
<th>base.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6374*</td>
<td></td>
<td></td>
<td>82</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6867</td>
<td></td>
<td></td>
<td>95</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7662*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9204</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10231</td>
<td>2</td>
<td>67</td>
<td>21</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>.5</td>
<td>85.5</td>
<td>17</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

The peritoneal differential leukocyte 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 monocytes were differentiated. Marked neutrophilia and lymphopenia was observed in all 4 chronic traumatic reticuloperitonitis 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 reticulo-peritonitis case No. 6374.

a. Segmenter  b. Lymphocyte (X 500)

EXPLANATION OF PLATE II

Photograph of peritoneal fluid film in chronic traumatic reticulo-peritonitis case No. 6374.

a. Segmenter  b. Lymphocyte (X 1250)
EXPLANATION OF PLATE III

Photograph of peritoneal fluid film in chronic traumatic reticulo-pancreatitis case No. 6867.

a. Segmenter  b. Lymphocyte (X 500)
Monocytes were not observed in case No. 6857. It is of interest that neither eosinophils nor basophils were seen in the peritoneal fluid of these cases by counting 100 cells.

The peritoneal fluid differential leukocyte count could not be accomplished in three chronic traumatic reticuloperitonitis cases No. 3408, 6368 and 7662 due to the inadequate peritoneal fluid available in these cases. Well organised adhesions, apparently of long duration, were observed on exploratory operations in these cases. It is considered that well organised adhesions were associated with inadequate peritoneal fluid for aspiration and cellular study.

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

While comparing the average differential leukocyte counts of the peritoneal fluid and circulating 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 leukocyte counts of the peritoneal fluid and blood as both were indicative for a stress reaction in chronic traumatic reticuloperitonitis cases.

Traumatic Pericarditis. The blood leukocyte total and differential counts of 3 cases of traumatic pericarditis are shown in Table 4(a).
Table 4(a). Blood leukocyte total and differential counts in traumatic pericarditis cases.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Total WBC</th>
<th>Bands</th>
<th>Seg.</th>
<th>Differential count percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lyn.</td>
</tr>
<tr>
<td>5513</td>
<td>2 yr.</td>
<td>10,326</td>
<td>2</td>
<td>70</td>
<td>24</td>
</tr>
<tr>
<td>6608</td>
<td>5 yr.</td>
<td>6,600</td>
<td>79</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>9108</td>
<td>2 yr.</td>
<td>25,000</td>
<td>65</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>Average</td>
<td>3 yr.</td>
<td>13,975</td>
<td>71</td>
<td>24</td>
<td>4</td>
</tr>
</tbody>
</table>

Marked leukocytosis was observed in case No. 9108. Normal (Table 2) total leukocyte count was seen in case No. 5513 and 6608.

The differential leukocyte 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 leukocytosis 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.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Bands</th>
<th>Seg.</th>
<th>lym.</th>
<th>mono.</th>
<th>eos.</th>
<th>cos.</th>
<th>base.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5513*</td>
<td>Clear fluid—inefficient cells for differential counts.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6808</td>
<td>85</td>
<td>10</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9108**</td>
<td>Clear fluid—inefficient cells for differential count.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*wire penetrated through anterior portion of reticulum—slight peritoneal adhesions between reticulum and diaphragm.

**Long term condition.

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 leukocyte 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 inefficient leukocytes 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 diaphragm. It is postulated that the differential leukocyte count could not be accomplished in this case, due to inefficiency of the leukocytes in the peritoneal fluid. It is probable that due to the slight peritoneal reaction, less numbers of leukocytes were concentrated at the site of adhesions and migration into the peritoneal fluid did not occur. In case No. 9108 the differential leukocyte 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. 6808, it was observed
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 Reticuloperitonitis. The differential leukocyte counts in the blood and peritoneal fluids of seven cases, which produced clinical syndromes somewhat similar to traumatic peritonitis but were negative for this disease are shown in Table 5 as A and B respectively. The blood total leukocyte 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 leukemic blood picture. It is of interest to note that the total leukocyte 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 lymphopenia 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 lymphopenia was observed in cases No. 3144, 7664, and 10666. Schalm (1960) stated that a 'shift to the left' should not always be taken for traumatic reticuloperitonitis. 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 traumatic reticuloperitonitis.
Table 5. (A) Blood leukocyte total and differential counts, and (B) Peritoneal fluid leukocyte differential counts, in clinical conditions other than traumatic reticulo-peritonitis.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Clinical diagnosis</th>
<th>Total WBC</th>
<th>Bands</th>
<th>Seg.</th>
<th>lymph.</th>
<th>mono.</th>
<th>eos.</th>
<th>baso.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6635</td>
<td>2 yr.</td>
<td>Displaced abomasum</td>
<td>(A) 14,300</td>
<td>2</td>
<td>74</td>
<td>16</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11030</td>
<td>5 yr.</td>
<td>Displaced abomasum</td>
<td>(A) 4,921</td>
<td>26</td>
<td>60</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3144</td>
<td>5 yr.</td>
<td>Ketosis</td>
<td>(A) 12,700</td>
<td>2</td>
<td>62</td>
<td>22</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>6645</td>
<td>6 yr.</td>
<td>Malignant lymphoma</td>
<td>(B) 27,341</td>
<td>2</td>
<td>44</td>
<td>46</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7664**</td>
<td>5 yr.</td>
<td>Indigestion rumen impaction</td>
<td>(A)* 7,427</td>
<td>51</td>
<td>44</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10666</td>
<td>4 yr.</td>
<td>Indigestion</td>
<td>(A) 9,000</td>
<td>69</td>
<td>26</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9111</td>
<td>6 yr.</td>
<td>Enteritis</td>
<td>(A) 40,000</td>
<td>12</td>
<td>73</td>
<td>7</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Inadequate peritoneal fluid or total cells for differential count.

**Male Hereford.

The differential leukocyte count of the peritoneal specimens of suspected cases for traumatic reticulo-peritonitis were accomplished in case No. 11030 (Plate IV) and 6645 (Plate V, VI). Neutrophilia and lymphopenia was observed in the case of displaced abomasum (No. 11030). The picture was quite similar to that of a traumatic reticulo-peritonitis case and did indicate a stress reaction. The blood differential leukocyte count 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
EXPLANATION OF PLATE IV

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

a. Segmenter    b. Lymphocyte (X 500)
EXPLANATION OF PLATE V

Photograph of peritoneal fluid film malignant lymphoma 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)
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 accomplished 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 traumatic reticuloperitonitis. 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 those which produced physical stress in the peritoneal cavity such as displaced abomasum and malignant lymphoma of abomasum and simple localized penetrating cases of reticuloperitonitis, apparently will not cause sufficient peritoneal 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 reticuloperitonitis.

SUMMARY

A technique for the diagnosis of traumatic reticuloperitonitis (TRP) 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 reticuloperitonitis 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 abomasum 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 reticuloperitonitis cases which produced a slight peritonitis, and 3 chronic cases of traumatic reticuloperitonitis with circumscribed and well organized peritonitis 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 reticulo- peritonitis.
ACKNOWLEDGMENTS

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THE DIFFERENTIAL LEUKOCYTE COUNT OF PERITONEAL FLUID AS ASSOCIATED WITH TRAUMATIC RETICULOPERITONITIS 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
Manhattan, Kansas

1964
A technique was studied to assist the veterinary clinicians to detect traumatic reticuloperitonitis (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 puncturing into the space dorsal and lateral to ziphoid cartilage, below the reticulum and in front of the anterior blind sac of the rumen. Peritoneal specimens were centrifuged and after discarding the supernatant fluid, microslide films were prepared by spreading a portion of the remaining specimen. Wright's method was used for staining. A differential leukocyte count was made with the microscope using the oil immersion lens.

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

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

Peritoneal fluid leukocyte differential counts were accomplished in seven out of twenty-one cases. Four cases were confirmed as chronic traumatic reticuloperitonitis. The other three cases were confirmed as traumatic pericarditis, displaced abomasum and malignant lymphoma of the abomasum. The differential leukocyte 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 lymphopenia 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 acute 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 cellular study of the peritoneal fluid is most useful in determining late acute diffuse cases of traumatic reticuloperitonitis.