

## Peritoneal Fluid Cytodiagnosis for Abdominal Diseases of Cattle: A Review

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**Abstract:** Peritoneal fluid reflects the pathophysiological state of the parietal and visceral meso-thelial surfaces of the peritoneum. Today, peritoneal fluid analysis in cattle is a highly sensitive indicator of peritoneal diseases. Specifically, peritoneal fluid cytology can help to distinguish the causes of effusion and often assists the clinician in initiating appropriate therapy. Technically, after proper abdominocentesis smears are prepared, stained and dried off for evaluation. Through cytologic examination, mainly inflammatory reactions and hyperplastic responses of the mesothelial lining can be identified. Peritoneal fluid samples collected from cattle are having bacterial peritonitis (septic peritonitis) contained and revealed degenerated neutrophils and bacteria. Peritoneal fluid samples collected from cattle having non-septic peritonitis like with cases of traumatic reticuloperitonitis (TRP) showed predominately non-degenerated neutrophils. The morphology and the different cell types present in the peritoneal fluid samples also varied according to the status of urinary bladder of bovines with clinical cases of obstructive urolithiasis. Finally, on the basis of the facts mentioned, we can come to the conclusion that peritoneal fluid cytologic analysis has significant diagnostic, therapeutic and prognostic value for several abdominal diseases in cattle.

**Key words:** Abdominal Diseases • Cattle • Cytology • Peritoneal Fluid

### INTRODUCTION

Abdominal problems are one of the most important diseases in cattle around the world [1]. Peritoneal fluid reflects the pathophysiological state of the parietal and visceral mesothelial surfaces of the peritoneum. Today abnormal peritoneal fluid in cattle is a highly sensitive indicator of peritoneal diseases, but not a good indicator of the nature of the diseases [2]. The use of abdominal fluid as an aid to diagnose abdominal diseases has been documented [1]. A retrospective study on the analysis of bovine peritoneal fluids showed a non-septic peritonitis, acute bacterial peritonitis, ascites and miscellaneous disorders such as abomasal impaction, enteritis and lymphosarcoma [3].

Peritoneal fluid changes quantitatively as well as qualitatively, especially in more serious diseases of abdominal organs [4]. Normal peritoneal fluid is amber and crystal clear transudate [1]. Peritoneal fluid analysis was a very useful aid in clinical examinations and the correct diagnosis of abdominal disorders, especially if it was not possible to carry out blood tests [2].

Specifically, cytological analysis of the peritoneal fluid can help distinguish from fungal, bacterial, sterile, inflammatory or neoplastic causes of effusion and often assists the clinician in initiating appropriate therapy while waiting for the results of culture, antimicrobial susceptibility testing and histological examination [5]. When peritoneal fluid from clinically normal cattle is examined cytologically, mature non-degenerate neutrophils and macrophages predominate. Low numbers of small lymphocytes are expected [6]. Mesothelial cells commonly exfoliate into the cavity fluid of normal animals. Eosinophils are commonly seen in bovine peritoneal fluid [5].

Performing cytologic evaluation of abdominal fluid from a patient with abdominal disease is essential for rapid determination of the disease etiology, often assisting the clinician in initiating appropriate therapy. In many cases, analysis and cytology of the abdominal fluid provide valuable information necessary for deciding whether medical or surgical intervention is most appropriate [7]. This technique was used for the diagnosis of traumatic reticuloperitonitis (TRP), acute abdominal crises in cattle

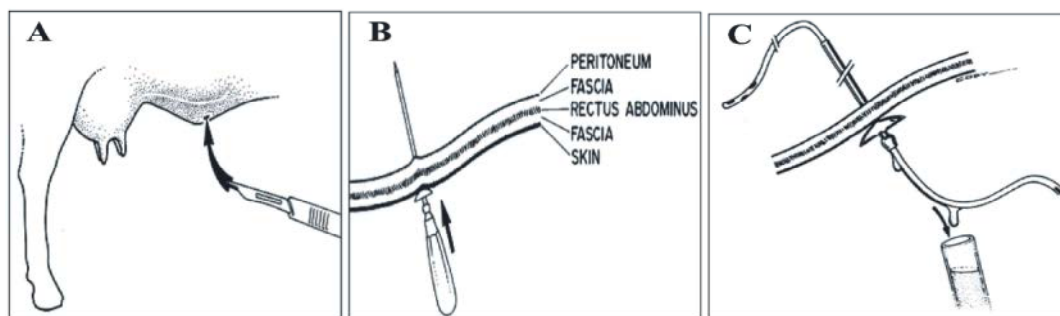


Fig. 1: Site of abdominocentesis in cattle using the trocar and cannula method (A). Collection of bovine peritoneal fluid showing the tissues penetrated by trocar and cannula (B). Collection of peritoneal fluid using trocar and cannula and fenestrated tube showing sites of collection (arrows), through and around the tube (C) [10].

and may be valuable for the diagnosis of peritonitis in cattle [8, 9]. Therefore, based on the above facts, the objectives of this paper are to review the techniques of peritoneal fluid sample collection and smear preparation from cattle, to review important points on cytological evaluation and interpretation of the results and to review cytological features of major abdominal diseases in cattle.

#### **Techniques of Peritoneal Fluid Sample Collection and Smear Preparation:**

The trocar and cannula method of collecting peritoneal fluid has been proved to be reliable [5]. Fluid is collected from the cattle in the standing position. The recommended site for collection is about 4 cm medial and 5 to 7 cm cranial to the point where the milk vein enters the abdomen (Figure 1A). The site is clipped, surgically prepared and locally anesthetized. A small stab incision is made through the skin and external fascia and a 9 cm blunt ended bovine teat cannula passed through the incision. The cannula is then gently pushed through the rectus abdominis muscle and with a quick, short thrust popped through the peritoneum (Figure 1B). To prevent blood contamination, the shaft of the cannula is wrapped with a sterile gauze sponge. If no fluid is obtained from this site, the tap is repeated a few centimeters caudal to the site at the most dependent aspect of the abdomen. The fluid obtained is collected in a small tube containing dipotassium ethylene diamine tetra acetic acid (EDTA) (Figure 1C) and analyzed as soon as possible after collection [10, 11].

Smears from cloudy, highly cellular, well-mixed fluids can be made directly via the blood smear technique or line smear technique. If there are any floccules of particulate matter grossly visible in the fluid at the time of collection, then these should be included in the smears as well. Aliquots of clear or slightly turbid fluids should be concentrated via centrifugation to increase the cellularity

of prepared smears. Following centrifugation, the majority of the supernatant is decanted, the pellet (cellular material) is then re-suspended in the minimal remaining supernatant and smears are made via either the blood smear or line smear techniques. The line smear technique is similar to the blood smear technique; however, the spreader slide is abruptly stopped and lifted off the specimen slide prior to creating a feathered edge, resulting in a higher concentration of cells present in the terminal line, than within the remainder of the smear [12].

**Staining of the Smears:** Romanowsky-type stains (Wright's, Giemsa and Diff-Quik® stains) are commonly used. Romanowsky-stains are inexpensive, easy to use and they are readily available to veterinary practitioners. They provide good nuclear detail, excellent cytoplasmic detail and infectious organisms are readily visualized. In clinical practice, the most cost effective, quickest and easiest stain to use is the Diff-Quik® stain. The recommended staining procedures outlined on the product should be followed, however, as a general rule, the thinner the material on the smear, the less time needed for staining and the thicker the material, the more time required for staining [13].

#### **Cytologic Evaluation and Interpretation of the Results**

**Cytologic Evaluation:** After the smear has been stained and dried, it should first be evaluated at low power using the 4 [times] and/or 10 [times] objective. This degree of magnification allows the clinician to assess the adequacy of staining and to identify areas of high cellularity or areas with unique staining features. In addition, larger objects, including some crystals, parasites and plant debris, may be seen while scanning at low magnification. Magnification can then be increased to the 10 times or 20 times objective. At this degree of magnification, the

clinician can gain an impression of the overall cellularity and cellular composition. When an area of increased or unique cellularity is identified, the smear is viewed with either the 40 times or 50 times objective. To improve resolution when using the 40 times objective, one can place a drop of oil on the smear and cover it with a coverslip. At this magnification, individual cells are examined and compared with other cells and most microscopic organisms can be seen. A differential cell count can also be performed at 40 times to 50 times magnification. Finally, the smear should be evaluated using the 100 times using the oil-immersion to definitively identify organisms and cellular inclusions and examine cellular morphology [14].

**Interpretation of the Results:** 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 [5]. Fluid with a WBC count greater than 1000 cells per micro-litre in a patient that has not recently had an exploratory laparotomy and that contains many degenerative neutrophils is suggestive of peritoneal inflammation or suppuration with possible sepsis; an exploratory laparotomy is indicated. Other indications for surgical exploration of the abdomen include the presence of intracellular bacteria, a finding that is suggestive of bacterial peritonitis and vegetable fibers that indicate visceral perforation with leakage of bowel contents. Patients with chemical peritonitis secondary to biliary rupture or uro-abdomen will often benefit from medical stabilization. Surgery can be performed later when the patient's cardiovascular status is more stable, rather than on an emergency basis [15].

#### Cytologic Features of Specific Diseases

**Peritonitis:** Peritonitis is the inflammation of the peritoneum which is usually characterized by accumulation of clear fluid known as hydroperitoneum or ascites. Its main etiologies include bacteria (*Staphylococci*, *Mycobacterium sp.*), viruses, parasites and neoplasia [16]. Peritonitis can occur as a primary disease or secondarily as a part of a specific disease. As a primary disease it commonly results from injury of the gut serosal surface allowing the gut contents to enter the peritoneal cavity [2]. Less commonly there is perforation of abdominal wall from exterior by penetrating foreign bodies or introduction of pathogens or irritating substances as a result of injections into peritoneal cavity or exploratory laparotomy [17]. The leakage of gut

contents may also occur through perforating abomasoduodenal ulceration resulting into diffuse peritonitis [18].

**Cytologic Features:** Peritoneal fluid samples collected from cattle having bacterial peritonitis contained degenerate neutrophils and bacteria, both within neutrophils and extracellularly (Figure 2A). In the cases with a perforation of the gastrointestinal tract, the fluid contained plant fibers, squamous cells and a mixed bacterial population (Figure 2B). These fluids could be differentiated from gut contents by the elevated protein content and the presence of degenerate neutrophils [3].

In the contrary, peritoneal fluid samples collected from cattle having non-septic peritonitis with cases of traumatic reticulo-peritonitis (TRP) showed predominantly non-degenerate neutrophils (Figure 3A). Mononuclear cells were usually actively phagocytic macrophages (Figure 3B) and reactive mesothelial cells were also observed (Figure 3C). Eosinophils were not commonly seen. No bacteria were found on cytological preparations. Those cattle with functional disorders such as abomasal impaction had a mononuclear to neutrophil ratio of approximately 1:1, consisting of non-degenerate neutrophils, lymphocytes and mesothelial cells (Figure 4A), similar to the cattle with ascites. Cytological preparations of fluid from the cow with lympho-sarcoma revealed many large bizarre epithelial cells and mitotic figures (Figure 4B). A population of large, pleomorphic, epithelial cells in clusters predominated in peritoneal fluid from a cow with disseminated squamous cell carcinoma [3, 15].

**Bovine Obstructive Urolithiasis:** Urinary calculi (uroliths) are concretions formed anywhere in the urinary collecting system and although some clearly originate in the lower urinary tract or as microscopic calculi in the renal collecting tubules, the point of development of most is not known. Uroliths are commonly found in the ureter, followed by any portion of the lower urinary tract and least commonly in the renal pelvis. The diseases caused by uroliths are among the most important urinary tract problems of domesticated animals, Urolithiasis can cause urinary obstruction or traumatic injury to the urinary bladder mucosa [19]. Urolithiasis may affect any species of the animals but is considered of great economic importance in fattening steers being fed heavy concentrate ration and in castrated lambs [2]. Urolithiasis has been reported more frequently in feed lot and grazing cattle [20].

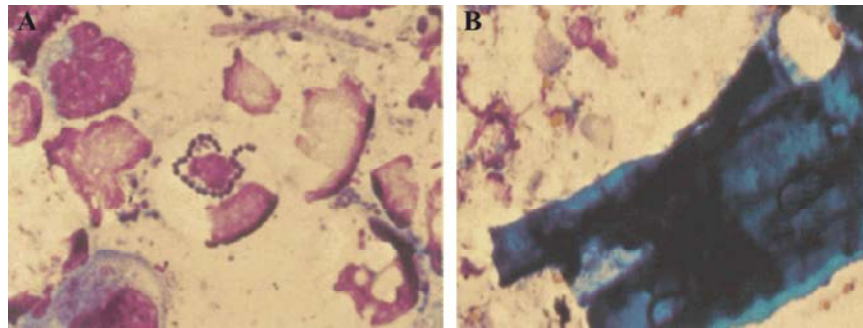


Fig. 2: Peritoneal fluid from a cow with a septic peritonitis due to a perforated abomasal ulcer and severely degenerate neutrophils with swollen pink nuclei and indistinct cytoplasm which contain bacteria. Some free bacteria also present. X880 (A). Low power view of same fluid with plant fiber is evident (Wright's-Giemsa stain). X90 (B) [3].

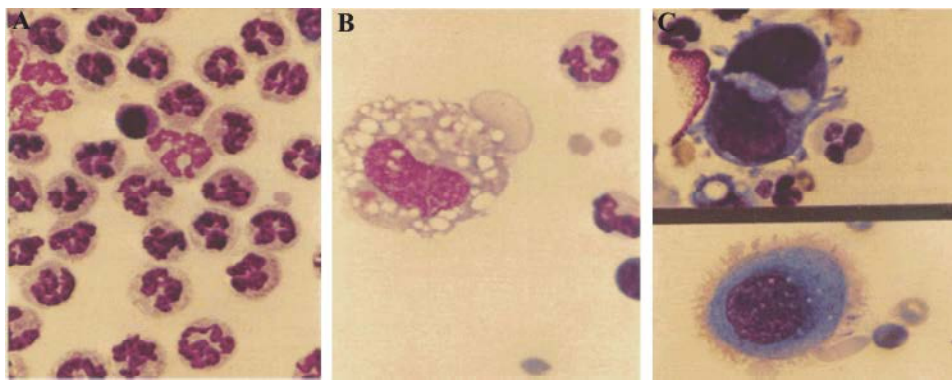


Fig. 3: Peritoneal fluid cytology from a cow with non-septic peritonitis due to TRP showing non-degenerate neutrophils. Bacteria cannot be seen (Wright's-Giemsa stain). X880 (A). An actively phagocytic macrophage in a cow with TRP (Wright's-Giemsa stain). X880 (B). Reactive mesothelial cells. Both have a ruffled border and one cell has an eosinophilic brush border. Note the large size in comparison to neutrophils (Wright's-Giemsa stain). X880 (C) [3].

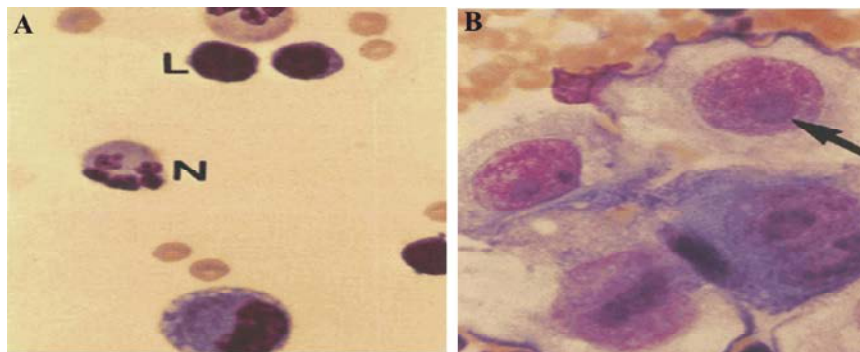


Fig. 4: Wright's-Giemsa stained smear of peritoneal fluid from a cow with abomasal impaction and non-degenerative neutrophils (N), lymphocytes (L) and a mesothelial cell are present. X880 (A). Large bizarre epithelial cells with nucleoli from peritoneal fluid in a case of disseminated squamous cell carcinoma. Arrow indicates a prominent nucleolus (Wright's-Giemsa stain) X880 (B) [3].

**Cytologic Features:** Peritoneal fluid cytology of bovines with clinical cases of obstructive urolithiasis showed higher percentage of neutrophils than the normal reference range reported for cattle and with slight

decrease in lymphocyte percentage. The value for neutrophil percent was almost similar in both intact and ruptured urinary bladder cases. The monocyte/mesothelial cells/macrophage percent, though increased than the

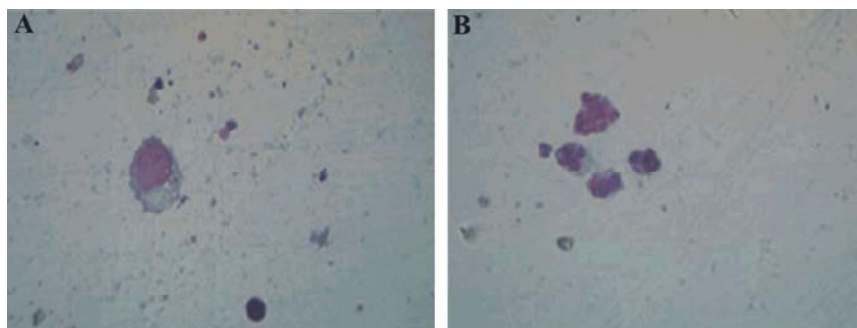


Fig. 5: Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing activated monocyte with vacuolated cytoplasm (A). Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing degenerated and hyper-segmented neutrophils (B) [21].

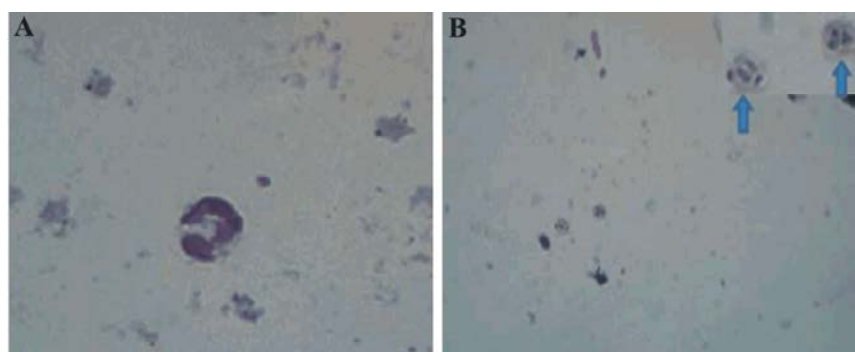


Fig. 6: Slide made from the peritoneal fluid sample obtained from intact urinary bladder case showing intact neutrophils (A). Slide made from the peritoneal fluid sample obtained from ruptured urinary bladder case showing bacteria intra-cellularly (arrows) (B) [21].

normal reference range were identical in both the groups. There was a predominant decrease in eosinophil percentage with more decrease in intact urinary bladder cases. The morphology and the different cell type present in the peritoneal fluid samples also varied according to the status of urinary bladder (Figure 5 and 6) [21].

### CONCLUSION

Abdominocentesis and abdominal fluid analyses are quick, simple and reliable. Through cytologic examination of peritoneal fluid, inflammatory reactions and hyperplastic responses of the mesothelial lining can be identified. Cytologically, peritonitis is characterized by high cellularity with a predominant cytological cell population of degenerated or non-degenerated neutrophils based on whether it is non-septic peritonitis or septic peritonitis. Peritoneal fluid cytology of bovines with obstructive urolithiasis may reveal highly increased neutrophils and the morphology and the different cell type present in the peritoneal fluid samples may also vary

according to the status of urinary bladder. Generally, peritoneal fluid cytologic analysis has significant diagnostic, therapeutic and prognostic value for several abdominal diseases of cattle.

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