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Review Article

Peritoneal Fluid Analysis in Canine Disease Diagnosis

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Abstract

Abdominal effusion is a relatively common problem in small animal practice. Proper collection and evaluation of peritoneal effusion can provide valuable information about the disease which is responsible for the fluid accumulation in the body cavity. The classification of effusions based on their underlying etio-pathology is clinically useful for the clinician to ensure proper diagnosis and treatment. Present article reports briefly regarding pathophysiology of effusion, sample collection, physical, microscopic, biochemical changes, and their clinical significance in various disease conditions.

Keywords: Analysis; Ascitis; Dog; Peritoneal fluid

Introduction

An effusion is a large amount of fluid, which may alleviate the symptom of a greater disease. Normally, a small amount of lubricating fluid is found in the peritoneum, which comes from the surrounding tissue and vessels. If the fluid exceeds the normal amount, an individual may have an effusion. If an effusion is present, the collection and retention of fluid inhibits the function of the organ. Abdominal pain can signal an effusion, though pain may also be associated with other conditions of the pelvic region and should be ruled out during thorough examination. Peritoneal effusion is abnormal accumulation of fluid in peritoneal cavity. Patients can either presented to veterinarians for clinical signs secondary to fluid accumulation or simply due to change in appearance because of fluid build-up (Rizzi *et al.*, 2008). Abdominal effusion is a relatively common presenting problem in veterinary practice and analysis of fluid helps the clinician to obtain further information regarding the understanding of disease (Alleman, 2003; Kruth, 2005).

Normal and abnormal peritoneal effusion

The abdominal cavity is lined with thin watery membrane known as peritoneum. Its serous membrane comprising a single layer of squamous mesothelial cells resting on a deeper layer of loose connective tissue (Mc Grafty and Doust, 2004). The layer of peritoneum that lines the inner surface of the abdomen is termed as "parietal peritoneum" and the abdominal organs lined by "visceral peritoneum". The total surface areas of the peritoneum are roughly one and a half that of the skin size (Ross and Labato, 2006).The peritoneal fluid plays an important role in the peritoneum of abdominal infection. The total amount of fluid that passes across the peritoneal membrane in a 24hr period is about 80ml/kg body weight in normal animals (Nagy and Jackman, 2000). Fluid absorbed from the abdominal cavity by lymphatic vessels lying beneath the mesothelial basement membrane on the surface of the diaphragm. Small stromas of 8-12 µm between the mesothelial cells provides an opening into efficient lymphatics. This stoma decides the size of foreign materials that can be absorbed by the lymphatics. Mesothelial cells secrete glycosaminoglycans including hyaluronic acid, proteoglycans such as decorin, bioglycan and phosphatidylcholine containing lamellar bodies (Nagy and Jackman, 2000). Mesothelial cells also affect the blood flow through peritoneal capillaries by secretion of various substances like nitric oxide and endothelin. The mechanisms of fluid and solutes transport across the peritoneal membrane that involves several physical processes, including diffu-

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sion, convection and ultra filtration (Burkhart, 2000).

In a normal animal, the peritoneal space contains small amount of fluid (less than 1mg/kg body weight), which moistens the opposing surface and serves to reduce friction between the abdominal organs (Alleman, 2003) and that the effusions are abnormal or increased accumulation of fluid in any of the body cavity, which was lined by mesothelial cells. The characteristics of normal peritoneal fluid are clear to slightly yellow with specific gravity less than 1.016 and protein (2g/dl, mainly albumin), total white cell count (2000- 2500/ml, 50% macrophage),some eosinophils, mast cells, and few polymorph neutrophils without fibrinogen (does not clots on standing) and fibronectin (Bray, 1996). The inciting event in the development of peritoneal fluid accumulation is activation of hepatic stellate cells (Ito cells) with subsequent perisunoidal collagen accumulation and fibrosis that results in sinusoidal hypertension followed by portal hypertension. Neuronal and hormonal activation are also involved in the development of peritoneal fluid accumulation (Cardens and Arroyo, 2003; Levy, 1994). Accumulation of fluid within a body cavity results when the rate of filtration of fluid into space is greater than the rate of fluid resorption from the space (Zocchi, 2002). An increase in interstitial hydrostatic pressure narrows the hydrostatic gradient between the interstitium resulting in decreased fluid resorption.

Peritoneal fluid analysis

It is a laboratory test to examine fluid that has collected in the abdominal cavity. Proper collection and evaluation of body fluids can provide valuable information about the disease process, responsible for the fluid accumulation in the body cavity (Alleman, 2003). Generally, peritoneal fluid is analyzed for the condition with peritoneal effusions include septic peritonitis, nonseptic peritonitis, hemoabdomen, uroabdomen, pancreatitis, bile peritonitis, chylous effusion, neoplasia, etc (Hunt, 2002).

Sample collection

Abdominocentesis usually performed with the patient restrained in standing position or in left lateral recumbency and the area surrounding the umbilicus subjected to clipping and full surgical preparation. A site should be chosen 2 to 3 cm caudal to the umbilicus and 2 to 3 cm left of the midline for sampling. The bladder should be emptied before fluid collection (Hall, 2005) and insert an 18 – 20 gauze needle or intravenous catheter just caudal and lateral to the umbilicus at 30o to 40o angle (Alleman, 2003; Mc`Groty and Doust, 2004) (Fig 1). If simple paracentesis fail to provide results, a four quadrant paracentesis may be useful. In this method of paracentesis, four sites viz. right cranial quadrant, left cranial quadrant, right caudal quadrant and left caudal quadrant are used for needle placement utilizing the umbilicus as center point. Abdominal centesis / abdominal paracentesis are preferred to puncture into peritoneal cavity for the purpose of the fluid collection. Abdominal paracentesis is a sensitive technique for fluid collection as long as more than 6ml /kg body weight of fluid usually present within the abdominal cavity (Ford and Mazzaferro, 2006). It can be done either to obtain fluid for evaluation or to remove large amounts of fluid if it is interfering with the pet's ability to breath. Proper collection and evaluation of ascitic fluids can provide practitioners with valuable information that assists the clinician in identifying the disease process responsible for the fluid accumulation.

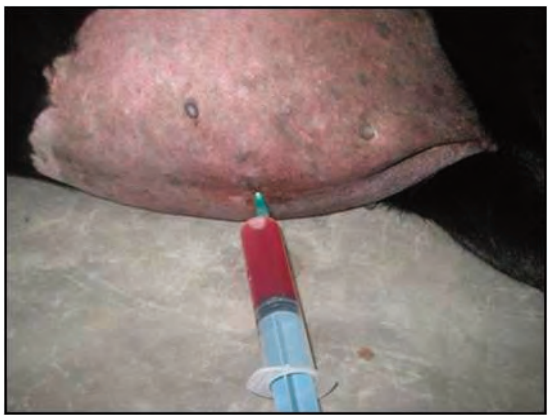

Fig. 1. Peritoneal fluid collection at caudal umbilicus.

Fluid can be collected in ethylene-diamine-tetraacetic acid (EDTA, lavender top) tubes, serum (red top) tubes, sterile tubes (for culture), and/or other tubes for effusion-specific tests such as PCR. Samples should be prioritized according to the volume of fluid available and to the suspected underlying disease process. Fluid collected in EDTA should be submitted for total nucleated cell counts (TNCC), RBCs count or PCV if the fluid is hemorrhagic or serosanguinous, cytology and further analysis as clinically indicated (Dempsey and Ewing, 2011). Fluid collected in serum tubes can

be used for analysis of albumin, bilirubin, creatinine, potassium, triglyceride, glucose, lactate and lipase levels. Fluid sample collected in sterile tubes can be stored for aerobic and anaerobic bacteria, mycoplasma and fungal culture. Anaerobic cultures are not refrigerated and should be processed within 24 hour of collection.

Ascitic fluid analysis

a) Physical examination

Gross examination is performed for transparency or turbidity, color, odor (if any), clots, fibrin, pH and specific gravity. Clear and colorless peritoneal fluid in transudate (Fig. 2A), clear and colorless as pure transudate and slightly turbid in modified transudate (Fig. 2B) and exudates (Fig. 2C) may vary in color from white to amber to pink, but they are usually turbid in nature. Fifteen liter of colorless but slightly cloudy exudates was reported in 10 months old Alsatian bitch with recurrent ascites (Nottidge *et al.*, 2003). Fluid turbidity (due to lipids, hemolysis, and/or cellular debris) and refrigeration of fluid samples may also falsely increase total protein measurements, determined by refractometry. Turbid samples can be centrifuged and total protein can subsequently measured using the supernatant at or near room temperature. Clear yellow in ruptured bladder, acute diffuse peritonitis (yellow, turbid) infarction or necrosis of gut wall (thin red tinged). Milk-colored peritoneal fluid may indicate disease conditions such as carcinoma, lymphoma, tuberculosis or infection. Blood stained fluid usually due to traumatic tap, peritoneal carcinoma, and ascitic fluids, which remain homogeneous blood stained fluid throughout the tap and could indicate malignancy, pancreatitis, intestinal infarction and tuberculosis (Runyon *et al*., 1988). Bloody fluid may indicate tumor or trauma. Bile-stained fluid may indicate gallbladder problems. High specific gravity and high protein content are indicative of vascular damage and leakage of plasma protein as in peritonitis.

b) Microscopic examination

Smear for cytology should be prepared from sample with anticoagulant. Direct smears of the anticoagulant fluid can be made if it is flocculent or turbid; however smear made from the sediment of centrifuged sample of the fluid is preferred, particularly if the fluid is clear or hazy. Cytology of the fluid stained with Diff-Quik or other Romanovsky type stains is performed immediately to determine if there is an obvious cause for the effusion and additional unstained smears can be prepared and submitted with original fluid to a laboratory. The slide is examined at low power followed by high power oil objective lens. The predominant cell type present should be noted, as well as the distribution of other cell types, and the presence or absence of other cell types. Cells seen in effusions are those encountered in blood films or other types of cytological specimens, additionally, mesothelial cells that lines the body cavities are often present.

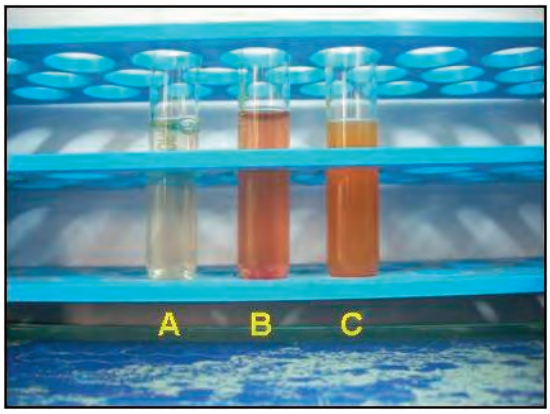

Fig. 2. Peritoneal fluid A) Transudate B) Modified transudate C) Exudate.

i) Neutrophils

Normal neutrophils (Fig. 3) appear much as they do in peripheral blood. Neutrophils are usually well preserved (non-degenerate) in non-septic inflammatory lesions. As the neutrophils age, the nuclei become hyper segmented and eventually pyknotic. In septic inflammatory lesions, the neutrophils undergo rapid degeneration and eventual rupture. This cellulitic process is called karyolysis and is characterized by nuclear swelling in which the nucleus become pink staining and smudged in appearance.

ii) Lymphocytes

Normal lymphocytes appear much as they do in peripheral blood. Immature lymphoid cells (lymphoblasts) are characterized by their large size and the presence of nucleolus.

iii) Plasma cells

Plasma cells are similar in size to small lympho-

cytes, but the nuclear chromatin is denser, the cytoplasm is blue and abundant and a peripheral clear area (Golgi apparatus) is usually apparent.

iv) Eosinophils

Eosinophils appear similar to eosinophils in peripheral blood and are associated with various types of lesions, including allergic inflammation, parasitic inflammation, eosinophilic granuloma, collagen necrosis, and mast cell tumors.

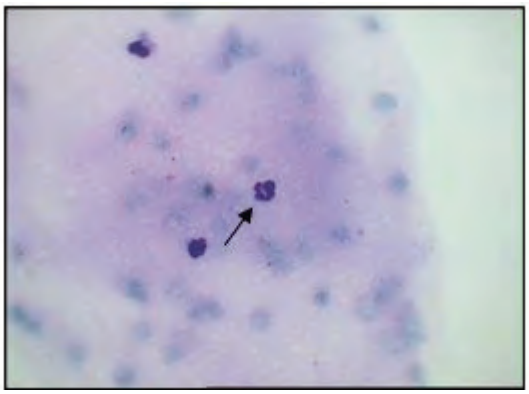

Fig. 3. Normal neutrophils (arrow) from peritoneal fluid of dog. Wright-Giemsa stain, original magnification x1000.

v) Mast cell

Mast cell may be present in low concentration in many types of inflammatory disorders, but if present in high concentrations, mast cell neoplasia may be suspected. Mast cells are round cells with round to oval nucleus and cytoplasm that contains purple granules.

vi) Macrophages

Macrophages (Fig 4) in tissue are derived from blood monocytes. Macrophages have a round to oval nucleus that may contain an apparent nucleolus, and have light blue, usually vacuolated cytoplasm.

viI) Mesothelial cells

Mesothelial cells (Fig. 5) tend to proliferate and exfoliate when fluid accumulates in the body cavity. They may appear singly or clusters of 2, 4, 8 or 16 cells. They are large with light to dark basophilic cytoplasm, and have single or multiple, round to oval nuclei with one or more nucleoli. The cytoplasmic border may appear to have a pink "fringe" round it. Most helpful in differentiating mesothelial cells from carcinoma cells is the sheer number of cells, as well as the size of clusters. When carcinoma cells are exfoliating, large numbers are usually observed, and cluster of up to 100 cells may be observed.

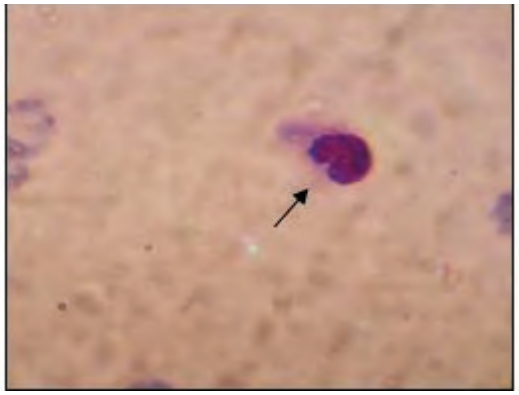

Fig. 4. Normal macrophage (arrow) from peritoneal fluid of dog. Wright-Giemsa stain, original magnification x1000

viii) Microorganisms

Bacteria stain blue with Romanowsky stains, and must be distinguished from background protein and stain sediments. They are usually somewhat uniform in size, present within the cytoplasm of neutrophils, and if present in large numbers, may be both free and phagocytosed. Even the presence of small number of bacteria is significant.

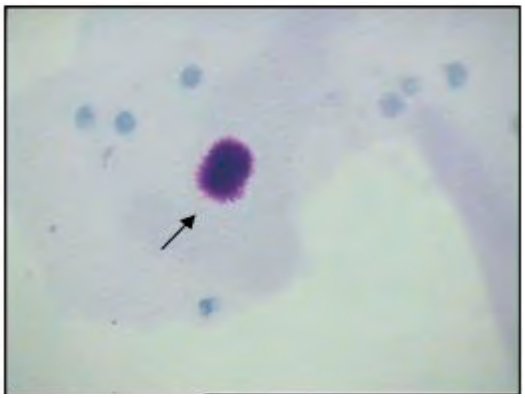

Fig. 5. Mesothelial cells (arrow) from peritoneal fluid of a ascetic dog. Wright-Giemsa stain, original magnification x1000

Clinical significance

An increase in total white cell counts of the fluid including a disproportionate number of polymorph nuclear cells indicates acute inflammation, which may have an infectious origin or else be sterile. An increase in mononuclear phagocytes from the peritoneum is an indication of chronic peritonitis. If neutrophil is predominant, the inflammation is considered suppurative (purulent). If neutrophils are

degenerative (Fig. 6), sepsis should be considered, and the slide to be examined carefully for the presence of microorganism. If both neutrophils and monocytes are present inflammation is considered of mixed origin. Degenerative toxic neutrophils suggest probability of infection being present. An increase in number of mesothelial cells with the distinctive presence of actively dividing mitotic figure suggests neoplasm. Bacteria found as phagocytosed inclusions of leukocytes or by culture of fluid, indicate an infective peritonitis which may arise by haematogenous spread in which case infection is likely to be specific one. If there is a leakage from peritoneal abscess, the same comment implies, but if there is leakage through segment of devitalized or perforated bowel wall there may be presence of mixed infection with the possibility of particulate matter from bowel contents. Highly increased white blood cell counts may indicate peritonitis or cirrhosis. Ascites of non inflammatory origin due to inadequate cardiac function may depict red blood cells, neutrophils, mesothelial cells and macrophage in the absence of bacteria. Cytological analysis of the abdominal fluid revealing mixed inflammatory exudates with numerous calcareous corpuscles (clear, yellow gold, round to oval structures) is typical of mesocestoides species infection (Parker, 2002).

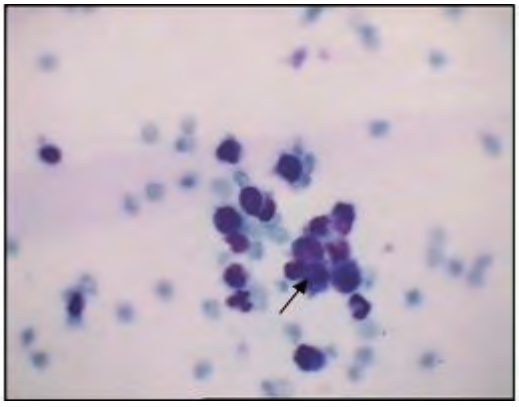

Fig. 6. Degenerated neutrophils (arrow) from peritoneal fluid of a ascitic dog. Wright-Giemsa stain, original magnification x1000

Biochemical test:

Total protein, albumin (Gupta *et al.*, 1995), glucose, triglycerides, amylase, bilirubin, lactate dehydrogenase (Garrett *et al*., 2004), urea, creatinine, cholesterol, adenosine deaminase (Alleman, 2003; Tarn and Lapworth, 2010) depicts variation in peritoneal effusion. Lipase activity in dogs that develops ascites is useful in complementing the diagnosis of acute pancreatitis. In dogs, the diagnostic accuracy of the peritoneal fluid lactate concentration and the blood to fluid lactate variation in differentiating septic peritoneal effusion was 95% and 90%, respectively (Levin *et al*., 2004). Amylase estimation is useful in pancreatic or perforative disease diagnosis while glucose level (<50 mg/dl) or increased LDH may be observed in secondary peritonitis (Garrett *et al.*, 2004).

Serum ascitic albumin gradient (SAAG)

Serum ascitic albumin gradient is calculated by subtracting the albumin concentration of the ascitic fluid from the albumin concentration of a serum specimen obtained on the same day. SAAG used to differentiate ascitic fluid into two main categories i.e. patient with ascites related to portal hypertension have a SAAG ≥ 1.1 g/dl e.g. cirrhosis, alcoholic hepatitis, CHF, massive liver metastasis, fulminant liver failure, portal vein thrombosis etc. Ascites associated with normal portal pressure have a SAAG < 1.1 g/dl e.g. peritoneal carcinomatosis, TB, pancreatic ascites, biliary ascites, nephrotic syndrome, bowel obstruction or infarction etc. (Hoefs, 1983; Beg *et al*., 2001). Specifically, higher serum effusion albumin ratios are associated with portal hypertension, and lower ratios are more typically associated with disorders of vascular leakage and inflammation (Pembleton-Corbett *et al*., 2000).

Based on above said analysis peritoneal effusions are broadly categorized as transudate, modified transudate, exudates, hemorrhagic effusion or neoplastic effusion. Exudates are further classified to septic or nonseptic exudates.

Transudate are effusions of low protein content and they are typically clear and colorless with protein concentration of less than 2.5 g / dl and less than 1000 nucleated cells/ml (Table 1). Cytologically these fluid contain mostly mononuclear cells such as lymphocytes, macrophage and mesothelial cells with lower number of non-degenerate neutrophils (Alleman, 2003). Burgess (2004) reported that transudate resulting from hypoalbunemia alone usually have plasma albumin concentration to be less than or equal to 1.0g /dl. If portal hypertension were present with liver disease, transudate may accumulate when albumin concentrations are greater than 1.0 g/dl. Modified transudates (James *et al.*, 2008) are effusion that often clear and colourless

like pure transudate and it may be slightly turbid to pink or opaque and white depending on the etiological factors. The protein content ranges from 2.5 –5.0g /dl (Table 1) with total nucleated cell count of 1000- 8000 cells/ml and cytological examination may reveal mononuclear nucleated cells, either macrophages or lymphocytes and even combination of both (Shelly, 2001). Exudates may vary in colour from white to amber pink, but they are usually turbid in nature. The protein content usually high (more than 3 g/dl) and cell counts are typically higher than 3000 cells/ml (Table 1). But in case of uroperitoneum exudates, low cell count and protein may depict due to accumulation of urine in the body cavity (Coldric, 2008). Classification of peritoneal effusion helps in the diagnosis of different pathological condition in dogs (Table 2).

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Table 1. Peritoneal fluid classification and characteristic in dogs

Effusion Type	Color/Turbidity	Total protein (g/dl)	Specific Gravity	WBC (/µL)	Predominant cell type (s)
Transudate	Colorless/Clear	<2.5	<1.017	<1,000	Mesothelium, Mononuclear phagocytes
Modified Transudate	Light yellow to medium yellow/clear	>2.5	<1.017 - 1.025	>1,000	Mononuclear
Exudates	Medium yellow to tan/cloudy	>3.0	>1.025	>5,000	Neutrophils (non-septic: nondegerate, septic: degenerate)

Table 2. Peritoneal effusion in various disease condition

Classification	Various Disease Conditions
Transudate	• Liver cirrhosis, Portal hypertension, Congestive heart failure, Hepatic vein obstruction, Nephrotic syndrome, Congestive • pericarditis, Hepatic vein obstruction, Nephrotic syndrome, • Constructive pericarditis, Inferior vena cava obstruction, Protein • losing enteropathy/nephropathy, Intestinal parasitism, • Lymphangiectasia, Hypoalbuminaemia
Modified Exudate	• Congestive heart failure, Lung atelectasis, thoracic pericardial • effusion-trauma, Partial/complete obstruction of caudal vena cava • or hepatic vein, Intra hepatic portal hypertension, Neoplasia
Exudate	• Pancreatitis, Neoplasia, Septic peritonitis, Infections (bacterial, • virus, fungi and protozoa), Bile peritonitis, Haemoperitoneum

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