Abdominal Fluid Collection and Analysis Made Easy
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Introduction
Dogs and cats often present to veterinary clinics because of the appearance of a pendulous or enlarged abdomen or for clinical signs secondary to abdominal fluid accumulation. Abdominal fluid can accumulate from a variety of disease processes including many serious conditions that require immediate intervention. Sometimes the condition that led to the accumulation of abdominal fluid is obvious, such as the collapsed elderly dog with the history of a large splenic mass, presumably ruptured and causing hemorrhage. Conversely, the underlying condition or cause of fluid accumulation is not always apparent. For example, a dog that was hit by a car could develop abdominal fluid because of a splenic or liver tear (hemoabdomen) or a bladder rupture (uroabdomen). In that situation, the treatment required differs depending on the underlying cause. In order to accurately determine the etiology of the effusion and advise the owner about the treatment options and prognosis for their pet, a sample of the fluid should be obtained by abdominocentesis and analysis of the fluid performed. Sometimes the analyses are easy and can be performed in-hospital, while other times cytologic assessment by a clinical pathologist is warranted. In any scenario, the collection and analysis of abdominal fluid will give a significant amount of information to help guide the management of these patients.

Normal Abdominal Fluid Physiology
A small amount of abdominal fluid is normally present to allow for lubrication between abdominal organs during movement. Normal abdominal fluid is a low protein, serous ultrafiltrate of blood that flows out of arteriolar capillaries into the abdominal cavity and is mostly reabsorbed by the venous capillaries. The portion that is not reabsorbed, approximately 10%, is resorbed by the lymphatic system. The rate of fluid that exists within the abdominal cavity is largely determined by Starling’s forces, which are the hydrostatic and oncotic pressures within the blood vessels and body cavity. The degree of lymphatic drainage, as well as mesothelial and endothelial permeability, also dictate the amount of abdominal fluid accumulation. Mesothelial and endothelial cells line the abdominal wall and abdominal organs and the permeability across the surfaces are affected by hormones and cytokines released during different disease processes.

Abdominal Fluid Pathophysiology
Abdominal fluid accumulates when the rate of fluid filtration into the abdominal cavity exceeds the rate of fluid resorption from the abdominal cavity. The rate of abdominal fluid accumulation is increased with increased capillary hydrostatic pressure (e.g., heart failure), decreased capillary oncotic pressure (i.e., hypoalbuminemia), increased endothelial permeability (e.g., peritonitis), and decreased lymphatic drainage (e.g., lymphatic obstruction). If abdominal fluid accumulation is severe, abdominal compartment syndrome can occur resulting in abdominal organ dysfunction (e.g., kidney failure). Abdominal fluid is resolved by restoring the pressure balance, lymphatic drainage, and permeability of the endothelium and mesothelium.
Physical Examination

It is important to perform a complete physical examination on all patients with confirmation or suspicion of abdominal fluid. Signs of abdominal fluid can be obvious including a distended or taught abdomen or palpation of a fluid wave. However, some patients will present with severe systemic illness (e.g., collapse, shock) secondary to blood or fluid loss, which leads to a suspicion of abdominal fluid accumulation. Findings from the complete physical exam might help to determine the underlying etiology of the abdominal fluid. For example, lymph node palpation might detect lymphadenomegaly and increase the suspicion of lymphosarcoma. Alternatively, if a heart murmur is detected and the jugular veins appear distended, the abdominal fluid might be secondary to congestive heart failure. Conversely, if the abdomen is painful during palpation, this suggests that the abdominal fluid is likely secondary to a primary abdominal condition such as a ruptured gastrointestinal tract or severe pancreatitis.

Diagnostic Imaging

Abdominal radiographs are often the first diagnostic imaging modality chosen in patients suspected to have abdominal effusion, probably because radiography is an available modality for most practitioners. If performed, abdominal radiographs must be adequate to assess all intra-abdominal structures so that the abdomen can be thoroughly investigated for the presence of masses, intestinal obstructions, organ enlargement, absent abdominal organs (e.g., non-visible urinary bladder), or free peritoneal gas (i.e., ruptured abdominal viscous). These signs, when seen with concurrent evidence of abdominal fluid such as loss of abdominal serosal (organ) margin detail, can assist in determining the cause of the abdominal fluid.

When abdominal fluid is suspected, ultrasound can be a secondary diagnostic modality used to confirm the presence of the fluid and assist with obtaining a sample for analysis. Focused assessment using sonography for trauma (FAST) is a simple and rapid ultrasound examination that can be performed to detect free fluid. Veterinarians with limited previous ultrasound experience can perform a FAST examination in less than five minutes. It can be used with a scoring system (score 0 to 4 out of 4) to evaluate for the presence of fluid in four areas. With the patient in right lateral recumbency, use the ultrasound probe to assess for fluid (anechoic area around organs) in the following locations:

1) Diaphragmatico-hepatic (DH) view – just caudal to the xiphoid process
2) Spleno-renal (SR) view – left flank region
3) Cysto-colic (CC) view – on the midline over the urinary bladder
4) Hepato-renal (HR) view – right flank (most dependent) region

During the FAST examination, the urinary bladder and gall bladder should also be visualized. Note that even though an organ can be seen on radiographs or ultrasound and appear intact, organ rupture can still be present. FAST can be performed on initial presentation and serially thereafter to monitor for increases in abdominal fluid (i.e., increasing abdominal fluid score). In patients with lower abdominal fluid scores (1 or 2 out of 4), the DH and CC views are the most likely to reveal fluid.

Diagnostic Abdominocentesis

An abdominal fluid sample is generally accomplished by blind abdominocentesis, four-quadrant abdominocentesis, or ideally, ultrasound-guided abdominocentesis. With the patient in right lateral recumbency, the area around the umbilicus should be clipped and steriley prepped. When performing blind abdominocentesis, the ideal site is 2-3 cm caudal to the
umbilicus and 2-3 cm from midline in the dependent region. With the patient in right lateral recumbency, accidental puncture of the spleen is less likely in this region. Standing abdominocentesis is not recommended, as it increases the chance of splenic puncture. Abdominocentesis should be performed using a needle (20-22G) and syringe (3-6 cc) advanced perpendicular to the skin. This closed technique is preferable to open techniques (using needles without attached syringes), which introduce air into the abdominal cavity thus making additional diagnostic imaging tests more difficult to interpret.

Once the needle is through the skin, apply suction on the syringe while advancing through the subcutaneous tissue and abdominal wall. Fluid should enter the syringe as soon as the abdominal wall is penetrated, if abdominal effusion is present. Alternatively, suction can be performed intermittently during advancement. If single blind abdominocentesis is unsuccessful, four-quadrant abdominocentesis can be performed by repeating the blind abdominocentesis in areas 2-3 cm cranial and caudal to the umbilicus and lateral to midline (in a total of 4 quadrants). Finally, if ultrasound is available, the ideal method of abdominocentesis is ultrasound-guided into a pocket of fluid (i.e., anechoic region).

**Abdominal Fluid Diagnostic Tests**

Once an abdominal fluid sample is obtained, it can be placed into an EDTA (lavender top) tube, serum (red top) tube, and additional sterile (red top or other) tubes. Samples might need to be prioritized depending on the volume of fluid obtained and the suspected underlying disease. If the sample appears to be blood, it should be placed in a serum (red top) tube to evaluate for clotting. If the fluid was obtained from the abdominal cavity, it should not clot unless the hemorrhage is active/ongoing or the fluid has a high fibrinogen concentration. If the fluid was obtained from an accidental splenic or other vascular puncture, it will clot, similar to a venous sample.

**In-Hospital**

Fluid collected in EDTA tubes can have the PCV and total solids (TS) measured using hematocrit tubes and a refractometer. This is indicated immediately if the fluid appears hemorrhagic or serosanguinous. Unfortunately, some EDTA tubes contain additives that falsely increase the TS concentration. Similarly, if only a small amount of fluid was added to the tube, the PCV might be diluted. Therefore, these measurements can also be performed on the fluid in the syringe (prior to putting it into a tube) to avoid erroneous interpretation. Fluid turbidity from lipids, hemolysis, or cellular debris and refrigeration of fluid samples can also falsely increase the TS measurements. Therefore, turbid samples can be centrifuged and the TS measured on the supernatant. Analyses should always be performed on fluid samples at or near room temperature. Smears prepared for cytology (microscopic assessment) can be made directly from the fluid in the EDTA tube, especially if the fluid appears flocculent or turbid. However, if the fluid is clear or hazy, the tube should be centrifuged and smears made from the sediment of the sample. The smears can be stained with Diff-Quik or other Romanovsky-type stains for analysis.

Fluid collected into serum (red top) tubes can be used for measurement of total protein, albumin, bilirubin, creatinine, potassium, triglyceride, glucose, lactate, and lipase concentrations using an in-house laboratory or handheld device, if available. These measurements can be helpful in determining the underlying disease process, especially in comparison to the concurrent peripheral blood (serum, plasma) measurements. Note that a delay in sample
processing can affect the results; fluid glucose levels will decrease and lactate levels increase as samples sit in the tube.

**Send-Out**

Fluid collected in EDTA tubes can be submitted for RBC count, total nucleated cell counts (TNCC), cytology (microscopic review by a clinical pathologist), or other advanced analyses if indicated (e.g., flow cytometry [neoplasia], PCR [feline infectious peritonitis]). Smears can be prepared (as outlined above) and unstained smears can also be submitted with the EDTA tube fluid to the laboratory. Preparation of slides at the time of sample collection is ideal to reduce artifactual changes in cell morphology.

Fluid samples collected into other sterile tubes (preferably red top or culture tubes) can be stored for aerobic and anaerobic bacterial, mycoplasma, and fungal cultures. Anaerobic cultures should not be refrigerated and are ideally processed within 24 hours of collection. Culture results are typically not available for 48-72 hours due to the length of time required to grow the organisms. Purple top tubes should not be submitted for culture as the EDTA is bacteriostatic.

**Classification and Etiology of Abdominal Effusions**

Abdominal fluid is traditionally categorized by its protein concentration and cellularity (TNCC). Unfortunately, there is some overlap between the categories used (Table 1), which usually necessitates additional analysis of the fluid. However, when combined with the patient’s clinical picture, this information might be helpful in determining the etiology of the abdominal fluid, especially in patients with pure or modified transudates. For patients with exudates or more highly cellular fluids, cytology will help determine the predominant cell type(s) and underlying etiology. For example, exudates are often primarily composed of neutrophils; if the neutrophils appear degenerate, the smear should be thoroughly assessed for evidence of bacteria (intra-cellular), which would indicate a septic effusion. If bacteria are not readily seen, other laboratory tests can be performed in-hospital (i.e., lactate, glucose measurements) to determine the likelihood of the effusion being septic. Additionally, there are many useful diagnostic techniques to differentiate non-septic effusions, depending on the suspected underlying etiology.

**Septic effusion** is typically the result of a ruptured gastrointestinal tract or penetrating wound to the abdomen. Patients with a septic effusion require immediate intervention including antibiotic administration and an exploratory laparotomy. Therefore, diagnosis of a septic effusion must happen as soon as possible. The TNCC for septic abdominal fluid is usually > 13.0 × 10⁹/L. If intra-cellular bacteria are not seen, additional tests can be performed to increase the index of suspicion of infection, including measurement and comparison of abdominal fluid and peripheral blood lactate and glucose concentrations. Specifically, if a dog’s abdominal fluid lactate is 1.5 mmol/L higher than the concurrent peripheral blood lactate or if the dog’s abdominal fluid glucose is more than 1.1 mmol/L less than the concurrent peripheral blood glucose concentration (without IV dextrose supplementation), this suggests that the effusion is septic. Unfortunately, these measurements are unreliable in cats, making cytology still the best test if a septic effusion is suspected.

**Non-septic effusions** include abdominal fluid resulting from pancreatitis, feline infectious peritonitis (FIP), hemorrhage, chyle, neoplasia, or a ruptured abdominal viscous.

**Pancreatitis** causes a non-septic suppurative inflammation that can have degenerate or non-degenerate neutrophils. It is often diagnosed in combination with clinical signs and
ultrasound examination findings. A greater than 4-fold increase in abdominal fluid lipase compared to the upper end of the reference interval or more than twice the peripheral blood lipase activity suggest pancreatitis, in a patient that has not experienced trauma.

_Feline infectious peritonitis_ (wet form) causes a non-septic effusion. The total protein of the fluid is typically 35-45 g/L and an effusion total protein ≥ 80 g/L is 90% specific and 55% sensitive for FIP. The TNCC is usually low (2.0 – 6.0 × 10⁹/L). Unfortunately, FIP is very difficult to definitively diagnose and is typically a diagnosis of exclusion (i.e., ruling out bacterial infection and neoplasia). Abdominal fluid feline coronavirus antibodies (1:1600), gamma-globulin concentrations > 10 g/L, and albumin-globulin ratios ≤ 0.9 can be diagnostic but with only 75-85% sensitivity and specificity.

_Hemorrhagic_ effusions typically have a PCV > 10% and can appear hemolyzed. If the cytology is consistent with peripheral blood including platelets and no erythrophagocytosis, then inadvertent splenic aspiration, venipuncture, or acute severe hemorrhage should be suspected. Hemorrhagic abdominal fluid is typically secondary to blunt trauma (e.g., hit by car, fall from height), coagulopathies (e.g., anticoagulant rodenticides), or neoplasia (e.g., ruptured splenic hemangiosarcoma). If there is no history of trauma, coagulation testing and diagnostic imaging should be performed to differentiate coagulopathies and neoplasia. Unfortunately, cytologic evaluation of hemorrhagic effusions is usually of low diagnostic yield due to hemodilution.

_Chylous_ effusions are typically milky or opaque and result from reduced lymphatic drainage from the gastrointestinal tract into the cranial vena cava. Chylous effusions are characterized by an abdominal fluid triglyceride concentration > 1.1 mmol/L, abdominal fluid triglyceride concentration greater than the concurrent serum triglyceride concentration, or abdominal fluid cholesterol concentration less than the abdominal fluid triglyceride concentration. Chylous abdominal fluid can be the result of trauma, obstructed lymphatic drainage (secondary to masses), heart failure, or idiopathic.

_Neoplastic_ effusions are caused by exfoliation of neoplastic cells into the abdomen, most typically due to carcinomas, mesotheliomas, and round cell neoplasms (e.g., lymphoma, mast cell tumors, malignant histiocytes). Lymphosarcoma is one of the more common forms of neoplasia in small animals that can cause neoplastic abdominal effusions and is typically characterized by a monomorphic population of immature or atypical lymphoid cells. These are usually larger than neutrophils and have a moderate amount of clear-blue cytoplasm, variably shaped nuclei with prominent and sometimes bizarre/angular nucleoli, and finely stippled nuclear chromatin. Clinical pathologists are often adept at diagnosing lymphosarcoma based on cytology, but additional diagnostic tests such as flow cytometry or PCR for analysis of antigen receptor rearrangements (PARR) can also be helpful and require an abdominal fluid sample stored in an EDTA (lavender top) tube.

A ruptured abdominal viscous can result in a septic effusion (described above), uroabdomen, or bile peritonitis. When urine leaks from the urinary tract and into the abdomen, the resulting abdominal fluid is initially classified as a pure transudate. However, as inflammation progresses, especially if the urine is infected, neutrophils will accumulate. Creatinine, urea, and potassium are all concentrated in the urine compared to the peripheral blood, but because urea is a small molecule and easily passes across the mesothelium of the abdomen, abdominal fluid to peripheral blood creatinine or potassium ratios are recommended for diagnosing a uroabdomen. An abdominal fluid to peripheral blood ratio of creatinine > 2.0 or potassium > 1.4 is consistent with a uroabdomen.
Bile peritonitis is an inflammatory response to bile in the abdominal cavity, which usually occurs secondary to trauma, cholangitis, obstruction of the bile duct, or surgery (iatrogenic). Septic bile peritonitis results from the leakage of contaminated bile, which increases the severity of inflammation and has a worse prognosis. Bile peritonitis should be suspected when the abdominal fluid obtained from a patient is green, orange, or yellow-tinged, or the patient has a history of gall bladder disease, pancreatitis, trauma, or cholelithiasis. Cytology of bile peritonitis effusion often reveals gold, green, or black-brown pigment within macrophages or free in the background; however, measurement of fluid bilirubin concentrations is necessary to confirm the diagnosis. An abdominal fluid concentration that is more than 2-fold the peripheral blood bilirubin concentration is diagnostic. Cytologic evaluation for bacterial organisms should also be performed since septic bile peritonitis has a poorer prognosis for recovery.

<table>
<thead>
<tr>
<th></th>
<th>Pure Transudate</th>
<th>Modified Transudate</th>
<th>Exudate</th>
<th>Chyle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colour</strong></td>
<td>Transparent – straw yellow</td>
<td>Transparent – yellow – reddish</td>
<td>Yellow – red</td>
<td>White – pink Cloudy</td>
</tr>
<tr>
<td><strong>Protein (g/L)</strong></td>
<td>≤ 25</td>
<td>25-75</td>
<td>≥ 30</td>
<td>≥ 25</td>
</tr>
<tr>
<td><strong>Total nucleated cell count (TNCC) × 10⁹/L</strong></td>
<td>≤ 1.5</td>
<td>1.5 – 7.0</td>
<td>&gt; 7.0</td>
<td>0.5 – 20.0</td>
</tr>
<tr>
<td><strong>Predominant cells</strong></td>
<td>Rare monocytes and mesothelial cells</td>
<td>Variable (monocytes, lymphocytes)</td>
<td>Polymorphonuclear neutrophils (PMNs) (possibly degenerative)</td>
<td>Mature lymphocytes, PMNs, macrophages</td>
</tr>
<tr>
<td><strong>Common causes</strong></td>
<td>Hypoalbuminemia, cirrhosis, portal hypertension</td>
<td>Heart failure, vasculitis, diaphragmatic hernia, portal hypertension</td>
<td>Bacterial or fungal infection, neoplasia, FIP, pancreatitis</td>
<td>Trauma, lymphatic obstruction, heart failure, idiopathic</td>
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