



Practical Techniques for Emergency Patients Mini Series

Session Two: Emergency Diagnostic Procedures

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Practical Techniques in the Emergency Room: Emergency Diagnostic Procedures

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Following on from the previous webinar where we covered initial patient assessment, we will now focus on diagnostic procedures that may be used to assess emergency patients, detect life-threatening and/or management changing abnormalities and assess response to treatment.

Emergency bloodwork

A minimum data base (MDB) should be obtained from all emergency patients. This is a simple, two-minute, rapid assessment that consists of measurement of a patient's:

1. Packed cell volume (normal 35-45 %)
2. Total solids (normal 6.0-8.5 g/dL)
3. Glucose
4. Azostix (BUN)

Only a small volume of blood is required and may even be collected from the hub of an IV catheter during catheter placement. A MDB is a cheap way of improving patient care, allows the clinician to recognise how sick the patient is, and may also help to fine tune differential diagnoses for the owner to help guide decision making. With a MDB it is possible to rapidly diagnose kidney injury, diabetes mellitus, diabetic ketoacidosis, anaemia, severe dehydration, severe hypovolaemia, blood loss, or disease such as protein losing enteropathy (PLE) or nephropathy (PLN) immediately.

Using the following chart, assessment of the PCV and TS on presentation, prior to any fluid therapy, may help refine the most likely differential diagnoses and hence most useful treatment.

Change in PCV and TS	Common possible causes
Decreased PCV, normal TS	Haemolytic anaemia, aplastic anaemia, pure red blood cell aplasia, anaemia of chronic disease
Increased PCV, normal TS	Polycythemia vera, hyperthyroidism, Cushing's disease, HGE, renal EPO-producing tumour
Normal PCV, decreased TS	PLE, PLN, liver failure, acute blood loss, third spacing
Normal PCV, increased TS	FIP, multiple myeloma, chronic globulin stimulation, severe dehydration and anaemia (CKD), lipaemic serum
Increased PCV, increased TS	Haemoconcentration
Decreased PCV, decreased TS	Chronic blood loss (melena), blood loss (subacute)

One of the most useful outcomes of measurement of the PCV and TS is the early detection of patient with internal bleeding that not is immediately apparent from patient assessment. The presence of a low TS with a normal PCV in a patient with signs of shock is strongly suggestive of acute bleeding in dogs. The reason for this is that, in the presence of early bleeding in dogs, splenic contraction releases pooled red blood cells into the circulation thereby preventing a drop in PCV initially. There is no such pool of plasma proteins to be released and so the TS will drop first. With ongoing bleeding, this compensatory mechanism is depleted and the PCV will drop in conjunction with the TS. Any dog presenting with a TS < 60g/L without a known underlying cause should be rechecked (major body system assessment and repeat PCV and TS measurement) to catch any internal bleeding before it becomes life-threatening.

Another benefit to the assessment of PCV and TS is that the colour of the serum in the haematocrit tube may be informative and diagnostic without having to wait for a full biochemical analysis. Any indicator of haemolysis, icterus or lipaemia can add to the clinical picture. For example, a haemolysed sample in a dog with a regenerative anaemia would be suggestive of IMHA and warrant a saline agglutination test to be performed for confirmation of the diagnosis. A severely lipaemic serum for a dog presenting with acute vomiting is suggestive of pancreatitis. An anorexic cat presenting for lethargy, fever and vomiting with icteric serum would likely be investigated specifically for underlying liver disease (cholangiohepatitis, triaditis etc). In any severely hyperglycaemic patient, the serum in the haematocrit tube may be used to evaluate for the presence of serum ketones (using a urine dipstick), yielding an easy diagnosis of diabetic keto(acid)osis from a drop of blood. The presence of a marked buffy coat is consistent with a marked inflammatory, infectious or neoplastic condition and warrants examination of a blood smear to characterise this further.

A saline agglutination test may be performed by mixing one drop of blood, either fresh or from an EDTA sample, with one drop of saline on a slide. The slide should be examined with the naked eye for any evidence of agglutination, and subsequently under the microscope to differentiate between the random clumping of agglutination (consistent with immune-mediated disease) and the stacking seen in rouleaux.

Measurement of the blood glucose as part of the MDB will reveal the presence of euglycaemia, hypoglycaemia or hyperglycaemia. It should be checked in any patient with general malaise and any juvenile or paediatric patient regardless of the presenting complaint. Common differential diagnoses for marked hyperglycaemia include diabetes mellitus, stress hyperglycaemia in cats, inappropriate dextrose supplementation, hypovolaemia and traumatic brain injury (TBI). The patient history should be checked for historical changes in weight, appetite, and polyuria/polydipsia for supportive findings consistent with diabetes mellitus. Stress hormone release (cortisol, epinephrine) in severe hypovolaemia and TBI can lead to marked transient elevations in blood glucose – the so called ‘stress of dying’. The presence of hyperglycaemia in the absence of probable diabetes mellitus should prompt the clinician to reassess the cardiovascular and neurologic systems for evidence of compromise which should be addressed promptly.

Hypoglycaemia may be life-threatening but rapidly, cheaply and easily corrected in the short term. Adult patients should never become hypoglycaemic from malnutrition alone except in extreme cases of prolonged starvation, and an underlying cause sought in all cases. Possible causes include sepsis, insulinoma or exogenous insulin overdose, hunting dog hypoglycaemia, neoplasia (typically liver in origin, that may be benign or malignant), juvenile patient hypoglycaemia or laboratory error. Polycythemia may also cause an artificial hypoglycaemia due to increased cellular metabolism of glucose by the increased red cell mass in vitro. True hypoglycaemia should be treated with a meal (provided this is not contraindicated – such as in the presence of vomiting, or an increased risk of aspiration pneumonia from an altered mentation etc), or by administration of an IV dextrose bolus (0.5-1.5 ml/kg of a 50% dextrose diluted 1:2 with saline) followed by a CRI of 2.5-5% dextrose in the maintenance IV fluids.

Measurement of glucose may also be useful in cases with suspected thromboembolic disease to help confirm a diagnosis. A blood glucose measured from an affected limb will be lower than that of a normal limb. Although most cats with a saddle thrombus do not present a diagnostic challenge, measurement glucose may be helpful in cats with isolated forelimb lameness where thromboembolic disease is a consideration.

An Azostix provides a gross estimate of BUN and so may also be used to measure preliminary renal function. The dipstick provides a semi-quantitative measure of blood BUN levels although does not differentiate from pre-renal, renal or post-renal causes of azotaemia.

An abnormal result would justify measurement of further blood work to assess creatinine, and a urine sample should be collected to assess urine specific gravity prior to any fluid therapy to help determine the ability to concentrate urine appropriately. The Azostix can provide a useful 'heads-up' and can expedite investigation of the correct body system.

Blood smear evaluation is another useful in-practice test that can cheaply provide valuable information on how sick a patient is and the likely underlying causes of their presenting complaint. The blood smear may be a stand alone diagnostic or may be used to complement a complete blood count. In order to make a good blood smear, very little equipment is needed (aside from practice in how to create a good smear, immersion oil and a microscope with 100 x magnification).

The technique is as follows:

1. On one slide (the sample slide) place a small drop of blood using a haematocrit tube
2. Use the second slide as a spreader slide
3. Place the end of the spreader slide on the sample slide so that the short edge of the spreader is just below the drop of blood
4. Hold the spreader slide at an angle of 30° to 45° (relative to the sample slide) and bring the spreader back against the drop of blood so that the blood spreads in a thin line via capillary action
5. Rapidly, but gently, drag the spreader along the entire length of the sample slide in a single fluid motion
6. If the technique was performed correctly, the smear should end before the end of the sample slide in a 'feathered edge'
7. Air dry the sample
8. Fix and stain the slide using a Romanowsky type stain such as Diff-Quik

Once the blood smear is made, it should be examined for abnormalities in all cell lines.

- At low power (10 x ocular/objective) the quality of the smear can be assessed to see if it is of diagnostic quality. A subjective assessment of the number of WBCs, RBCs, cell distribution and morphology can be made (eg. platelet clumps, leukocyte clumps, agglutination).
- At a 40 x objective an approximate leukocyte count can be obtained by counting the number of leukocytes in at least 3 fields and the average per field obtained. This number can then be multiplied by 1500 to get the approximate total WBC count.
- At a 100 x objective using oil, the morphology of RBCs, WBCs and platelet can be assessed. To obtain an estimate of the platelet count, the number of platelets seen in at least 5 separate fields should be averaged, and this figure multiplied by 10,000-15,000 to get the approximate total number of platelets. A normal platelet count equates to approximately 8-15 platelets per high-powered (100 x) field. The presence of platelet clumps in the feathered edge of the smear is a common cause of thrombocytopenia being diagnosed falsely.

The techniques of making and assessing blood smears become easier with continued practice. It can be a very useful clinical option, and even alternative to the more-costly haematology assessment, in patients with financial constraints. In particular, a patient clinically suspected to have parvo-viral enteritis may be assessed for leukopenia, a patient receiving chemotherapy who is not doing well can be assessed for leukopenia and antibiotic therapy appropriately commenced early if their WBC count is < 1000-2000, and a patient presenting with epistaxis or other primary haemostatic bleeding can be assessed for thrombocytopenia.

Red blood cell morphology can provide useful information as to the cause of any anaemia. Indications of a regenerative anaemia include anisocytosis, polychromasia (polychromatophils), and nucleated red blood cells. Indications of a non-regenerative anaemia include microcytosis, hypochromasia and a lack of polychromasia in the face of an established anaemia. Whilst regeneration can be more accurately quantified by performing a reticulocyte count using a supra-vital stain, such as new methylene blue, this is not often an option in an emergency situation. The presence of spherocytes and/or ghost cells are strongly suggestive of immune-mediated destruction of red blood cells, and in conjunction with an anaemia and positive saline agglutination test, can confirm a diagnosis of immune-mediated haemolytic anaemia (IMHA).

As well as estimating total white blood cell numbers, white cell morphology can provide valuable information as to the underlying disease process present. Toxic change to neutrophils may be characterised by basophilia (blueish granulation) of the cytoplasm, a foamy appearance to the cytoplasm, swelling of the nucleus, and Dohle bodies. Band neutrophils are characterised as immature cells with an unsegmented, horse-shoe shaped nucleus, which are released before fully matured due to increased demand. Their presence in a blood smear is termed a left-shift, which can be regenerative (where the mature neutrophils are present in greater numbers than the band neutrophils), or degenerative (a low to normal total neutrophil number with > 10% band neutrophils. This is generally a poor prognostic indicator).

When looking at blood smears in practice it is useful to have access to images of the various cell morphologies that may be seen. A useful online resource is the free online veterinary clinical pathology textbook produced by Cornell University that may be found at www.eclinpath.com

Diagnostic approach to the patient with an acute abdomen

Animals with an acute abdomen are characterised primarily as having abdominal pain. As with any emergency patient, the basic principles of patient assessment using the ABCs and a major body systems assessment should be applied when initially presented with these patients. Stabilisation should then be performed to address any areas of concern. Once the patient is stable, a thorough diagnostic evaluation should be performed to determine the most likely underlying cause as soon as possible so definitive treatment can be commenced.

There are many differential diagnoses for the presenting complaint of acute abdominal pain, many of which require surgical for optimal patient outcome. If left untreated, many conditions could result in tissue necrosis and loss of function. The presence of an acute abdomen therefore requires prompt recognition and treatment of the underlying cause to minimise the risk of a more serious complication, such as septic peritonitis or systemic inflammatory response syndrome and multiple organ dysfunction.

Emergency bloodwork

Emergency clinical pathology may be useful in helping determine the underlying cause of the acute abdomen, or generate clinical concern for a surgical underlying cause. The PCV and TS should be assessed together as previously discussed. Parallel increases suggest haemoconcentration and fluid loss. A normal or increased PCV with a low to normal TS indicates protein loss from the vasculature, either from acute haemorrhage with splenic contraction, or protein loss from peritonitis. Haemorrhagic gastroenteritis (HGE) is associated with a higher PCV (typically 60% to 90%) and normal or low TS in dogs presenting with an acute onset of abdominal pain, vomiting and bloody diarrhoea.

Hypoglycaemia is often associated with sepsis and warrants an aggressive approach to find the underlying cause of the acute abdominal pain. Hypoadrenocorticism is another important differential diagnosis for abdominal pain and hypoglycaemia.

Reliable assessment of a blood smear may provide additional markers that may raise concern for a surgical cause. All cell lines should be systematically evaluated as previously described. Leukocytosis with a mature neutrophilia suggests an inflammatory or infectious process, although excessive numbers of any cell line may indicate neoplasia. Band cells indicate a more severe inflammatory or infectious process, and a concurrent low white blood cell or neutrophil count may represent severe consumption and a more severe disease process. The absence of a leukocytosis or left shift does not rule out an infectious or inflammatory process. Leukopenia may be due to decreased production or sequestration of white blood cells or a viral infection such as parvoviral enteritis.

A venous blood gas analysis may add additional cause for concern. The presence of a metabolic alkalosis with low chloride is strongly suspicious for an upper gastrointestinal obstruction and warrants immediate imaging to rule out foreign body and exploratory surgery. Other blood gas abnormalities tend to relate to perfusion abnormalities secondary to the underlying cause of the abdominal pain and have less diagnostic utility.

cPLI testing may be useful in the diagnosis of acute pancreatitis, especially where ultrasound is not available or practical to demonstrate consistent imaging findings. It is important to note however, that cPLI elevations or abnormalities do not exclude a concurrent surgical problem. A recent study showed cPLI elevations commonly occur in dogs with obstructive gastrointestinal foreign bodies and so reasonable efforts should be made to rule out surgical conditions, especially if medical management is failing.

AFAST (Abdominal Focused Assessment with Sonography for Trauma)

AFAST is a useful technique for evaluating the peritoneal cavity for free abdominal fluid that doesn't require extensive scanning skills. It is not an extensive examination of the internal abdominal organs and can typically be completed within 5 minutes. Free fluid detected in a patient with acute abdominal pain may be caused by haemorrhage (traumatic or neoplastic), uroabdomen, septic peritonitis or biliary peritonitis. Fluid may also represent non-surgical problems such as neoplasia, portal hypertension or hypoalbuminaemia. Any free fluid detected can subsequently be sampled to achieve a diagnosis.

The examination may be performed without clipping the fur and applying alcohol at the probe-skin interface, or by clipping a small 2 x 2 inch square of fur at each scanning site and using ultrasound gel at the probe-skin interface. The patient is positioned in right or left lateral recumbency. There are 4 standard views examined:

1. The subxiphoid or diaphragmaticohepatic view to evaluate the hepatodiaphragmatic interface, gallbladder region, pericardial sac and pleural space
2. The left flank or splenorenal view to assess the splenorenal interface and areas between the spleen and body wall
3. The midline bladder or cystocolic view to help assess the apex of the bladder
4. The right flank or hepatorenal view to assess the hepatorenal interface and the areas between the intestinal loops, right kidney and body wall.

The examination is usually completed using only the longitudinal view although adding the transverse view may be helpful if the initial results are equivocal. The ultrasound probe should be moved a few inches in several directions at each site, and fanned through an angle of 45 degrees until the target organs are identified to allow a greater area to be evaluated for the presence of free fluid.

The abdominal fluid score (AFS) technique can also be applied to record the number of sites (from 0 to 4) in which free fluid is detected with the animal in lateral recumbency. Progression in the underlying disease may be assessed objectively and monitored using this scoring system. Serial AFAST examinations should be performed every 2 to 4 hours, or more frequently as dictated by the clinical findings, such as difficulty stabilising the patient or a worsening haemodynamic status.

Abdominal radiography

Abdominal radiographs (2 views) should be performed in any animal that is presenting with abdominal pain to screen for surgical conditions. A thorough and systematic evaluation of the images should be performed, assessing the size, shape, density and location of all the abdominal organs. Attention should also be paid to extra-abdominal structures, and the retroperitoneal space which is often overlooked, looking for any loss of detail around the kidneys, a 'streaky' appearance or distention of the space which can all be evidence of fluid accumulation, a mass, or sublumbar lymphadenopathy. Subtle evidence of ureteroliths may also be apparent. The abdominal walls should also be checked for integrity to rule out herniation or rupture as may be seen with trauma.

Free gas in the peritoneal space (pneumoperitoneum) without prior abdominocentesis or recent abdominal surgery indicates either intestinal rupture or the presence of gas-producing organisms in the abdominal cavity and necessitates exploratory abdominal surgery as soon as the patient is stable. Other causes of pneumoperitoneum include gastric rupture with or without GDV, pneumocystography in the presence of a ruptured bladder, a ruptured vagina, or recent abdominal surgery. Free gas is most easily seen between the stomach and the liver, although small pockets may be detected throughout the abdomen where more detail than normal is seen. A horizontal beam radiograph may increase the sensitivity of radiography to detect free gas and is best performed with the patient in left lateral recumbency and focused on the least dependent area.

Plication of the small intestine may be seen with linear foreign body ingestion in dogs and cats and warrants surgical exploration where the surgeon needs to be prepared to perform multiple enterotomies and intestinal resection and anastomosis to remove all foreign material and devitalised intestine.

Signs of intestinal obstruction, another indication for exploratory surgery, includes segmental gas or fluid distension of the small intestine. Whilst there are several reported measurements confirming the presence of an obstructive pattern, a useful rule of thumb is:

- In cats, small intestinal loops should not exceed twice the height of the central portion of L4 vertebral body, or 1.2cm
- In dogs, all the intestinal loops should be of a similar diameter; it is abnormal for one segment to have a diameter 50% larger than other regions. The normal diameter is approximately 2-3 times the width of a rib, or less than the width of an intercostal space.

In many cases, the results of imaging can be equivocal and not definitive for the diagnosis of a surgical lesion. An approach to such cases is to repeat abdominal radiographs 3 hours later and look for repeatable focal intestinal distension which is consistent with obstruction. An upper GI contrast study with barium could also be considered as a cheap diagnostic tool. Contrast studies should not be performed in patients where there is concern for intestinal perforation since barium leakage into the peritoneal cavity can cause severe inflammation and granuloma formation. If small intestinal rupture is diagnosed via a contrast study, exploratory surgery should be performed ASAP and a thorough abdominal lavage performed to decrease such complications.

Abdominal ultrasound performed by an experienced practitioner may also be considered to rule out gastrointestinal obstruction. Studies have shown ultrasound to be more sensitive and specific than abdominal radiography for this purpose, but its use clinically may be limited by availability, experience and expense.

Abdominocentesis

If free peritoneal fluid is found on diagnostic evaluation or suspected, a diagnostic abdominocentesis should be performed rapidly. This may be performed blindly or using ultrasound guidance to target a focal area of peritoneal fluid accumulation. Positioning the patient in left lateral recumbency may be preferable to avoid accidental puncture or laceration of the spleen. A 22-gauge needle attached to a syringe is typically used, and inserted along the ventral midline for a blind tap, just caudal to the umbilicus, or slightly to the side in an attempt to avoid the falciform fat. Any haemorrhagic fluid should be examined for the presence of blood clots. Fluid from the abdominal cavity should not clot whilst haemorrhagic fluid obtained from puncture of the spleen, liver or any blood vessel will clot readily. If clotting fluid is obtained, the needle should be withdrawn and the abdominocentesis repeated at another site.

If closed abdominocentesis fails to retrieve any fluid present, an open approach may be considered in which a hypodermic needle alone is inserted into the peritoneal cavity and fluid collected directly into a sample container without the use of a syringe. This may have the advantage of preventing aspiration of falciform fat into the needle, and hence increase fluid yield. It is important to note that abdominocentesis, especially using an open technique, may introduce a small amount of air into the peritoneal cavity which may make interpretation of abdominal radiographs more challenging.

A four quadrant approach may be considered if single needle abdominocentesis is unsuccessful. Four needles are placed simultaneously into the peritoneal cavity, centred around the umbilicus, and kept open. Gravity dependency or changes in intra-abdominal pressure between the needles may increase the yield of any fluid recovered.

Any fluid obtained should be collected into plain and EDTA sample containers and analysed as detailed below to provide diagnostic information as to the cause of the abdominal pain.

Diagnostic peritoneal lavage

A diagnostic peritoneal lavage (DPL) should be performed in patients presenting with an acute abdomen that are suspected or confirmed to have free peritoneal fluid which cannot be tapped by standard abdominocentesis. The patient should be positioned in left lateral recumbency for this procedure and sedation and/or analgesia provided to improve patient comfort and tolerance of the procedure. A wide area centred around the umbilicus is clipped and prepared aseptically to avoid introducing infection. Local anaesthesia (2% lidocaine) is infused at the puncture site, either the umbilicus or 2 to 3 cm lateral to the midline to avoid the falciform fat. A small stab incision is made at the site of the local anaesthetic injection. An over the needle catheter (14-16 gauge) or dialysis catheter is then introduced through the incision and advanced into the abdominal cavity and any stylet completely withdrawn being careful not to kink the catheter. If desired, the over the needle catheter can have additional fenestrations made prior to use in order to increase the surface area of the catheter available for fluid retrieval.

These are made manually with a scalpel blade, but should be few in number, smooth, cover less than 50% of the catheter diameter and not compromise the integrity of the catheter in any way. A syringe may be attached to the catheter once it has been inserted into the peritoneal cavity to check for free fluid via the catheter. If no peritoneal fluid is obtained, a volume of 22 ml/kg of warmed 0.9% sodium chloride is infused through a drip set using gravity flow into the peritoneal cavity. The abdomen is gently massaged or the patient gently rolled, taking care not to dislodge the catheter. Fluid can then be gently aspirated via syringe or collected by gravity flow into the fluid bag. Large volumes are generally not obtained as the fluid given is dispersed throughout the abdomen. Any amount of fluid obtained should be submitted for microbiologic and cytologic analysis. DPL is not typically useful for detection of retroperitoneal disease.

Peritoneal fluid analysis

Once fluid has been sampled from the abdomen of a patient with abdominal pain, it is imperative the sample is examined as a matter of urgency. Usually this means assessment of the fluid in house although it is usually also advisable to submit the fluid to an external laboratory for confirmation of the diagnosis and additional testing such as bacterial culture and sensitivity testing. Cytology and biochemical analysis are both helpful to diagnose a variety of surgical conditions.

Although a diagnosis cannot be made on assessment of the gross appearance of the fluid alone, it should be looked at as part of the full fluid analysis. An abdominal fluid sample that is completely clear and colourless makes a diagnosis of peritonitis, severe intra-abdominal injury or perforation, or leakage from the gastrointestinal tract less likely. Fluid that appears opaque, cloudy or flocculent should be examined immediately and is concerning for a surgical diagnosis.

Test	Diagnostic criteria	Sensitivity	Specificity
Blood glucose minus peritoneal glucose for the diagnosis of septic peritonitis	> 20mg/dL (1.1 mmol/L)	Dogs: 100% Cats: 86%	Dogs: 100% Cats: 100%
Peritoneal fluid lactate minus blood lactate for the diagnosis of septic peritonitis	> 2.0 mmol/L	Dogs: 100% Cats: not reported	Dogs: 100% Cats: not reported
Fluid to blood potassium ratio for the diagnosis of uroabdomen	Dogs: ratio of 1.4:1 Cats: ratio of 1.9:1	Dogs: 100% Cats: unknown	Not reported but considered diagnostic for uroabdomen. Gastric perforation may cause similar findings.
Fluid to blood creatinine ratio for the diagnosis of uroabdomen	Dogs: ratio 2:1 Cats: ratio 2:1	Dogs: 86% Cats: unknown	Dogs: 100% Cats: unknown
Fluid to blood bilirubin ratio for diagnosis of bile peritonitis (also may see bile pigment or crystals in the abdominal fluid)	> 2:1	Dogs: 100% Cats: unknown	Not reported

(Table modified from Small Animal Critical Care Medicine, Ed Silverstein & Hopper)

A pure transudate will be grossly clear and is characterised by a protein of < 2.5g/dl and a low cell count (< 500 cells/ul). The cells present tend to be predominantly reactive mesothelial cells or non-degenerative neutrophils. Hypoalbuminaemia of any cause (PLE, PLN, hepatic failure) and portal venous obstruction are both cause of a pure transudate.

A modified transudate is usually serous to serosanguinous in appearance, with a protein level of 2.5 to 5 g/dl, and a moderate total cell count (300 to 5500 cells/ul). The cells present will depend on the underlying cause but may include red blood cells, mesothelial cells, non degenerate neutrophils, macrophages and lymphocytes. A modified transudate is typically a result of passive congestion of the liver and viscera and impaired lymphatic drainage. Common causes include right sided heart failure, neoplasia, and liver disease.

In contrast, an exudate is usually cloudy or turbid in appearance with a high protein level (> 3 g/dl) and cell count (> 5000 to 7000 cells/ul). The neutrophil is the predominant cell but other cell types may also be present. An exudate is the most common type of free peritoneal fluid found in association with abdominal pain. Exudates may be septic (and surgical) or non-septic (less likely surgical) and the diagnosis can be challenging in some cases. Septic exudates are characterised by the presence of intracellular and extracellular bacteria. A thorough systematic evaluation of a spun fluid sample sediment smear should be performed when looking for the presence of bacteria.

In cases with suspected septic peritoneal fluid, a sterile sample should be submitted to an external laboratory for full analysis in addition to bacterial culture and sensitivity testing. Prior antibiotic history should be listed on the submission form and an extended panel requested to check bacterial susceptibility to a wider range of antibiotics in cases where multi-drug resistance is a possibility. If the facilities are available, a gram stain of the fluid may be performed in house to get a heads up of the spectrum of bacteria likely present. In any case, broad spectrum antibiotic cover should be provided in these cases, with good coverage of gram negative bacteria which typically predominate in gastrointestinal derived sepsis.

In rare cases, septic peritonitis may be present despite the absence of cytologic evidence of bacteria in the peritoneal fluid. Some examples of such cases include high GI perforation (especially gastric) due to the lower bacterial load present in the stomach as compared to the intestines, or patients receiving antibiotic therapy, or those with localised infections. It is important that the result of the fluid analysis be interpreted in conjunction with the rest of the patient's history, diagnostic imaging and clinicopathologic data to ensure the patient is managed appropriately.

Fluid samples collected via diagnostic peritoneal lavage are not subject to the numerical cut-offs as detailed above owing to the dilution effect on the lavage fluid. Cytologic characteristics of the white blood cells are considered to be more useful than the biochemical measurements. Whilst fluid with a PCV > 5% collected this way is indicative of serious intra-abdominal haemorrhage, repeated patient assessment is probably a more accurate diagnostic tool for the presence of ongoing bleeding. Urinary contrast studies are necessary for confirmation of urinary tract rupture.

Airway sampling techniques

Collection of a sample from the airway and lung may be considered in patients with disease localising to the pulmonary parenchyma requiring further investigation. In cases where a community acquired pneumonia is suspected, empiric broad-spectrum antibiotic therapy is warranted initially and typically results in full clinical resolution in the majority of cases. Collection of an airway sample may be considered in cases with a poor response to therapy, a history of prior antimicrobial therapy, concern for a potentially resistant hospital-acquired infection, or an open diagnosis based on available information. Samples may be collected by an endotracheal wash (sometimes called a transoral wash), transtracheal wash, or a bronchoalveolar lavage (BAL).

An endoscopic guided BAL may be preferable to obtain a sample directly from the affected lung tissue, thereby increasing diagnostic yield, but is often unavailable on an emergency basis and requires additional equipment and expertise that may further limit its availability. The endotracheal and transtracheal washes are both performed blindly but require no special equipment or expertise, and can be performed readily in the emergency room. Both procedures involve the instillation of sterile saline into the airway to help retrieve airway secretions. It is important to note that either may therefore result in hypoxia and possible bronchospasm that may worsen patient respiratory function for several hours following the procedure. They should only therefore be performed in patients in which a thorough risk:benefit assessment has been performed. In patients with pre-existing respiratory distress and hypoxaemia, a wash should only be performed if the facilities are present for additional support afterwards should the patient's condition further decline.

Endotracheal wash (ETW)

The endotracheal wash is preferred over the transtracheal wash in cats, dogs of < 10 kg, brachycephalic dogs owing to the smaller tracheal diameter and thicker necks which can make tracheal palpation more difficult, and larger breed dogs in which the length of available through the needle catheter may limit sample collection with a transtracheal wash. Brief general anaesthesia is required in order for the patient to permit brief intubation whilst the wash is performed, as well as to limit coughing which can adversely affect the quality of the sample retrieved. Propofol is commonly used to facilitate intubation, has the potential benefits of a rapid recovery and is a good choice if concurrent evaluation of laryngeal function is planned. Patients should be pre-oxygenated prior to induction. A sterile ET tube should then be placed cleanly. Consideration should be given to using slightly smaller sized tube than would normally be used for a given patient so as to avoid the need for application of lubricant to the tube which may affect interpretation of the fluid cytology. If lubrication is required, a small amount only should be applied to the outside of the tube.

A 5- to 8-French red rubber catheter is passed down the ET tube to approximately the level of the carina (at the heart base). Sterile isotonic saline is then infused down the tube; volumes of 3 ml (small dogs and cats), 5 ml (medium sized dogs, 10-20 kg) and 10-15 ml (large dogs, > 20 kg) and followed with air to ensure all saline is instilled within the tube. The fluid is then manually aspirated and collected into a sterile container. Concurrent coughing may help increase fluid yield if not contraindicated. Drainage of fluid may also be facilitated by lifting the patient's hindquarters. Alternatively, a suction catheter or closed suction trap device may be used for sample collection. Suction devices may permit better recovery of airway cells but are less widely available than red rubber catheters.

The patient should be extubated once the procedure has been completed and is sufficiently awake to be able to protect the airway and minimise the risk of aspiration pneumonia. They should be closely monitored afterwards to ensure a good recovery. The clinician should be prepared to provide more aggressive respiratory support than was required prior to the procedure owing to the hypoxia and possible bronchospasm that may occur.

Transtracheal wash (TTW)

A transtracheal wash may be performed in medium to large breed dogs and has the potential advantage over the ETW as it can be performed in the awake or lightly sedated patient. The procedure involves the placement of a catheter between the tracheal rings to access the tracheal lumen and airway. Saline is infused as for the ETW to help retrieve airway secretions. Potential risks of the procedure include hypoxia as previously discussed, as well as subcutaneous emphysema, tracheal laceration and haemorrhage, although these are considered uncommon.

To perform a TTW, the patient is adequately restrained on the floor or a treatment table. It can be helpful to position the patient in a corner on the floor to prevent backwards movement. An area of the ventral neck, centred over the cricothyroid ligament or first couple of tracheal rings, is clipped and prepared. After carefully palpating the intended catheter placement site, a local anaesthetic block (lidocaine) is infused and the area re-prepared. It is often easier to isolate the cricothyroid ligament in dogs with thicker necks (for example Labrador Retrievers). With the needle bevel of the catheter directed downwards, the catheter is inserted through the skin and into the tracheal lumen. The catheter will typically be directed perpendicular to the trachea for the insertion. The stylet is removed once the catheter is in the desired location, taking care not to kink the catheter at the point of entry into the skin. A sample catheter of an appropriate size is fed through this catheter lumen and sterile isotonic saline aliquots of 5 to 10 mls are instilled into the airway and followed with air before the sample is collected. The instillation of saline and its aspiration may be repeated up to 3 times. Gentle coupage may help to improve the volume of sample obtained.

Airway sample analysis

Regardless of the technique used for airway fluid collection, the recovered fluid should appear cloudy with occasional flecks of mucus. Cytologic examination, looking for evidence of suppurative inflammation and intracellular bacteria, should be performed in-house immediately after sample collection. Ideally, the sample should be submitted to a veterinary clinical pathologist for interpretation and aerobic culture and sensitivity testing. Anaerobic culture should also be requested if a pulmonary abscess is suspected, and mycoplasma testing also requested separately if clinically suspected.

Samples should be evaluated cytologically for oropharyngeal contamination which is a relatively common occurrence during airway sampling. It can be recognised in cytologic preparations by the presence of squamous cells, a mixed bacterial population that is predominantly extracellular in location, as well as unusually large bacteria (*Simonsiella* sp.) that normally inhabit the oral cavity and pharynx.

Deep oral swabs

Deep oral swabs have been considered as an alternative means of airway sampling in patients with suspected pneumonia. They have the potential benefit of being performed in the conscious or lightly sedated patient, without the risk of worsening hypoxaemia. A recent study compared the results obtained from DOS with ETW in both puppies and adult dogs with community or hospital acquired pneumonia. Positive cultures were obtained from all samples taken, but there was no agreement between the sampling techniques in puppies. Whilst there was complete agreement in 2 adult dogs with hospital acquired pneumonia, the results obtained from cases of community acquired infections were completely different. At this time the DOS cannot be used as an alternative to either the ETW or TTW based on the results of this study.

VetBLUE

Lung ultrasound has a higher sensitivity than lung auscultation and supine thoracic radiography for many acute and potentially life-threatening conditions in people. Lung ultrasound is based on the observation of ultrasonographic artefacts looking for evidence of 'dry lung' (A-lines with a glide sign), versus 'wet lung' (ultrasound lung rockets or B-lines). Ultrasound does not transmit through aerated lung and the presence of ultrasound lung rockets represents forms of interstitial oedema. Whilst the standard TFAST protocol looks for these artefacts, it is only performed at the 'chest tube site' on each side the thorax. The Veterinary Bedside Lung Ultrasound Examination (VetBLUE) has been developed to provide a more comprehensive lung ultrasound survey. The VetBLUE examination consists of four bilaterally applied lung views (eight total acoustic windows), referred to as the caudodorsal lung lobe region, the perihilar lung lobe region, the middle lung lobe region, and the cranial lung lobe region. The ultrasound probe is placed horizontally at each site, with the starting point of the caudodorsal lung lobe region, the equivalent of the chest tube site of the TFAST assessment. Each of these sites is observed for lung ultrasound artefacts using the same wet versus dry lung principal as described above. Each site is given a score based on the number of B-lines seen. A recent study showed that lung ultrasound using the VetBLUE protocol showed good diagnostic accuracy to identify cardiogenic pulmonary oedema in dogs and that it may be helpful in staging dogs with chronic valvular heart disease. Further research into the application of VetBLUE in veterinary patients is needed, but it shows initial promise as a readily available, non-invasive, patient-side diagnostic tool for patients with respiratory dysfunction.

Assessment of oxygenation

The anatomic approach to the localisation of the cause of respiratory distress states that respiratory distress may originate from problems in:

- Upper airways
- Lower airways
- Pulmonary parenchyma
- Pleural space
- Chest wall and diaphragm
- Abdominal distension
- Respiratory look-a-likes

Assessment of patient oxygenation can be useful to determine whether a respiratory problem is causing hypoxia, thereby originating from the respiratory system itself, or not and hence more likely caused by a look-a-like as discussed in the previous webinar. Pulse oximetry and arterial blood gas analysis are both techniques that may be used in veterinary patients for the assessment of oxygenation.

Pulse oximetry

Pulse oximeters measure the saturation of peripheral blood (SpO_2) which can be assumed to represent the saturation of arterial blood. It is measured by placing a sensor device on a thin part of the patient's body. The device passes 2 wavelengths of light through the body to a photodetector. It measures the changing absorbance at each of the wavelengths, allowing it to determine the absorbances due to the pulsatile arterial blood alone, excluding venous blood and other tissues. In order to obtain a reliable reading, the area the sensor device is placed needs to be movement free and non-pigmented. The heart rate reported by the pulse oximeter should be checked to ensure it matches that of the patient, as the appearance of the waveform also assessed where present to ensure reading is likely to be reliable. A normal SpO_2 on room air is $> 95\%$. A patient is considered to be hypoxaemic if the SpO_2 is $< 95\%$ and severely hypoxaemic if the SpO_2 is $< 90\%$.

Arterial blood gas analysis

The data collected by analysis of an arterial blood gas sample includes the partial pressure of oxygen (PaO_2) and carbon dioxide (PaCO_2) in arterial blood. It can therefore be used to assess a patient's oxygenation and ventilation status. In order for correct interpretation of the PaO_2 , it is important that the fraction of inspired oxygen (FiO_2) is noted. A normal PaO_2 on room air is > 80 mmHg. A patient is considered to be hypoxaemic if the PaO_2 is < 80 mmHg and severely hypoxaemic if the PaO_2 is < 60 mmHg. The PaO_2 should be approximately five times the inspired oxygen level and so respiratory function can also be assessed in patients receiving supplemental oxygen as long as the FiO_2 is known at the time of sampling.

In order to take an arterial blood gas sample, the following steps should be taken:

- The blood gas analyser and correct cartridge should be prepared for use. The sample should be collected directly into a heparinised syringe. Pre-anticoagulated syringes can be purchased for this purpose and eliminates the concern for over or under anticoagulation of syringes that can affect the results obtained.
- The dorsal metatarsal (pedal) artery is used most commonly, although the femoral artery or lingual artery (in an anaesthetised patient) may also be used. The pulse should be palpated and the area over it clipped as necessary and gently prepped to prevent spasm of the underlying artery.
- The patient should be positioned in lateral recumbency, with the limb to be sampled placed downwards for ease of access to the artery. The patient should be prevented from withdrawing the limb during sampling by ensuring the hock is adequately restrained.
- The pulse should be re-palpated using 1-2 fingers from the non-dominant hand. It is not possible to directly visualise the artery and so sampling needs to be based on palpation alone.
- The needle should be inserted initially through the skin alone. This gives the animal time to react to any associated discomfort without damage to the arterial vessel.
- Using the pulse palpation as a guide, the needle should be advanced parallel to where the pulse is felt, with the needle angled at approximately 45 degrees.
- Arterial puncture results in the pulsatile filling of the pre-drawn collection syringe to the desired volume.
- After the sample is collected, the needle is withdrawn and pressure applied to the area for approximately 5 minutes. The site should be monitored for any haematoma formation.
- The arterial blood gas sample should be run through the analyser ASAP, any bubbles removed, and the top of the syringe covered to prevent exposure of the sample to room air. If there is any delay in running the sample, it should be stored in an ice water bath to limit ongoing cell metabolism which may affect the results obtained.

Common mistakes associated with arterial blood gas sample collection and storage include:

- Inappropriate collection syringe anticoagulation. Anticoagulation of the collection syringe is required for correct sample running. Over-heparinisation of the syringe may affect the results of the arterial blood gas analysis and several other biochemical values that may also be reported. Use of pre-anticoagulated syringes avoids this problem.
- If the sample is left uncapped for a prolonged period, the PaCO_2 and PaO_2 may be lower. The PaO_2 may increase if the sample PaO_2 is less than the partial pressure of oxygen in room air.
- If the sample is not run immediately, and not chilled for a long period of time, ongoing cellular metabolism within the blood sample causes a lower PaO_2 and a higher PaCO_2 .

Coagulation testing for the diagnosis and management of anticoagulant rodenticide intoxication

Anticoagulant rodenticide (ACR) ingestion, including agents such as brodifacoum, bromodialone and difenacoum, is a common toxicity in dogs. Animals that consume a sufficient amount of ACR develop clinical signs secondary to a coagulopathy and resultant internal bleeding. The development of a coagulopathy occurs secondary to the fact that activation of clotting factors II, VII, IX and X requires the presence of vitamin K. Anticoagulant rodenticides antagonise the enzyme vitamin K epoxide reductase which is needed to maintain vitamin K in an active form, preventing the ongoing activation of these vitamin K-dependent clotting factors. When the factor levels become depleted, a coagulopathy occurs. There is usually a lag period of 3-5 days between exposure of the patient to an ACR and the appearance of clinical signs of bleeding. Bleeding typically occurs in body cavities (commonly including the pleural space and peritoneal cavity) and the lung but bleeding at other sites is also reported.

The acute management of recent ACR intoxication should include the induction of emesis as long as there are no contraindications (seizures, depression, airway problems). In dogs apomorphine is the agent of choice and should be administered IV or IM as soon as possible. Activated charcoal should also be administered to adsorb any remaining rodenticide within the gastrointestinal tract. A single dose of a cathartic such as sorbitol may also be considered to reduce the possibility of systemic toxin absorption by decreasing intestinal transit time.

Prolongations of the prothrombin time (PT) are the earliest indicator commonly available that a coagulopathy is developing as a result of intoxication. The PT is a measure of the extrinsic pathway and includes factor VII. Factor VII has the shortest half-life of all the vitamin K-dependent clotting factors, and as a result, the PT will be prolonged before the activated partial thromboplastin time (aPTT) and before the development of clinical signs of bleeding in cases of ACR intoxication. Factor VII levels will be depleted 48 hours following ingestion, resulting in a sole prolongation of the PT prior to clinical signs of bleeding.

A study published in 2008 by Pachtinger *et al*, examined gastric decontamination using emetics and/or activated charcoal following ACR ingestion in dogs. In dogs that had these treatments within 6 hours of ingestion, PT prolongations were only seen in 8.6% of patients 2 days later. These patients were considered to require oral vitamin K treatment and no adverse effects or bleeding events were seen. More than 90% of patients had no PT prolongation and received no further treatment with no apparent clinical problems.