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The laboratory diagnostic approach to thoracic and abdominal effusions in the dog, cat, and horse

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Η εργαστηριακή προσέγγιση των υπεζωκοτικών και περιτοναϊκών συλλογών στο σκύλο, τη γάτα και το άλογο

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ABSTRACT: Cases involving pleural and peritoneal effusions occur relatively frequently in clinical practice. Determining the underlying etiology in these cases relies mainly on fluid analysis. The technique used for obtaining the pleural or peritoneal fluid can impact greatly the results of the analysis. Most often used diagnostic tools include evaluation of gross appearance, Total Nucleated Cell Count / Total Protein (TNCC/TP) measurement, chemical/biochemical analysis (Lactate dehydrogenase and lactate, cholesterol, triglycerides, glucose, creatinine, pH, pO₂, pCO₂, and K measurements), cytology (identification of septic and non-septic inflammation and neoplasia), microbiology (Gram stain, culture, molecular techniques), and specific tests for certain clinical conditions and diseases. Classifying an effusion as transudate, modified transudate and exudate is traditionally based on the TNCC and TP values. New diagnostic methods encourage the clinician to approach the effusion etiologically instead of strictly following this traditional classification. Many of the diagnostic tests described in this review are simple and can be performed in-house, providing the clinician quickly with information about the cause of the effusion, essential for an effective treatment plan without wasting valuable time.

Keywords: effusion, pleural, peritoneal, laboratory, diagnostic approach

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ΠΕΡΙΛΗΨΗ: Περιστατικά στα οποία διαπιστώνονται υπεζωκοτικές και περιτοναϊκές συλλογές είναι αρκετά συχνά στην κλινική πράξη. Ο καθορισμός της υποκείμενης αιτιολογίας σε αυτές τις περιπτώσεις βασίζεται κυρίως στην ανάλυση του υγρού της συλλογής. Η τεχνική που χρησιμοποιείται για τη λήψη του υγρού της υπεζωκοτικής ή περιτοναϊκής συλλογής μπορεί να επηρεάσει σε μεγάλο βαθμό τα αποτελέσματα της ανάλυσης. Στις πιο συχνά χρησιμοποιούμενες διαγνωστικές εξετάσεις περιλαμβάνονται η αξιολόγηση των φυσικών ιδιοτήτων του υγρού, ο προσδιορισμός του συνολικού αριθμού εμπύρηνων κυττάρων / ολικών πρωτεϊνών (TNCC / TP), ο προσδιορισμός χημικών / βιοχημικών παραμέτρων (γαλακτική αφυδρογονάση και γαλακτικό οξύ, χολοστερόλη, τριγλυκερίδια, γλυκόζη, κρεατινίνη, pH, pO₂, pCO₂, K), κυτταρολογική εξέταση (διάγνωση σηπτικών και μη σηπτικών φλεγμονών και νεοπλασμάτων), μικροβιολογικές εξετάσεις (επιχρίσματα με χρώση Gram, καλλιέργεια, μοριακές τεχνικές) και ειδικές διαγνωστικές εξετάσεις για ορισμένες παθολογικές καταστάσεις και νοσήματα. Η ταξινόμηση μιας συλλογής ως διδρώμα, τροποποιημένο διδρώμα και εξιδρώμα βασίζεται παραδοσιακά στις τιμές των TNCC και TP. Νέες διαγνωστικές μέθοδοι συνεισφέρουν στην αιτιολογική διάγνωση χωρίς απαραίτητα να ακολουθηθεί αυστηρά η παραδοσιακή ταξινόμηση. Πολλές από τις διαγνωστικές εξετάσεις που περιγράφονται σε αυτήν την ανασκόπηση είναι απλές και μπορούν να πραγματοποιηθούν στο ιατρείο, παρέχοντας γρήγορα πληροφορίες στον κλινικό κτηνίατρο σχετικές με την αιτία της συλλογής. Η γνώση αυτή είναι απαραίτητη για την έγκαιρη και αποτελεσματική θεραπευτική αντιμετώπιση της υποκείμενης παθολογικής κατάστασης.

Λέξεις ευρετηρίασης: συλλογή, υπεζωκοτική, περιτοναϊκή, εργαστηριακές εξετάσεις, διαγνωστική προσέγγιση

INTRODUCTION

The pleura is a membrane lined by a single layer of mesothelial cells surrounded by elastic connective tissue that contains vascular and lymphatic channels. The pleural space exists as a cavity only when there is fluid or gas collected between the parietal and visceral pleura. It normally contains 2 to 3 ml of fluid in dogs and cats, with low cell numbers (<500 cells/μl) and protein content (<1.5 g/dl). Regarding horses, it has been reported that 2 to 8mL of thoracic fluid could be aspirated by 17 out of 18 clinically normal horses, however multiple attempts were made in order to obtain the fluid (Wagner and Bennett, 1982). Its production depends mainly on the colloid osmotic pressure and the hydrostatic pressure within the capillary and lymphatic beds which means that lymphatics gather the surplus left after fluid filtration by the capillaries. In dogs and cats increased fluid can be absorbed by the visceral pleura, while horses have to rely on their lymphatic system, a fact that may explain the quick development of parapneumonic effusions in horses (Mazan, 2018). Pleural fluid production is normally favored because systemic and pulmonary capillary hydrostatic pressures are greater than those of the pleural space (Radlinsky, 2012).

The peritoneal cavity is a potential space between the visceral and parietal peritoneum. It normally contains a very small amount of fluid produced by the mesothelial cells to act as a lubricant. The peritoneum is capable of both absorption and exudation or transudation. It allows exchange between peritoneal fluid

and plasma. Normal peritoneal fluid is almost acellular, containing <300 cells/μl, with protein content <3 g/dl and does not clot, as it lacks fibrinogen. In an early study about peritoneal lymphatic drainage in dogs (Kirby, 2012), intraperitoneally injected particles cleared from the peritoneal cavity appeared quickly in the systemic circulation and in the lungs, which is why in bacterial peritonitis, bacteremia is an early and consistent finding. Contamination or injuries of the peritoneum activate a strong inflammatory response consisting of an inflow of protein-rich fluid along with macrophages and neutrophils. Mast cells and macrophages release histamine and prostaglandin, cause vasodilation and increase the permeability of peritoneal capillaries, leading to the massive entrance of fluid abundant in complement, immunoglobulins, clotting factors, and fibrin into the peritoneal cavity.

There are 4 main mechanisms by which a pleural or peritoneal effusion can occur; (1) it can be the result of an increase in hydrostatic pressure (e.g. right sided congestive heart failure), (2) reduction of osmotic pressure (hypoalbuminemia), (3) increased vascular permeability (e.g. inflammations in the pleural space), and (4) obstruction of lymphatics that can decrease fluid resorption (Kirby, 2012; Mazan, 2018).

Apart from the aortic, the caval and the esophageal hiatus, dorsally to the diaphragm and ventrally to the psoas muscles, the pleural and peritoneal cavities are separated only by a thin layer of fused endothoracic and transversalis fascia (Kirby, 2012). This explains how even when the diaphragm is intact, cases like

pneumothorax/pneumoperitoneum, pyothorax/septic peritonitis, or even uroperitoneum/urothorax can develop (Tsompanidou, 2015).

INDICATIONS AND COLLECTION TECHNIQUES

Fluid analysis can quickly narrow the differential diagnosis of an effusion. Thoracocentesis/abdominocentesis is not always strictly diagnostic as lessening the amount of fluid is essential for alleviating the symptoms caused by fluid accumulation. Ultrasound-guided paracentesis is the safest option to obtain a sample for analysis, especially in the presence of a small amount of fluid, or when the effusion is unilateral or compartmentalized. Furthermore, it has much higher yield than blind tapping (Valenciano et al., 2014). The procedure and the materials needed are described in Fig. 1 and Table 1, respectively.

Table 1. Materials used for sampling and collection

| Sampling | Collection |
|---|--|
| Needle 19-21 G (cats, small dogs) | EDTA tube (TNCC, PCV, TP, cytology) |
| Needle 18-20 G (medium and large sized dogs) | Plastic or glass serum tube (chemical, biochemical analysis) |
| Needle 14 G (horse) | Sterile tube or culturette (PCR, culture) |
| Over-the-needle catheter | |
| Extension tube | |
| 3-way stop-cock | |
| Tomcat catheter | |
| Peritoneal dialysis catheter (abdominocentesis) | |
| Teat cannula (horse) | |

TNCC: Total nucleated cells, PCV: Packed Cell Volume, TP: Total protein

1. Preparation of the insertion site. Clipping and surgical scrubbing. Care should be given to the strict aseptic conditions of the chosen technique, especially in surgery patients. Preoperative thoracocentesis or intrathoracic biopsy were found to be risk factors that contributed to the development of pyothorax in animals following thoracic surgery (Meakin et al., 2013).



2. The animal is left standing (dog, cat, horse) or put in sternal recumbency (dog, cat). Restraining is crucial, especially if sedation or anesthesia is not used.



3. **Dog, cat:** insert a butterfly needle or an over-the-needle catheter approximately two thirds down the chest, near the costochondral junction between the 6th-8th intercostal space, close to the cranial surface of the rib. The caudal border should be avoided to minimize the possibility of lacerating the vessels. Use a 19-21 gauge needle for cats and small dogs and an 18-20 gauge for larger breeds. An over-the-needle catheter allows for the needle to be withdrawn before attaching the syringe, thus minimizing the risk of injuring the organs of the thoracic cavity. If the removal of a large amount of fluid is needed, the catheter should be attached to an extension tube and a three-way stopcock (Kirby, 2012). **Horse:** insert a 14-gauge catheter between the 7th-8th intercostal space, 4cm above the costochondral junction. Care needs to be taken not to lacerate the lateral thoracic vein. A stab incision needs to be made first, for a chest tube or a teat cannula placement. Due to the anatomical characteristics of the horse's mediastinum, to obtain a diagnostic sample for analysis it is important to sample the respected chest side (the condition of the two chest sides may differ) (Mazan, 2018).

Fig. 1. Synoptic presentation of thoracocentesis/abdominocentesis

Table 2. Traditional effusion classification

| | Transudate (pure) | Modified transudate | Exudate |
|-------------|--------------------------|----------------------------|----------------------|
| TP | <2.5 g/dL | 2.5 – 7.5 g/dL | >3.0 g/dL |
| TNCC | <1500 cells/ μ L | 1000 – 7000 cells/ μ L | >7000 cells/ μ L |

TP: Total protein, TNCC: Total nucleated cells

Abdominocentesis is performed with similar materials as thoracocentesis. A tomcat catheter or a peritoneal dialysis catheter can also be used, especially if only a small amount of fluid is present. When a syringe is not attached the technique is considered more sensitive. Preparation also includes emptying the urinary bladder to avoid accidental cystocentesis. The animal is left standing or put in lateral recumbency and the puncture is made along the ventral midline to avoid the presence of falciform fat that can block the needle barrel, except in the case of a previous surgical incision. When a surgical scar is present, the insertion should be made at least 1.5 cm away to avoid any abdominal viscera that may have adhered in the area.

Method selection depends on factors such as time, cost, safety, and amount of fluid present. Using only a syringe is less expensive than an over-the-needle catheter but also less safe, while using a peritoneal dialysis catheter is the best method to collect a sample from an effusion with a very small amount of fluid -and it further allows for abdominal lavage but is also the most expensive. In dogs with septic peritonitis, the comparison among needle paracentesis, catheter paracentesis, and diagnostic peritoneal lavage showed an accuracy rate of 94.6% for the later, and less than 50% for the first (Culp and Holt, 2010). The needle and the cannula technique were compared in horses using repeated abdominocentesis and were found to be similar in the time needed and results yielded with no technique being superior over the other (Duesterdieck-Zellmer et al., 2014).

THORACIC/ABDOMINAL FLUID ANALYSIS

A. Gross appearance

Fluid appearance can sometimes be indicative of both the process that resulted in its accumulation and the Total Nucleated Cell Count (TNCC). An opaque sample is usually expected to have a higher nucleated cell number than a colorless clear sample. Transudates, which are low cellularity effusions usually produce more clear and colorless specimens. Modified transudates of moderate cellularity are often of amber color and clear to slightly opaque. However, fluids with more or less opaque appearance are most commonly exudates (high cellularity fluids). Whether the fluid is clear or turbid will also affect the laboratory process. More opaque fluid smears

can be made directly with well-mixed, uncentrifuged samples, while non-turbid fluid specimens need to be centrifuged at 1000-1500 rpm for 5 minutes before making a smear from the sediment (or if centrifugation is not available, a line smear can be made instead of a traditional pull or squash smear). A milky appearance is often observed when chylous or, less often, pseudo-chylous effusions are present and the cause of this appearance is their consistence of chylomicron rich lymph fluid. Of course, the milky macroscopic appearance is only indicative, as there are 'white' appearing effusions that are not chylous, as well as chylous effusions that do not have a milky appearance (especially when the animal is fasted or anorectic). Determining the Total Protein (TP) concentration in clear fluids can be done without centrifugation but in opaque fluids is best estimated by using the supernatant after centrifugation to avoid falsely high TP readings. Comparing the appearance of the specimen before and after the centrifugation can give the clinician important clues. A turbid sample that yields clear supernatant indicates that the turbidity was a result of cell and debris accumulation (may be suggestive of empyema), whereas turbidity that persists despite the centrifugation may suggest high lipid concentration.

B. TNCC and TP measurement

Effusion classification as transudate, modified transudate, or exudate is traditionally based on these two tests (Table 2). TNCC measurement can be performed either by automated cell counters or manually with the use of a haemocytometer. It should be noted that TNCC measurement whether is performed manually or automatically, does not substitute cytological evaluation which is necessary to confirm cell counts (Brudvig and Swenson, 2015). When using an automated counter that also counts RBC numbers, the information obtained can be useful to determine blood contamination of the sample, haemorrhage, or increased capillary permeability. Counting errors can occur with either automated or manual methods by factors such as debris, cell fragmentation and clumping. Delaying of the analysis can also alter TNCC, as well as cell morphology (Valenciano et al., 2014; Hughes et al., 2016; Mazan, 2018). The TP measurement can be performed either with a biochemical analyser or refractometer. Refractometry has in fact shown acceptable results

for TP measurement in canine pleural and abdominal fluid samples, providing a quicker, convenient and relatively inexpensive method, compared to the biuret biochemical assay (Rose et al., 2016).

C. Chemical and biochemical analysis

- Lactate dehydrogenase (LDH) and Lactate: Difference between blood lactate and effusion lactate concentrations < 1.5 mmol/dL in canine and feline effusions was found to be indicative of septic exudates (Dempsey and Ewing, 2011). Elevated lactate concentrations in fluid from effusions supports the diagnosis of septic exudates (lactate increase due to an anaerobic microenvironment, production of lactate by neutrophilic glycolysis, and from the presence of bacterial metabolites). It has been observed that LDH activity, LDH effusion/serum relationship, lactate concentration as well as lactate effusion/serum gradient present a high correlation with the classification of an effusion as exudate. On the other hand, an LDH effusion:serum ratio of < 0.5 has been associated with transudates. Lactate dehydrogenase activity seems to be significantly different depending on the method of measurement, therefore it is necessary that the method used is known and remains constant for comparison (Smuts et al., 2015; Smuts et al., 2016).

In horses with colic, higher levels of LDH activity may be present in the peritoneal fluid when the condition is caused by sepsis or neoplasia, rather than those caused by mechanical obstructions or non-septic inflammatory conditions, with the latter ones more probable to respond to treatment. Again, the use of a consistent method of determination seems to be crucial, as great variation exists among the results. A moderate to good correlation appears between LDH activity and lactate concentration (Smuts et al., 2015). Peritoneal fluid lactic acid concentration was found to be an indicator of intestinal ischemia, and higher concentrations correlate with a poor prognosis (not surprising, as there is likely a relationship between the amount of necrotic tissue and peritoneal fluid lactate concentration). Assessing survivability, serum lactate concentrations were shown to be significantly higher in non-surviving colic horses than those that eventually lived (Boom et al., 2010), and lower serum LDH levels were found in dogs with better survival time amongst animals suffering from lymphoma (independently of the clinical stage or cytological evaluation) (Zanatta et al., 2003).

- Cholesterol: This measurement has been proposed as a way to increase the diagnostic accuracy when classifying effusions as transudates or exudates.

Although measuring cholesterol on its own is not suggested because of low sensitivity, it can be used in combination with other markers e.g. LDH (Zoja et al., 2009). The exact mechanism by which cholesterol levels are found higher in exudates remains poorly understood (serum leakage suggesting increased permeability and release due to cellular degeneration have been proposed but not yet proven).

- Triglycerides: Effusion triglyceride concentration on its own, and also in combination with effusion cholesterol and serum triglyceride concentration are methods used for cavitory fluid analysis. Effusion triglyceride concentration $>$ serum triglyceride concentration, effusion cholesterol $<$ triglyceride concentration, and effusion triglyceride concentrations > 100 mg/dL suggest the presence of a chylous effusion. Triglyceride measurement in the pleural fluid has been compared to - and has shown good correlation to - the lipoprotein electrophoresis, the latter being used in humans for the differentiation between chylous and non-chylous effusions as it provides the detection of chylomicrons (Waddle and Giger, 1990).

- Glucose: As a bacterial infection develops in a cavity, the effusion glucose levels begin to decrease, either because of glucose usage by the bacteria and the inflammatory cells, possible glycolysis in the fluid, or low blood to fluid glucose transport. Measuring effusion glucose levels alone, despite the established concentration of 50mg/dL being specific for septic effusion, is not recommended due to low sensitivity. Comparing serum and fluid levels provides a rapid and more accurate screening test. A concentration difference between blood glucose and effusion glucose concentrations > 20 mg/dL is a quick and reliable diagnostic tool in differentiating septic from non-septic effusions (Dempsey and Ewing, 2011; Bonczynski et al., 2003). Cytology and additional diagnostics (e.g. culture) are however essential in the definitive diagnosis of a septic effusion. Veterinary point-of-care glucometers can be useful in the sense of a more rapid diagnosis of septic peritonitis, but diagnostic accuracy is limited, and cut-off points need to be different for maximizing sensitivity (Koenig and Verlander, 2015).

- Creatinine: In dogs, measuring creatinine levels in the peritoneal fluid can help diagnose uroabdomen. Effusion concentration 2 times above serum levels is considered indicative of the condition. In a case reported in 2015, the same criteria were also used to detect urothorax in a dog in which the accumulation of urine in the thoracic cavity was found to be secondary

to uroperitoneum (Tsompanidou et al., 2015). Fluid urea is not useful in the diagnosis of uroabdomen as it diffuses rapidly across membranes given its small size.

- pH, pO_2 , and pCO_2 : Attempts have been made in humans and animals to link these markers with bacterial and malignant effusions, often with ambiguous results. A low pH value in an effusion suggests high metabolic activity and indicates an inflammatory or an infiltrative process. In humans, low pH value in pleural fluid is used to facilitate the decision of fluid drainage in parapneumonic effusions (since clinical findings and radiographic imaging often provide poor diagnostic accuracy in determining which effusions need surgical treatment). In malignant effusions, low pH values were linked to shorter survival time and poorer response to chemical pleurodesis. A study analyzing the pH, pO_2 and pCO_2 values in the peritoneal fluid of dogs with ascites, showed statistically significant differences in the group of dogs with bacterial peritonitis (Glińska-Suchocka et al., 2016). As an inflammatory process presents within the peritoneal cavity, fibrin accumulation leads to decreased oxygen supply causing non-oxidative glucose metabolism that results in a decreased pH value, and also creates perfect conditions for the growth of anaerobic bacteria (e.g. preventing phagocytosis, activating proteolytic enzymes). The peculiarity of this measurement, which could be the cause of the differing results in many previous studies, seems to involve the sample collection and handling. Maximizing diagnostic accuracy requires recognizing factors that easily occur in a clinical practice and can have an impact in these results. In humans it has been demonstrated that residual air in the collection tube, topical anesthetics such as lidocaine (commonly used in hospitals as well as animal practices), and delay of analysis significantly alter the values measured (Rahman et al., 2008). In horses, exposure of peritoneal fluid to room air was shown to have significant effects on pH, PCO_2 , PO_2 , HCO_3 and ionized Ca (Romero et al., 2011). Not acknowledging the need for anaerobic collection and containment, the importance of rapid analysis or the possibility of clot formation in the blood gas analyzer leads to diagnostic inaccuracy that can influence case management.

Potassium: The effusion:serum potassium value has been used in the diagnosis of uroabdomen (Dempsey and Ewing, 2011; Fry, 2011; Tsompanidou et al., 2015, Ben Oz et al., 2016) but it was recently proposed that it could also be indicative of gastric perforation (Ben Oz et al., 2016).

More parameters such as leukocyte esterase reagent strips (Porcel, 2013; Thomovsky and Johnson, 2014), serum amino-terminal pro-C-type natriuretic peptide (Guieu et al., 2015), adenosine deaminase, growth factors as cancer-related biomarkers, the albumin gradient (ALBg) for distinguishing transudates from exudates (Zoia and Drigo, 2015) are currently being evaluated in human and veterinary medicine with promising results.

D. Cytology

Cavitary effusions are routinely examined cytologically to evaluate the presence of an inflammatory process and/or microorganisms and extracellular material (e.g. ingesta in peritoneal fluid resulting from gastrointestinal tract rupture as shown in Fig. 2a and Fig. 2b), to observe the cells included, or to determine if there is an underlying neoplastic cause for fluid accumulation.

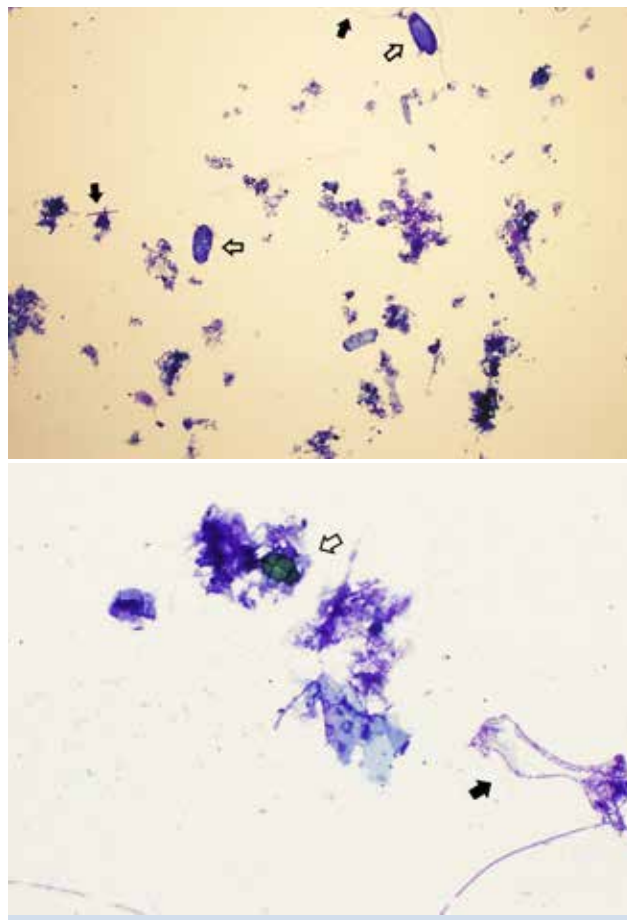


Fig. 2a and 2b. Accidental enterocentesis in a horse. Note the presence of aggregates of mixed, extracellular bacteria (black arrows) and amorphous plant material (intestinal contents). There are also large ciliated protozoa (white arrows) which are part of the normal gut microbiota in horses. An inflammatory response is not observed. Repeat abdominocentesis in this horse revealed a transudate with no obvious sign of gut rupture. Wright-Giemsa stain, 10x objective (2a) and 50x objective (2b)

A negative cytological result for malignancy cannot completely exclude the possibility from the differential, as cytology is a method that can be influenced by several factors. Paucity of representative cells (Fig. 3), cell overcrowding, cell changes and abundance of inflammatory cells can pose great difficulties. To overcome these difficulties many methods have been described in literature. From the more ‘traditional’ pull smears to the most recently described cell block, there are many methods to choose from, depending on various factors such as fluid quantity and character.

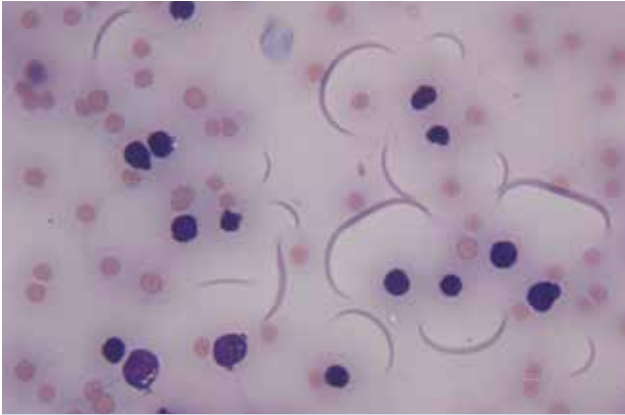


Fig. 3. Pleural fluid from a 6-year-old DSH cat with mediastinal lymphoma. Note the eosinophilic background and the crescent formations, as a result of the highly proteinaceous fluid. Cell morphology cannot be assessed due to the cells being too condensed – the definitive diagnosis in this case was reached post-mortem through histopathology (the cat was euthanized). Cytoцентрифуг preparation, 50x objective

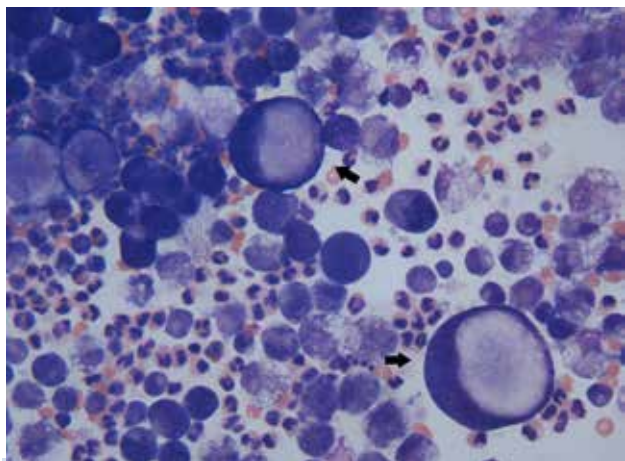


Fig. 4. Pleural fluid from a 14-year-old Collie dog. The cytological diagnosis was mesothelioma or carcinoma, however the possibility that these are reactive mesothelial cells could not be ruled out. There is also marked pyogranulomatous inflammation (probably secondary to the neoplastic process). Amongst high numbers of neutrophils and reactive macrophages there are cells of epithelial/mesothelial origin with marked pleomorphism, including two ‘signet ring’ cells (arrows), each with a single large vacuole that displaces the nucleus to the margin of the cell. Cytoцентрифуг preparation, 50x objective

Despite its limitations, cytology is extremely valuable in evaluating effusions for the presence of malignant cells (Fig. 4 and Fig. 5). Diagnostic accuracy depends mainly on the observer’s ability and experience (Brudvig and Swenson, 2015), and on whether the causative neoplasia is exfoliative. The most challenging cases to diagnose are those in which no primary neoplasm can be found anywhere else (e.g. in the lungs, or the abdomen), like in cases with mesothelioma. Most dogs with mesothelioma require immunological testing or histology, apart from cytological evaluation, and even computed tomography has begun to be evaluated as a non-invasive diagnostic tool for this purpose (Watton et al., 2017). A flow chart of a basic microscopic examination of an effusion is presented in Fig. 6.

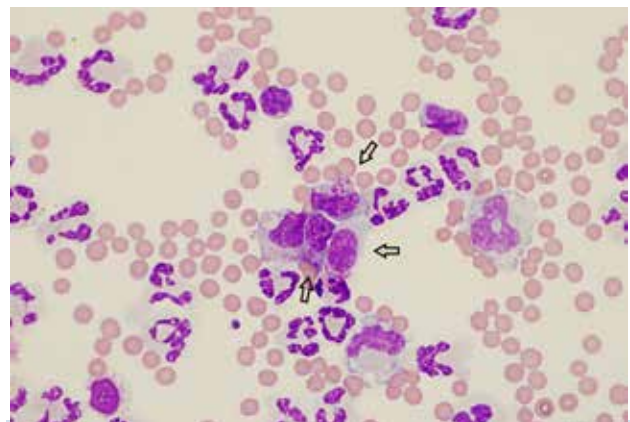


Fig. 5. Abdominal fluid (exudate) from a horse with intestinal lymphoma. Note the presence of intermediate-sized lymphocytes with large, magenta, cytoplasmic granules in the center of the image (arrows). Wright-Giemsa stain, 100x objective

Slide preparation: Opaque fluids usually just need a direct smear (pull or squash prep from well-mixed uncentrifuged sample) because of their high cellularity. Staining can be done using a variety of choices, as long as the cellular elements are depicted well and there is as little precipitate as possible to be confused with bacteria. It is important to prepare as many slides as possible, especially if they are going to be submitted in another laboratory. Generally, when submitting samples, the clinician should include an EDTA tube and at least one fixed stained slide. The slides should be prepared as soon as possible to avoid cell hypersegmentation and morphology alteration due to prolonged storage in the EDTA tube. However, unstained and/or unfixed slides are commonly requested by several laboratories.

The most difficult samples to prepare are those of low cellularity specimens. If centrifugation is not possible, a line smear can be made instead of the more traditional pull smear. This allows for the majority of

cells to appear more concentrated and thus more easily observed under the microscope. If centrifugation is possible, after removing the supernatant (which can be used for TP measurements as referred above) slide preparation can proceed with the known techniques.

The cell block method was first reported in human medicine more than 100 years ago but started to be used widely just in the mid-20th century. The technique was

developed as a way for liquid samples to be converted in solid material, thus provide sections in a cytologic sample that can be used for histopathology, immunohistochemistry and molecular biology testing. In veterinary medicine, cell block began to be used only in the last few years. It is not recommended as a replacement, but when used in combination with conventional cytology it increases the diagnostic accuracy (Koksal et al., 2013; Wallace et al., 2015; Assawasaksakul et al., 2017).

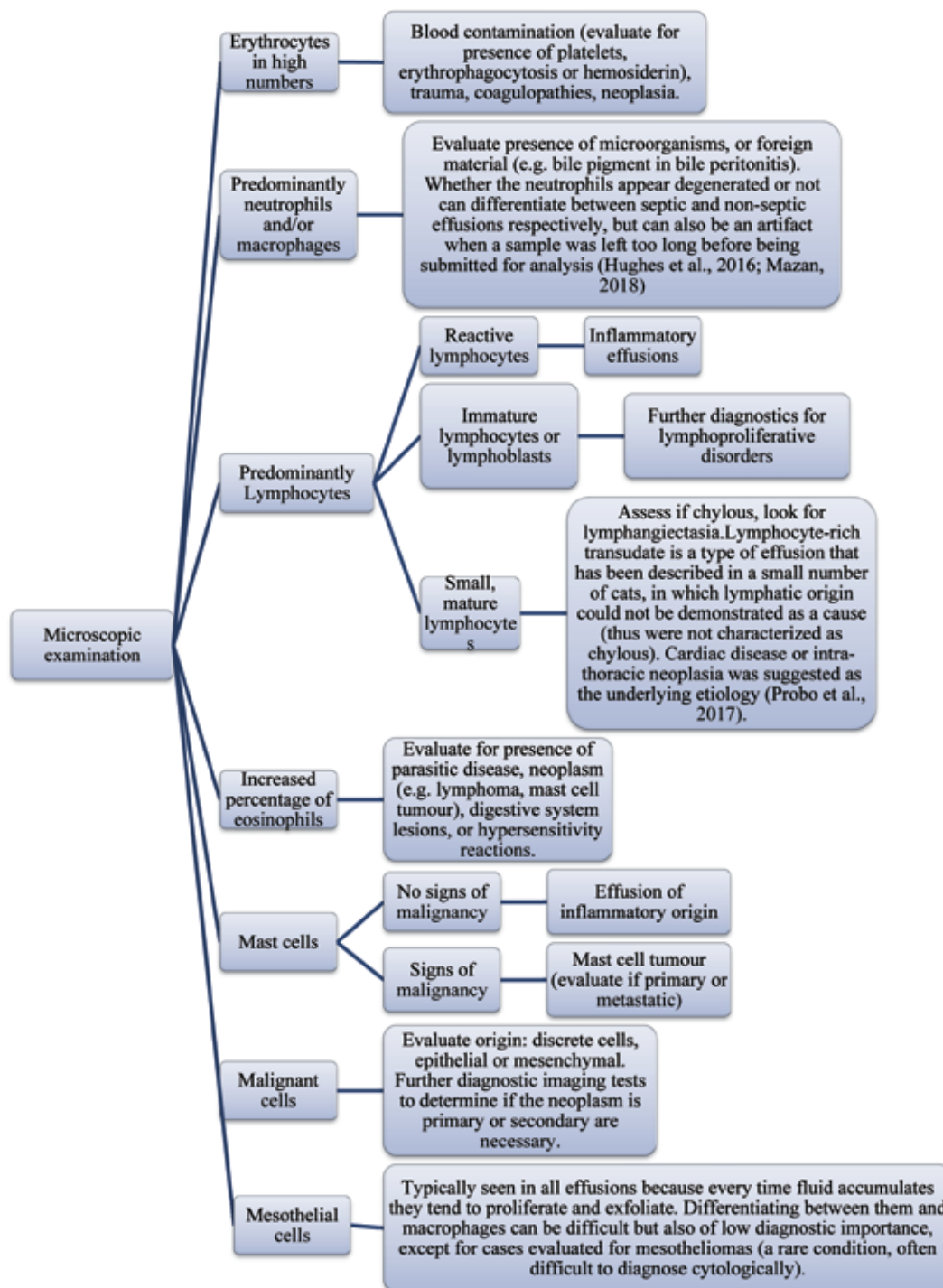


Fig. 6. Flow chart of the microscopic examination of an effusion

Table 3. Specific diagnostic tests for characteristic effusions

| Condition | Specific diagnostic tests |
|--|--|
| Haemorrhagic effusions | Effusion PCV > 10% |
| Pancreatitis | Effusion lipase activity > 4 times the upper reference limit for serum lipase activity Effusion/serum lipase ratio > 2 |
| Feline infectious peritonitis (FIP) | Rivalta's test (Fischer et al., 2012) Feline Coronavirus (FCoV) antibodies 1:1600 Effusion γ -globulin concentration >1.0 g/dL Effusion albumin/globulin ratio < 0.9 g/dL Immunofluorescent staining of FCoV antigens in effusion macrophages Nested reverse transcription PCR of effusions for FCoV |
| Chylous effusion | Effusion triglyceride concentration > serum triglyceride concentration Effusion cholesterol < triglyceride levels Effusion triglyceride concentrations >100 mg/dL |
| Uroabdomen | Effusion/serum creatinine ratio >2.0 Effusion/serum potassium ratio >1.4 Effusion creatinine >4 times greater than normal serum creatinine concentration |
| Bile peritonitis | Bile pigment crystals in effusion fluid (cytological evaluation) Effusion/serum bilirubin > 2.0 |
| Lymphosarcoma | Flow cytometry PCR for Antigen Receptor Rearrangements (PARR) |
| Septic exudates | Effusion TNCC > 13,000 cells/ μ L (Dempsey and Ewing, 2011) Blood glucose - effusion glucose concentrations difference >20 mg/dL Effusion lactate - blood lactate concentrations < 1.5 mmol/dL Phagocytized bacteria on cytology |

PCV: Packed Cell Volume, TNCC: Total nucleated cells

Cell blocks can be used for all effusion samples but they are more useful for the low cellularity fluid specimens. Advantages include separation of erythrocytes from nucleated cells (especially important in haemodiluted specimens, e.g. when only a few neoplastic cells are present among large concentrations of erythrocytes), maintenance of cellular architecture even when cell clusters are present, use of histochemistry and immunohistochemistry, storage of material and possibility to be re-examined later, cost-effectiveness, and simplicity, as it can be done using equipment present in most practice environments (e.g. microhematocrit centrifuge) (Marcos et al., 2017).

E. Effusion pathology and classification, traditional criteria and beyond

In 1972, the first study about the Light criteria was published. It involved a combination of 3 tests: TNCC, TP and LDH to help clinicians in human medicine distinguish between transudates and exudates. Since then, numerous studies have built upon these criteria in order to classify effusions. Forty years later, the Light criteria remain relevant; however, although they can correctly identify the vast majority of exudates, they misclassify almost 25% of transudates as exudates (Light, 2013). Many additional tests have

been suggested to improve the misclassification ratio.

In veterinary medicine, effusions have traditionally been classified according to TNCC and TP measurements (Table 2), with cell types and a few other characteristics taken into account. However, the traditional classification algorithm is occasionally criticized (Bohn, 2017). Multiple cut-off points suggested (Dempsey and Ewing, 2011), confusion over how the published recommendations were established and a large number of cases that seem to defy the classic criteria often restrict the clinician rather than provide a coherent diagnostic tool. Furthermore, in horses, TNCC in the pleural space can be up to 5000 cells/ μ L (Piviani, 2014; Mazan, 2018).

Classification according to the underlying cause of the effusion instead of a numerical value seems to be a more useful tool for clinicians, enabling them to choose further diagnostic approach more easily (Dempsey and Ewing, 2011). Additional tests for etiological diagnosis are shown in Table 3. The traditional effusion classification helps narrowing down the differential list, as it provides an insight on the mechanism that has resulted in their formation. However, effusions are not static, can be influenced by various factors (e.g. multiple resampling attempts may increase the TNCC

and/or TP) and despite of their mechanism do not always “fit the box”. A characteristic example of the latter is FIP. In the “wet” form, a cat can present with a pleural, abdominal or even pericardial effusion. These effusions are usually highly proteinaceous and could be classified as exudates according to the TP value, but are often moderately or even poorly cellular, which would bring them in the modified transudate category (Tasker, 2018). Moreover, effusion classification is not always diagnostic of a specific disease. For all these reasons, a holistic approach should be implemented, taking into account the history, clinical examination and other clinicopathological findings (such as imaging), alongside the laboratory analysis.

-Transudates: As there is no compromise in capillary permeability involved in the formation of transudates, cells are not leaked into the cavity. Thus, cell numbers are not increased but instead appear decreased due to dilution as fluid accumulates. The mechanism behind a transudative effusion involves passive fluid shifting due to increased hydrostatic or decreased oncotic pressure. The cause of this is most commonly severe hypoalbuminemia (serum TP <1.0 g/dL) resulting from conditions such as hepatic insufficiency, protein losing enteropathy (PLE), renal glomerular disease, malnutrition/malabsorption, or even iatrogenic. Other causes can include heart failure, portal hypertension or portosystemic shunt, and very early stages of uroabdomen.

-Modified transudates: Generally, modified transudates are formed due to leakage from blood vessels or lymphatics carrying protein-rich fluid. Conditions resulting in modified transudative effusions are numerous and include cardiovascular disease, neoplasms, feline infectious peritonitis (FIP), early stages of uroabdomen, hepatic disease, and, less commonly, glomerulonephritis, lung lobe torsion, and diaphragmatic hernias. Effusions containing blood elements are also usually classified as modified transudates. In these cases, care must be given in distinguishing blood contamination of the sample from intracavitary haemorrhage. Most noncoagulopathic, nontraumatic, spontaneous haemothorax/haemoabdomen, have neoplasms as the underlying cause (in dogs most commonly lymphoma, hemangiosarcoma, pulmonary carcinoma, mesothelioma, osteosarcoma, in cats most commonly hemangiosarcoma, hepatic carcinoma, mast cell tumor).

Exudates: They are associated with a variety of conditions, such as infectious diseases and noninfectious conditions, but they are generally inflammatory

in nature and occur in response to a foreign material within a cavity (whether this material is endogenous e.g. urine, bile, pancreatic enzymes, or exogenous e.g. fungi, parasites, bacteria). Cytological evaluation will determine the cell type that predominates in the effusion (Fig. 7 and Fig. 8). Exudates are usually subcategorized in septic (Fig. 9) and non-septic (Fig. 10) depending on the presence or absence of an infectious cause. Although the gold standard method for the diagnosis of a septic effusion is bacterial isolation, when it comes to patient management there often is little time to wait for culture results before starting treatment. Diagnosis can be helped by analyzing certain biochemical markers and by cytology.

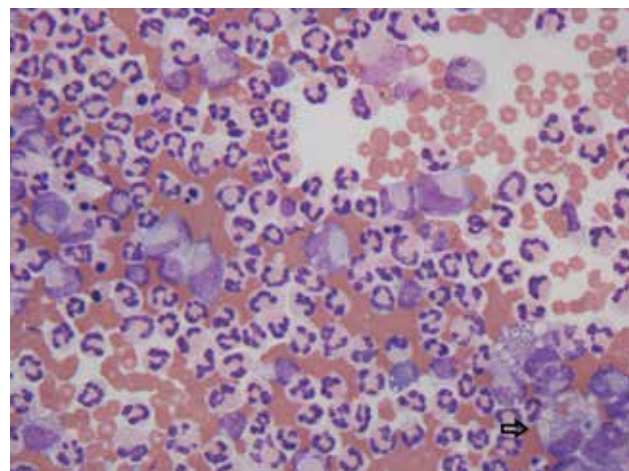


Fig. 7. Pleural fluid from a 12-year-old Golden Retriever dog with carcinoma. Mixed inflammation with non-degenerate neutrophils and reactive macrophages. Many pyknotic neutrophils are shown. Arrow showing a macrophage in the lower right corner exhibiting erythrophagia (evidence of recent or ongoing haemorrhage). Erythrophagia can also be present if the fluid is left to stand as macrophages can phagocytize erythrocytes in vitro. Cytoцентрифуг preparation, 50x objective

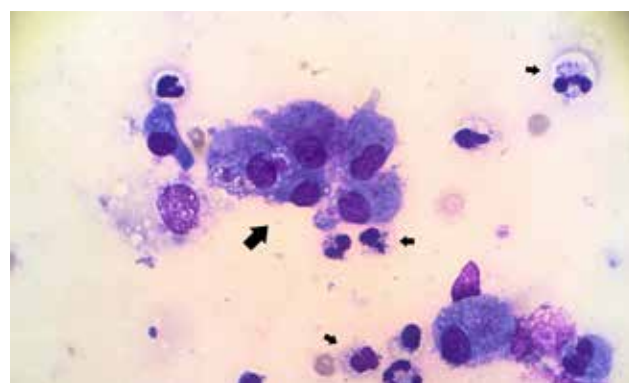


Fig. 8. Abdominal fluid from a dog with septic peritoneal and pleural effusion. Degenerate neutrophils with phagocytized rods (small arrows), and an aggregate of macrophages in the center (large arrow). Direct smear, 100x objective

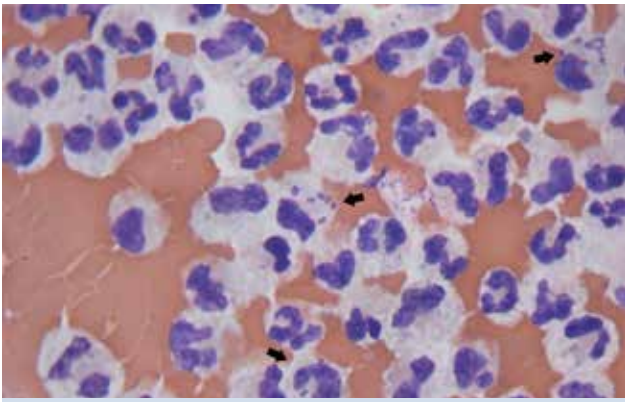


Fig. 9. Abdominal fluid from an 8-year-old Jack Russell with septic peritonitis. Neutrophils with signs of degeneration (nuclear swelling) and phagocytized cocci in a haemodiluted sample. Cytocentrifuged preparation, 100x objective

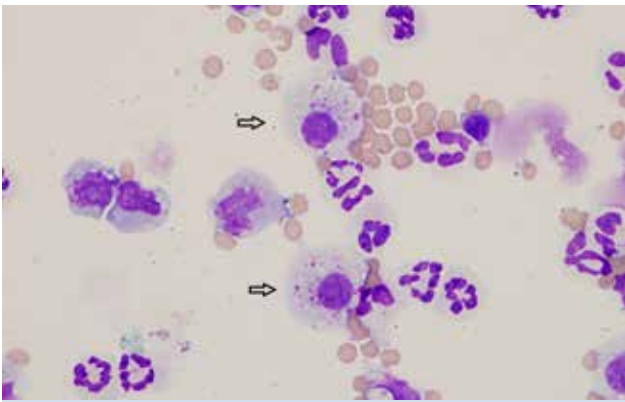


Fig. 10. Abdominal fluid from a horse with a history of colic surgery 3 days prior to abdominocentesis. Fluid analysis including cytology revealed a non-septic exudate with 12,000 leukocytes/ μ L, total protein 39 g/dL and predominantly non-degenerate neutrophils. Note the presence of vacuolated macrophages in the center of the image (arrows) which contain variably sized pink to purple cytoplasmic material, consistent with carboxymethyl cellulose (CMC) which was administered intraperitoneally during surgery to prevent adhesion. Wright-Giemsa stain, 100x objective

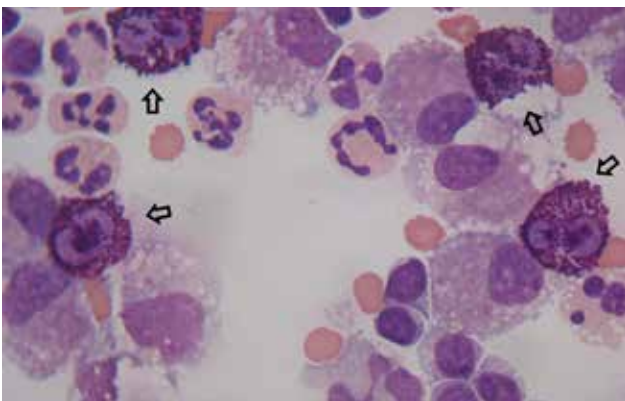


Fig. 11. Pleural fluid from a 4-year-old German Shorthaired Pointer with mast cell tumor. Four well-granulated, occasionally binucleated mast cells are noted. Mixed inflammation with neutrophils, macrophages and small lymphocytes is also evident. Cytocentrifuged preparation, 100x objective

Less commonly, an exudative effusion can form due to exfoliation of cells into a cavity, which can either be neoplastic (e.g. mesothelioma, mast cell tumors (Fig. 11), lymphomas) or reactive mesothelial (Fig. 12), or secondary to a chronic chylous effusion (Fig. 13).

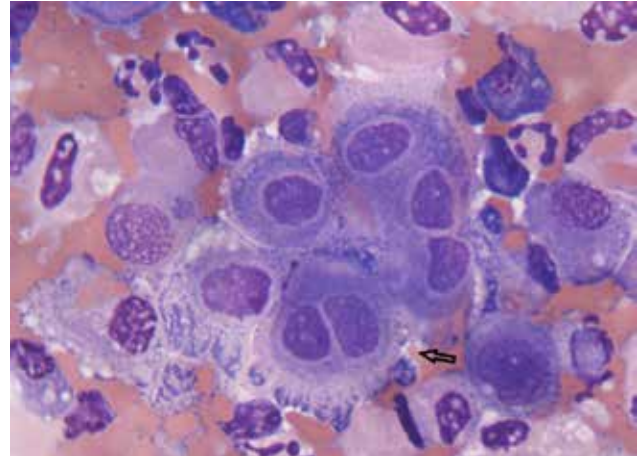


Fig. 12. Pleural fluid from a 6-year-old dog with a marked mixed inflammation secondary to a mast cell tumor. Poorly preserved neutrophils, and a cluster of reactive/dysplastic mesothelial cells with cytoplasmic basophilia are shown. Arrow showing a reactive binucleated mesothelial cell. Cytocentrifuged preparation, 100x objective

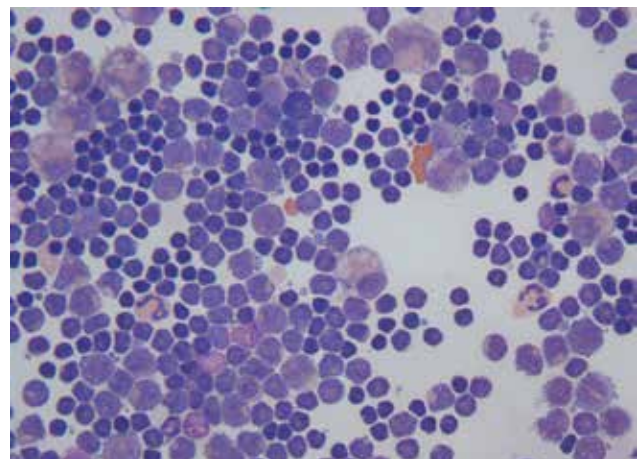


Fig. 13. Abdominal fluid from a 14-year-old DSH with chronic dyspnea, showing high numbers of lymphocytes (predominantly small) and occasional macrophages. The cat was diagnosed with cardiac disease. Cytocentrifuged preparation, 50x objective

F. Microbiology

Septic pleural effusions: The pathogenesis in the majority of pyothorax cases in companion animals, involves the aspiration of microorganisms that normally inhabit the oral cavity and upper respiratory tract. Most pleural effusions seem to be caused by polymicrobial infections. Most frequently involved microorganisms are presented in Table 4.

Table 4. Commonly isolated microorganisms in septic pleural and peritoneal effusions in dogs, cats and horses

| Pleural effusions | | |
|-----------------------------|---|--|
| | Dogs, Cats | Horses |
| Bacteria | <i>Escherichia coli</i> <i>Streptococcus spp.</i> <i>Staphylococcus spp.</i> <i>Corynebacterium spp.</i> <i>Pasteurella spp.</i> <i>Bacteroides spp.</i> <i>Fusobacterium spp.</i> <i>Actinomyces spp.</i> <i>Nocardia spp.</i> <i>Porphyromonas spp.</i> <i>Peptostreptococcus spp.</i> <i>Prevotella spp.</i> <i>Mycoplasma spp. (kittens, immunosuppressed adults)</i> | <i>Streptococcus equi subspecies zooepidemicus</i> (Stillion and Letendre, 2015; Reuss and Giguère, 2015) <i>Staphylococcus spp.</i> <i>Escherichia coli</i> <i>Pasteurella spp.</i> <i>Klebsiella spp.</i> <i>Enterobacter spp.</i> <i>Actinobacillus spp.</i> <i>Bordetella spp.</i> <i>Enterobacter spp.</i> <i>Salmonella enterica</i> <i>Clostridium spp.</i> <i>Bacteroides spp.</i> <i>Prevotella spp.</i> <i>Peptostreptococcus spp.</i> <i>Fusobacterium spp.</i> <i>Rhodococcus equi (immunosuppressed individuals)</i> <i>Mycoplasma spp. (controversial)</i> (Stillion and Letendre, 2015) |
| Fungi | <i>Candida albicans</i> (Bradford et al., 2013) <i>Cryptococcus spp.</i> <i>Blastomyces dermatitidis</i> | |
| Peritoneal effusions | | |
| Bacteria | <i>Escherichia coli</i> <i>Klebsiella spp.</i> <i>Bacteroides spp.</i> <i>Streptococcus spp.</i> <i>Clostridium spp.</i> <i>Enterococcus spp.</i> <i>Staphylococcus spp.</i> | Same as for dogs and cats |

Septic peritoneal effusions: In companion animals, most common etiology of septic peritonitis (Fig. 14 and Fig. 15) is perforation of an abdominal organ (usually the gastrointestinal tract) causing fluid containing bacteria to leak into the peritoneal space (Culp and Holt, 2010). Like in septic pleural effusions, in most septic peritonitis cases multiple microorganisms are often isolated, although Gram- bacteria are predominant (Table 4).

More recent case reports have indicated various other infectious microorganisms as potential causes for pleural effusions. Cytauxzoonosis is reported as an emerging tick-borne feline disease (Lloret et al., 2015; Alho et al., 2016). *Streptococcus equi* subsp. *zooepidemicus* (a commensal organism of horses) has been

reported as an emerging pathogen in dogs (Priestnall and Erles, 2011), having caused several outbreaks of haemorrhagic pneumonia. The particular subspecies has also been isolated from purulent pleural effusions in humans and seems to be of animal origin (Held et al., 2014). In dogs, *Bartonella henselae* was found in high prevalence in haemorrhagic pleural effusions but whether it plays a primary role in the pathogenesis, or it causes an opportunistic infection requires further research (Cherry et al., 2009; Weeden et al., 2017).

The diagnostic value of the microbiological analysis (Gram stain, culture, enrichment culture before PCR) is controversial. In human medicine, the routine use of microbiological testing of patients with parapneumonic effusions, was shown to be of very limited

value, having no impact whatsoever in treatment decision or mortality rate, and found to add very little to the diagnosis, compared to other diagnostic tools such as biochemical analysis (Jimenez et al., 2006).

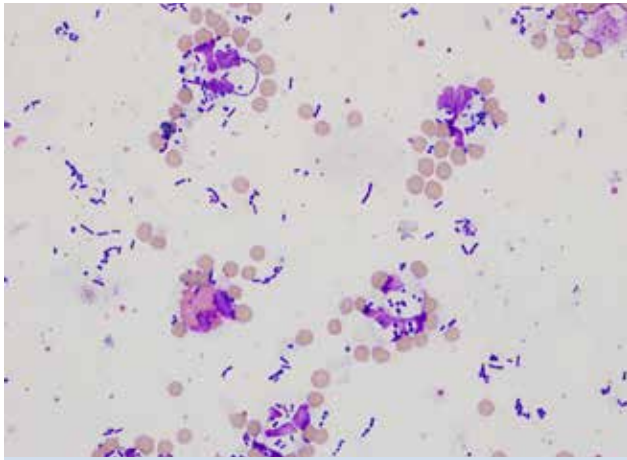


Fig. 14. Abdominal fluid from a horse, cytospin preparation. Septic exudate. Total nucleated cell count 89,000/ μ L, total protein 4.2 g/dL. Cytology revealed a predominance of frequently degenerated neutrophils with intracellular and extracellular bacteria (mixed population of cocci and rods). An eosinophil is also seen. The increased numbers of erythrocytes can be related to red cell diapedesis (due to serositis), secondary haemorrhage, and/or blood contamination during abdominocentesis. Wright-Giemsa stain, 100x objective

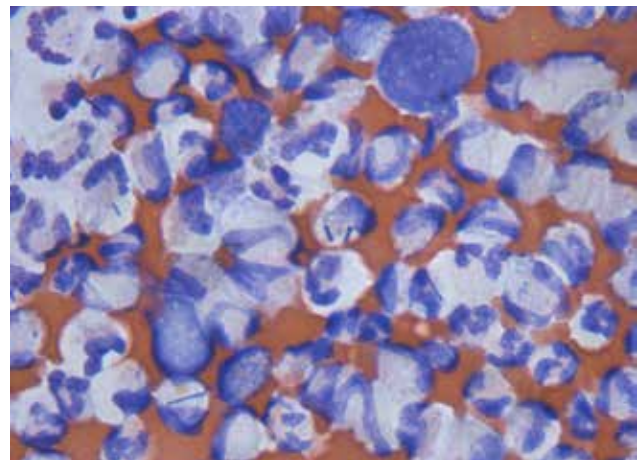


Fig. 15. Abdominal fluid from a 3-year-old Cocker Spaniel dog with septic peritonitis. Neutrophils with signs of degeneration and intracellular bacterial rods, against a bloody background (haemodiluted sample). Cytoцентрифуг preparation, 100x objective

CONCLUDING REMARKS

Cases involving thoracic and/or abdominal effusions occur frequently in a clinical setting and are often life threatening. Using the established algorithm for TNCC/TP parameters, is a useful initial step. The diagnostic tools described in this review are an important part of forming a complete picture that will help the practitioner to reach a diagnosis, develop effective therapeutic plans, and improve in-hospital stay and mortality rates.

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