Cytology in Ruminant and Camelid Practice

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Abstract

Cytology is often an overlooked diagnostic modality in food animal medicine. The reasons for this are varied but often revolve around unfamiliarity with the indications, procedures, methods of evaluation, or criteria for interpretation. Diseased tissues tend to respond with specific changes in cell type and architecture. Evaluation of these changes can aid in refining a diagnosis, establishing a prognosis and recommending specific treatment. Collection of cytological samples from both solid tissues and body fluids is a simple, efficient and economical procedure. The methods for collection and preparation of these samples including abdominocentesis, thoracocentesis, pericardiocentesis, tracheal wash aspirates and cerebral spinal fluid are described. Interpretation of cytological and fluid analysis is also discussed.

Résumé

La cytologie est un outil diagnostic souvent ignoré en médecine des animaux de consommation. Cette ignorance peut s'expliquer de plusieurs façons mais dénote généralement un manque de familiarité avec les procédures, les méthodes d'évaluation ou les critères d'interprétation de même que les situations les plus appropriées à son utilisation. Des changements spécifiques dans le type de cellules et leur architecture sont souvent associés aux tissus malades. L'évaluation de ces changements peut aider à raffiner le diagnostic, à établir un pronostic et à recommander un traitement particulier. La cueillette d'échantillons cytologiques à partir de tissus et de fluides corporels est une procédure simple, efficace et économique. Les méthodes pour la cueillette et la préparation des échantillons sont décrites pour l'abdominocentèse, la thoracocentèse, la péricardiocentèse, l'aspiration trachéale et les fluides cérébrospinaux. L'interprétation de l'analyse cytologique et des fluides est aussi discutée.

Introduction

A thorough review of diagnostic cytology in livestock was recently published and will serve as the primary reference for this paper.[1] The premise of cytological evaluation in clinical practice is that diseased tissues respond to injury and inflammation in a predictable manner represented by the fluid and cytological characteristics of the tissue. Injured or inflamed tissues respond with a common cascade of inflammatory mediators, including a diverse collection of cytokines and prostaglandins. These mediators direct the recruitment of inflammatory cells into the affected region, as well as initiate vascular changes resulting in altered blood flow and vascular leakage. Ultimately, this results in the classic signs of inflammation including hyperemia (redness and heat), extracellular fluid accumulation (swelling or effusion), nerve receptor sensitivity (pain) and inflammatory cell accumulation (exudates).

Fluid accumulation due to increased vascular permeability and decreased lymphatic drainage is a fundamental aspect of the inflammatory process. In solid tissues, this results in swelling. However, in body cavities this results in free fluid accumulation referred to as effusion. An effusion can be classified as a transudate, modified transudate, or an exudate based on the fluid characteristics (Table 1). A **transudate** is characterized as a fluid with a normal or low protein concentration and cell count. These primarily result from vascular leakage due to increased hydrostatic pressure without significant inflammation. **Modified transudates** have increased protein concentration but normal cell counts and cell type. An **exudate** is an effusion that has both an increased protein concentration and cell count. The percentage of inflammatory cells such as neutrophils is

Table 1. Characterization of effusions.

Effusion	Total Protein	Cell Count	Cell Type
Normal	<3 g/dl	1000-5000 /ul	Mesothelial/Macrophage
Transudate	<3 g/dl	<1000 /ul	Mesothelial/Macrophage
Modified Transudate	>3 g/dl	<5000 /ul	Mesothelial/Macrophage
Exudate	>3 g/dl	>5000 /ul	Neutrophil/Macrophage

also increased in an exudate. Exudates may be septic or non-septic. Septic exudates are characterized by degenerative neutrophils and the presence of intra- and/or extracellular bacteria.

Along with identifying inflammatory processes, cytology is very useful in diagnosing neoplastic conditions. Aspirates of solid tissue masses and fluid accumulations can lead to the identification of specific neoplastic cell types. Of all the uses for cytology, differentiating between a septic or inflammatory versus a neoplastic process may be one of the most clinically significant indications as this will lead to a very different assessment of treatment options and prognosis.

Sample Processing

Proper care and processing of samples is critical in obtaining relevant cytological evaluation. Fluid samples should be divided between an EDTA tube for cytology and a sterile container or other suitable bacterial culture storage media as soon as possible after collection. EDTA increases the refractive index of samples. Therefore, EDTA tubes must be filled at least one-quarter full to avoid false elevation of total protein determination by refractometer. Samples for aerobic bacterial culture may be placed in any sterile tube or may be kept in a capped syringe. The preferred method for submitting fluid samples for anaerobic culture is to apply the sample to a sterile swab and place in Port-A-Cul™ transport tube[a]. Alternatively, samples can be placed in a capped syringe with all of the air removed. In most cases, it is best to refrigerate samples until submission. Timely submission of culture samples is critical for obtaining accurate results.

Fluid smears and tissue impressions may be made in the field and await staining in the laboratory. Fluid smears are made in the same manner as blood smears. For samples with suspected low cellularity, a single drop may be placed on a slide and allowed to air dry without smearing. An alternative method relies on the fact that nucleated cells tend to be dragged with the leading edge of a smear. In this method, a normal smear is made but the slide is stopped before developing the feathered edge. This last portion is allowed to air dry and will often have higher cellularity. Alternatively, the sample may be centrifuged and the cellular sediment may be directly applied to the slide in the same manner as urine sediments are performed.

Impression smears can be very useful for rapid diagnosis of tissue masses. A freshly cut surface of the tissue mass is first blotted onto a paper towel and then blotted directly to the slide and allowed to air dry. Some tissues do not exfoliate well and it may be necessary to gently scrape the surface with a scalpel blade. The tissue is then blotted on a slide and the cellular debris on the scalpel blade may be applied to another slide. Squash preps are also useful in evaluating biopsy tissues. A small piece of tissue is applied to a slide and a second slide is placed over the specimen at right angles. The two slides are pressed together to compress the tissue and the top slide is moved off the end of the bottom slide.

Cytology specimens may be stained with either Wright's stain or a modified Wright's stain such as Diff-Quik[b]. These stains will not differentiate gram-negative and gram-positive bacteria, so it is useful to also have slides available for gram staining. Most private practices utilize Diff-Quik® stain in the field. Pathologists tend to prefer Wright's stain so it is useful to always prepare additional unstained slides for further review by a pathologist if necessary.

Tracheal Wash Aspirate

A tracheal wash aspirate is the single best way to accurately determine the pathological character of respiratory disease and determine a specific etiology. The transtracheal wash technique is a simple procedure that can be performed in the field with basic head restraint. To perform this procedure, the animal is restrained manually (small ruminants and young camelids) or in a head catch (adult camelids). The ventral neck is clipped in the mid-trachea region and prepared with a surgical scrub. While palpating the trachea, a lidocaine bleb is placed under the skin and in the subcutaneous tissue over the trachea. A stab incision is made with a #15 scalpel blade. A 10-gauge, 2-inch needle is placed through the tissue into the trachea. The tip of the needle is used to identify a space between tracheal cartilage rings. Once in the lumen of the trachea, polypropylene tubing is inserted through the needle and into the trachea down to a level approximately 10 cm beyond the thoracic inlet. Twenty to 30 mL of sterile saline or lactated Ringer's solution is infused into the trachea and then aspirated back into the syringe. You will typically recover only 5 to 10 mL of the solution. This can be repeated an additional two times if necessary without adverse effect. A portion of the sample can be transferred to an EDTA tube and the remaining sample is kept in the syringe or transferred to a sterile tube. Tracheal wash specimens can also be collected by endoscopy via the biopsy port.

Since tracheal wash specimens are diluted to differing degrees, total cell counts and protein measurements are not routinely performed. Cytologic evaluation is performed to evaluate possible inflammatory processes indicated by an increase in percentage of neutrophils. Normal tracheal wash samples will contain airway epithelial cells and predominantly macrophages. Neutrophils should be less than 30% of the total white blood cells. Elevated percentage of neutrophils is suggestive of an inflammatory process. This would suggest either

a septic or non-septic (toxic, immune-mediated, irritant, parasitic) process. Degenerative neutrophils are generally associated with septic processes. The presence of eosinophils suggests either parasitic pneumonia (*Dictyocaulus viviparous* in cattle, or *Dictyocaulus filarial*, *Protostrongylus rufescens* and *Muellerius capillaries* in small ruminants) or an immune-mediated process such as allergic pneumonitis. Bacterial cultures should routinely be submitted for any inflammatory wash. Virus isolation may be performed on tracheal wash specimens, and is a very good sample for the detection of bovine respiratory syncytial virus (BRSV). Plant material, squamous epithelial cells, pollen, rumen protozoa and fungal elements are often observed in tracheal wash aspirates of cattle. These are often natural contaminants or contaminants that occur due to aspiration of pharyngeal contents during the procedure.

Abdominocentesis

Collection of abdominal fluid for analysis is a useful procedure in assessing abdominal disease in small ruminants and camelids. It will aid in the diagnosis and differentiation of ascites, peritonitis, intestinal crisis, enteritis, uroperitoneum and abdominal neoplasia such as lymphosarcoma. The two common sites for collecting abdominal fluid are the cranial abdomen and the caudal flank. In small ruminants, cranial abdominal abdominocentesis is performed about 2-3 inches cranial to the umbilicus and 2-3 inches to the right of midline. In camelids, fluid may be more easily recovered either on midline or further lateral to midline due to a layer of intra-abdominal fat located just lateral to the linea alba. The area is clipped and prepped with surgical scrub. Lidocaine is locally infiltrated under the skin and into the deeper muscles. A stab incision is made through the skin and into the muscle layers, feeling the tougher fascial planes as you penetrate them. Typically, a 3-inch metal teat cannula is then inserted through the incision and into the abdomen. In many cases, fluid is not obtained with a teat cannula in normal animals. Sometimes, use of an 8- to 10-inch bitch catheter will allow access to distant pockets of fluid for collection. Alternatively, a sleeved trocar can be inserted into the abdomen. After removing the trocar, a flexible feeding tube can be placed through the sleeve and into the abdomen. Fluid will either flow out through the feeding tube or come out through the sleeve using the feeding tube as a wick. Samples are collected directly into purple-top and red-top tubes.

The alternative site is the caudal flank. The procedure can be performed on either the left or right sides but usually the right side is used. The caudal flank is clipped in the thin-haired region just cranial ventral to the stifle. Generally, no local anesthesia is used. The sample is collected by inserting multiple 18-gauge, 1-1/2-inch needles through the skin and body wall into the abdomen. Often, several needles will allow fluid to be collected more readily. Samples are collected directly into purple-top and red-top tubes.

Normal abdominal fluid is clear, colorless to straw-colored fluid. It should not clot in the EDTA tube, but may clot in the red-top tube. Clotting of the sample does not correlate well with either total protein or fibrinogen, and should not be used as criteria for assessing the fluid. Normal abdominal fluid will have a total protein <3 g/dl and a total WBC count <5000 /ul. The cells should consist of primarily mesothelial cells and macrophages with <30% neutrophils. The neutrophils should be intact and non-degenerate. Eosinophils are often present in normal abdominal fluid and may represent up to 70% of the WBCs. Pregnant animals may have a larger volume of fluid with lower total protein and cell count, consistent with a transudate. Modified transudates (increased TP and normal WBC) are often seen with enteritis. Exudates (increased TP and increased WBC) are observed with inflammatory processes and usually indicate peritonitis. They are commonly observed with traumatic reticuloperitonitis, perforating abomasal ulcers, abdominal abscess, uterine rupture, post-surgical peritonitis, and intestinal crisis such as obstruction, ulceration with perforation, intestinal volvulus or intussusception. Eosinophils <10% and neutrophils >40% are indicative of peritonitis in cattle, however, this has not been established in small ruminants or camelids. Normal abdominal fluid generally contains <20% lymphocytes. Lymphocyte counts >50% are suggestive of possible lymphosarcoma and should be submitted for pathologic examination.

The abdominal fluid following routine exploratory celiotomy will routinely show inflammatory exudates. This makes it difficult to evaluate whether or not post-operative sepsis has occurred. Generally, the post-operative abdominal fluid will contain non-degenerative neutrophils unless there is bacterial contamination, in which case it will contain degenerative neutrophils. The observation of intracellular and extracellular bacteria also indicates post-operative sepsis.

Thoracocentesis

Thoracocentesis should be performed any time breath sounds are attenuated in the ventral thorax or when pleural effusion is demonstrated by thoracic radiographs or ultrasound. Thoracocentesis is both a diagnostic and therapeutic procedure and is most commonly used in cases of septic pleuritis, either due to pleuropneumonia or to traumatic reticulopleuritis. Thoracocentesis is performed in a manner similar to abdominocentesis. A suitable area over the 6th or 7th

intercostal spaces is clipped and prepared with surgical scrub. Staying caudal to the 6th rib minimizes the risk of contacting the heart when performing the procedure. The site for thoracocentesis is best determined by ultrasound. Otherwise, choose a site in the 6th or 7th intercostal space, dorsal to the olecranon and ventral to the line of dullness observed on thoracic auscultation and percussion. The area is infiltrated with lidocaine and a stab incision is made on the cranial aspect of the 7th or 8th rib. A 3-inch teat canula is inserted through the incision and into the thorax. A painful response is often elicited upon piercing the pleura. Generally, fluid will freely flow from the cannula and can be collected into a purple top and red top tube. An extension set can be attached to the cannula and the fluid can be drained by gravity flow or by attaching a 3-way stopcock and a 60 mL syringe.

The fluid analysis for thoracic fluid is similar to peritoneal fluid, except that eosinophils are less commonly observed. Inflammatory exudates are suggestive of septic pleuritis caused by either pleuropneumonia, reticuloplеuritis, or penetrating wound. Drainage of the fluid will often improve the animal's condition and allow time to institute other appropriate treatment.

Pericardiocentesis

Pericardiocentesis is used to collect pericardial fluid in conditions where pericardial effusion is suspected such as infectious pericarditis, traumatic reticulopericarditis or cardiac lymphosarcoma. Clinical signs that are suggestive of pericardial effusion include tachycardia, distended jugular veins, brisket or ventral abdominal edema, muffled heart sound, "washing machine-like" cardiac murmur, or evidence of thoracic pain. Radiographs or cardiac ultrasound can confirm the presence of pericardial effusion.

Pericardiocentesis is performed at the left 5th intercostal space, 1-3 inches dorsal to the olecranon. The area is clipped and prepared with surgical scrub. Lidocaine is infiltrated into the area to the level of the pleura. A stab incision is made with a #15 scalpel blade through the skin and deep into the muscle just cranial to the 6th rib. A 3-inch teat cannula or a 16- or 18-gauge, 3-1/2- to 5-inch spinal needle is carefully inserted through the incision and into the pericardial space. Occasionally, the teat cannula may not be able to enter the pericardial space because the pericardial membrane is oftentimes thickened and very tough. In these cases, a spinal needle should be used. However, care must be taken when using a spinal needle as it is easier to inadvertently contact the ventricle and initiate an arrhythmia or to enter the left ventricle and cause a hemopericardium. Once in the pericardial space, fluid should freely flow from the canula or the needle. The sample may be drained by gravity flow, or an extension set and syringe may be connected to aspirate the fluid. Potential complications include acute cardiac arrhythmia or hemopericardium, both of which can result in sudden death. However, relief of pericardial effusion often dramatically improves the cardiac function of the patient and will allow time for additional treatment.

Fluid analysis of pericardial fluid is similar to thoracic and abdominal fluid. An exudate is consistent with septic pericarditis, most commonly observed with traumatic reticulopericarditis. The other common finding is a fluid with a high percentage of lymphocytes indicative of cardiac lymphosarcoma. In both cases, these patients are often severely compromised due to cardiac tamponade and the resulting cardiac failure. Removal of pericardial effusion will often dramatically improve stroke volume and cardiac function. While the long-term prognosis for these animals is poor, they can occasionally be maintained for weeks to months in order to salvage a valuable late term pregnancy.

Cerebrospinal Fluid Analysis

Cerebrospinal fluid analysis is used to help differentiate a variety of neurological conditions in small ruminants and camelids including septic, toxic, parasitic, neoplastic and traumatic causes of neurological disease. Lumbosacral CSF taps are typically performed, since they can be performed on conscious animals with no or minimal chemical restraint. The procedure is most easily performed with the animal standing or in dorsal recumbency. It can be done in lateral recumbency as well, but this tends to be more difficult to assess the positioning of the needle. The lumbosacral space is located on midline, just caudal to a line extending between the cranial aspects of the tuber coxae. You can palpate a noticeable depression at the lumbosacral space. An 18- or 20-gauge, 3-1/2 inch needle is used in small ruminants and alpacas. An 18-gauge, 5- or 7-inch needle may be required in larger llamas. The area is clipped and prepared with surgical scrub. The local site is anesthetized with lidocaine and a stab incision is made with a #15 scalpel blade in the middle or caudal third of the lumbosacral space. The spinal needle is positioned parallel to the longitudinal plane and tilted slightly caudally from the transverse plane. One hand holds the hub of the needle to steady the position and the other hand is used to gently insert the needle in 1/2-inch increments near the body. This technique helps minimize curving and misdirection of the needle. As the needle progresses through the tissues, one should feel the tough supraspinal ligament and then the interspinal ligament before you reach the dura mater sheath. The animal will typically flinch when the dura mater sheath is touched or pierced. At this time, the stylet is

removed and the hub of the needle is watched for free flowing spinal fluid. Twisting or slight repositioning of the needle may be required to get fluid. Fluid may be collected into a syringe and then placed in both purple top and red top tubes.

Because of the low protein and cell counts of CSF fluid, these samples are best analyzed at a medical laboratory. They should be refrigerated and submitted within one hour of collection. If a veterinary laboratory is not nearby, the samples may be submitted to a human clinical pathology laboratory at a nearby hospital. There are many referenced reports of normal protein and cell count values. The fluid should be clear and colorless. Cloudy fluid may indicate either increased protein concentration or cell count. In general, the total protein of normal CSF is <60-100 mg/dl and the cell count is <5-10 WBC/ul. Some amount of blood contamination is a frequent occurrence and may elevate the WBC count. While the contribution of WBCs from peripheral blood is debated, it should be less than 1 WBC per 500 RBCs. This correction will allow further assessment of the WBC count when peripheral blood contamination is encountered.

The interpretation of CSF must be made relative to the signalment, history and clinical signs of the animal. Many viral, toxic and metabolic CNS diseases may show no abnormalities in the CSF. Alternatively, at more advanced stages they may show increased protein and/or increased cell counts. Typically, if the cell count is elevated it will be a mononuclear pleocytosis (presence of greater than normal number of mononuclear cells in the CSF). With septic conditions, there will typically be an increase in neutrophils (>20%). A neutrophilic pleocytosis is observed with bacterial meningitis, and CNS or vertebral body abscesses. Listeriosis typically shows a mononuclear pleocytosis that can be either a combination of macrophages and lymphocytes, or in some cases a specific increase in lymphocytes. Eosinophils are not normally seen in CSF. The presence of eosinophils is highly suggestive of aberrant parasite migration such as *Parelaphostrongylus tenuis* (the meningeal worm of white tailed deer) in small ruminants and camelids.

Tissue Aspirates

Tissue aspirates are very helpful in diagnosing and differentiating inflammatory, septic and neoplastic conditions in tissues. Tissue aspirates are typically performed by two different techniques. In the first technique, a needle is attached to a 10 mL syringe and inserted into the tissue of interest. Gentle aspiration is applied with the syringe as the needle is repositioned into different areas. Needles ranging in size from 25- to 18-gauge are typically used. Smaller-gauge needles tend to result in less tissue bleeding and peripheral blood contamination of the sample. However, larger needles may sometimes facilitate obtaining appropriate cytological samples. Once the aspirate is obtained, the needle is separated from the syringe, the syringe is filled with air, reattached and used to apply the sample to a glass slide. A standard smear is then made, allowed to air dry and stained.

The second method for obtaining tissue aspirates is to use a larger size needle (22-18-gauge) and repeatedly insert it into the tissue site without using a syringe to aspirate. This method works well for solid tissue tumors and tends to cause less bleeding. The needle is then attached to a syringe filled with air and the contents of the needle are sprayed onto a glass slide. A standard smear is made, air dried and stained.

The primary goal of in-house tissue aspirates is to distinguish between an inflammatory and a neoplastic process. Inflammatory aspirates will contain numerous WBCs. Although these are typically neutrophilic, a preponderance of eosinophils is sometimes observed in non-specific cellulitis in cattle. An inflammatory aspirate should be examined closely for the presence of intracellular and extracellular bacteria. If present, it is advisable to also perform a gram stain and to submit an aspirate for bacterial culture. Tissue aspirates are especially helpful in quickly diagnosing or confirming clostridial cellulitis. The clostridial organisms tend to be large, blocky gram-positive rods that are present in fairly large numbers. It should be cautioned that the *Clostridia* obtained from many *in vivo* samples may not have the classic tennis racket or paperclip morphology associated with spore formation.

Tissue aspirates can also help confirm a diagnosis of neoplasia in small ruminants, llamas, and alpacas. The differentiation of normal cells and neoplastic cells is more difficult than the identification of an inflammatory process. Common neoplastic tumors observed in small ruminants and camelids include lymphosarcoma, squamous cell carcinoma, mammary adenocarcinoma and hepatic tumors. Aspirates of enlarged lymph nodes or tissue masses may be helpful in confirming a diagnosis of neoplasia. It is difficult to get good needle aspirates of squamous cell carcinomas. Instead, a small biopsy may be obtained for an impression smear, or a scraping of the cut surface of the tumor may be used for cytology. These samples will often show pleomorphic epithelial cells with an eosinophilic keratin background.

Liver aspirates and biopsies can be used for diagnosing hepatic disease including hepatitis, hepatic lipidosis and neoplasia. The location of the liver is variable between individual animals, and it is recommended that this be done following ultrasound identification of the liver. In general, the liver can be identified between the 9th and 11th rib spaces above the costochondral junction. Once the liver is identified, an area is clipped and

prepared with surgical scrub. For liver aspirates, a 22-gauge, 3-inch needle is passed through an 18-gauge guide needle through the skin and body wall into the liver. Resistance is felt as the needle enters the liver. Aspiration is applied with a 10 mL syringe and the needle is moved back and forth within the liver. Release the suction and remove the needle. The sample is then applied to a glass slide, smeared and stained. Liver biopsies are obtained from the same site using a tissue biopsy instrument. Normal hepatocytes have a diameter approximately 5-6 times that of an RBC and a nucleus about twice as large as a RBC. Hepatic lipidosis can be graded based on the accumulation of fat vacuoles and the development of pyknotic nuclei. Excessive fatty vacuoles and the presence of pyknotic nuclei are consistent with severe hepatic lipidosis and carry a poor prognosis. Occasionally, neutrophils are also observed in the aspirates. They are suggestive of hepatic abscessation, peritonitis, enteritis, or hepatitis.

Other Uses of Cytology

There are many other indications and uses for diagnostic fluid analysis and cytology in small ruminants and camelids. An assortment of these other techniques, indications, and clinical examples will be presented at the meeting.

Footnotes

[a]Port-A-Cul Transport Tube, BD, Franklin Lakes, NJ
[b]Diff-Quik Stain, International Medical Equipment, Inc. San Marcos, CA

References

1. Bohn AA, Callan RJ: Cytology in food animal practice. *Vet Clin North Am Food Anim Pract* 23(3):443-479, 2007.

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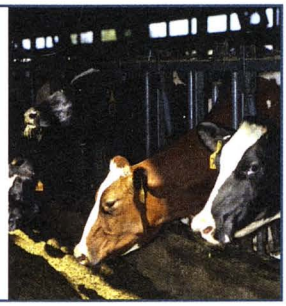

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