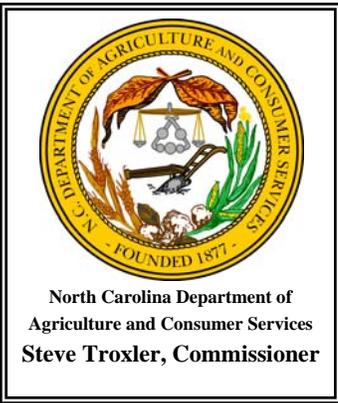


The NCVDLS REPORT



Veterinary News and Information From North Carolina's Diagnostic Laboratories



North Carolina Department of Agriculture and Consumer Services
Steve Troxler, Commissioner

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Holiday Closings...

April 2, 2010
May 31, 2010

Our laboratories will be closed on the above listed days.

Please e-mail NCVDL@ncagr.gov with any comments and/or suggestions concerning The NCVDLS Report
Editor - Dr. Tim McComb

Message from the Director

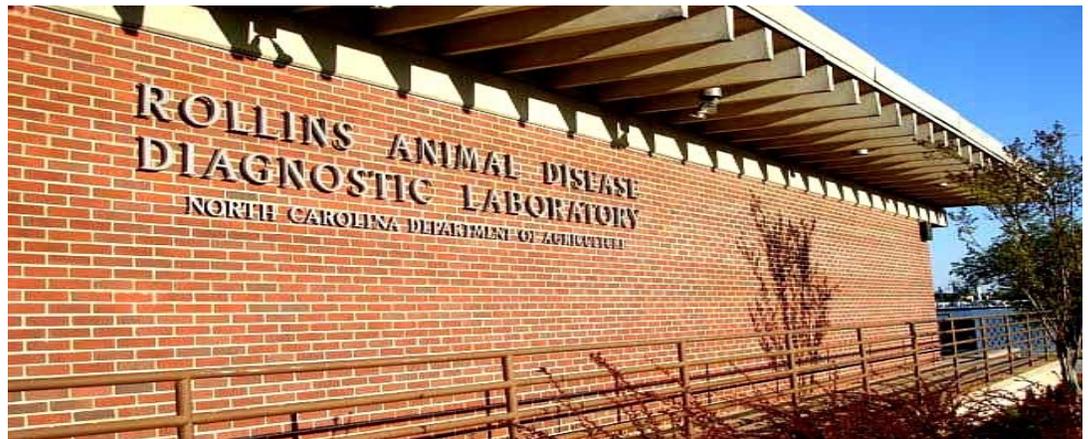
Welcome to this issue of THE NCVDLS Report, the first for the New Year and for a new decade. We hope our clients will find the information that we have gathered to be both interesting and informative.

We urge you to check out our newly revamped website at: <http://www.ncagr.gov/vet/ncvdl/>. Recently, the site underwent a major overhaul that included changing the color scheme to become aligned with that of the Department of Agriculture. For those clients that obtain results on the website, you will need to update the URL/address in your list of favorites in order to be able to access your account.

Since the last newsletter, a disease has been diagnosed for the first time in North Carolina hogs: pandemic H1N1 2009 influenza virus. This virus was and has continued to be erroneously referred to by the press as “swine flu”. The Molecular Diagnostic and Virology sections of the NCVDLS have various tests and levels of detail with which swine influenza is diagnosed. The initial diagnosis of respiratory disease in swine as influenza only determines that animals are infected with a type A virus, usually H1 or H3. Although the NCVDLS has the capability to isolate influenza viruses and determine if they are H1N1, viruses must be sent to a reference laboratory for DNA sequencing to determine if they are truly pandemic H1N1 2009. Please note that these referrals will not be done without the permission of the submitting veterinarian or client.

Wishing you all a happy, healthy and prosperous New Year!
Sincerely,

Karen W. Post DVM, MS



Client Corner

Equine Infectious Anemia submission forms

Dear NCVDL Client,

Due to the state budgetary crisis and in a further effort to comply with the Governor's mandate for state agencies to reduce their costs of operation, we will no longer be printing or routinely supplying specimen submission forms for equine infectious anemia testing to our clients. In the future, you may order VS 10-11 forms from the regional USDA/APHIS Veterinary Services office in Raleigh. The contact person is Mr. Jeff Denton and he may be reached at 919-855-7705. He will fax the order forms to accredited practitioners. During this transitory period, we will maintain a small supply of VS 10-11 forms for emergencies at our central Rollins Laboratory. Please contact the lab at 919-733-3986 for these types of requests.

Karen W. Post, DVM, MS, D-ACVM
Director of Laboratories

New In-house Immunohistochemistry Tests

In the first quarter of 2010, the NCVDLS biopsy service will be offering in-house immunohistochemical tests for T lymphocytes, B lymphocytes and histiocytes. These tests are based on detection of cell surface molecules (clusters of differentiation) and are useful in refining the diagnosis of round cell tumors, particularly poorly differentiated tumors that may not have an obvious tumor type based on cell morphology. In addition, lymphomas from B (bone marrow derived) and T (thymus derived) lymphocytes may have different biologic behavior and response to therapy but the neoplastic cells can be morphologically similar. Immunohistochemistry can be useful in differentiating the two. Recommendations for immunohistochemical testing will be made by the pathologist in the comment section of the biopsy report you receive. If you elect to pursue these tests, notify the pathologist on the case. Testing will be performed weekly, reducing the turnaround time you may have experienced previously when samples were sent to outside laboratories. Additional tests for melanocytes will be offered later in the spring. The tests will be available individually and in panels, at a cost of \$35.00 for one, \$55.00 to two, and \$75.00 for three on any particular section or tumor.

If you have any questions, please contact one of the pathologists at the NCVDL, Rollins Laboratory.

Alison Tucker, MA, VMD, Dipl. ACVP

Feature Article

Cytology: A valuable non-invasive diagnostic tool with limitations

Steven Rushton DVM, Diplomate American College of Veterinary Pathologists
Rollins Diagnostic Laboratory
Raleigh, North Carolina

Cytology can provide a quick and non-invasive way for diagnosing a number of common diseases involving the skin and subcutaneous tissues in large, small, and exotic animal practice. Cytology is able to provide reliable diagnoses of a number of benign and malignant tumors, such as: histiocytomas, squamous cell carcinomas and mast cell tumors as well as inflammatory reactions.

Cytology does however have major limitations including lack of tissue orientation (compared to histopathology) along with possibility of cells present not being representative of the lesion (inflammatory cells associated with a neoplasm).

Even in situations where the definitive diagnosis is difficult to make, cytology can often narrow the potential diagnoses down to epithelial, mesenchymal, or round cell tumors and may even help determine if the lesions are inflammatory.

The number one reason for a non-diagnostic sample or inconclusive diagnosis is the absence or scant number of nucleated cells on the slides submitted.

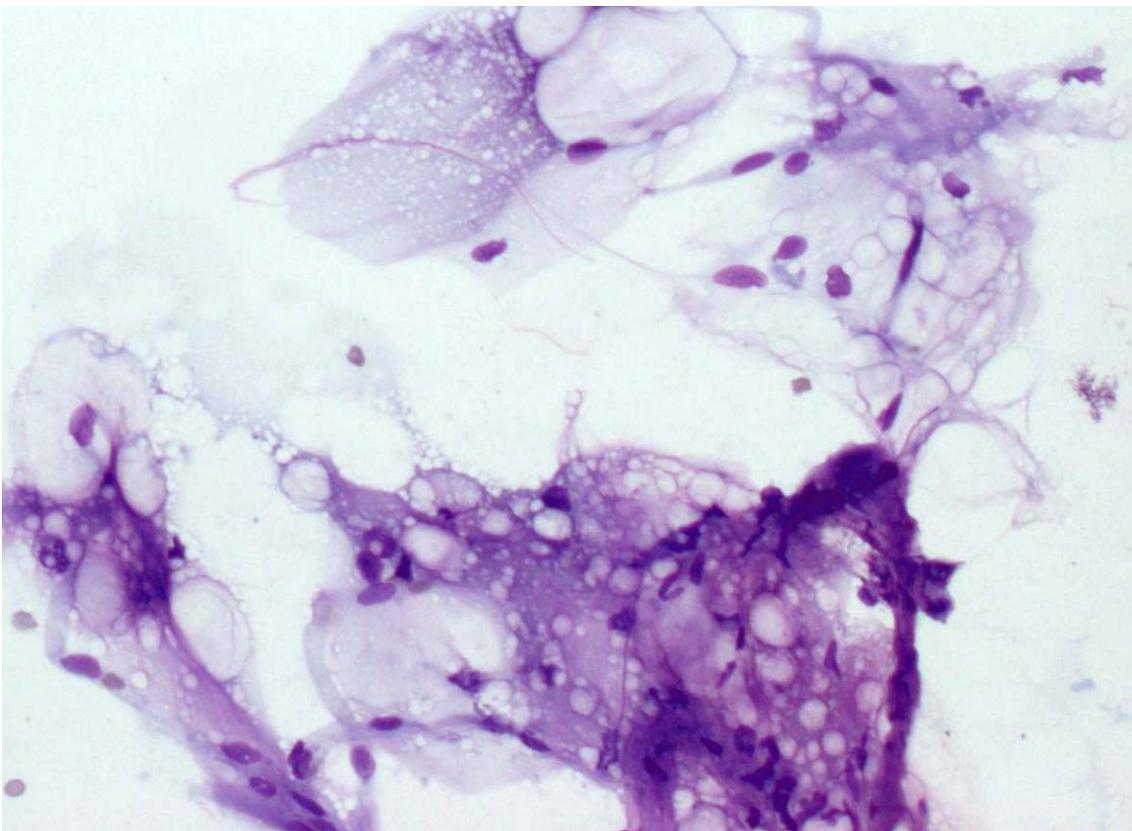


Figure 1: Lipoma, dog. Note the small clumps of well differentiated adipocytes. Many lipomas will not exfoliate cells, or produce only small aggregates such as this.

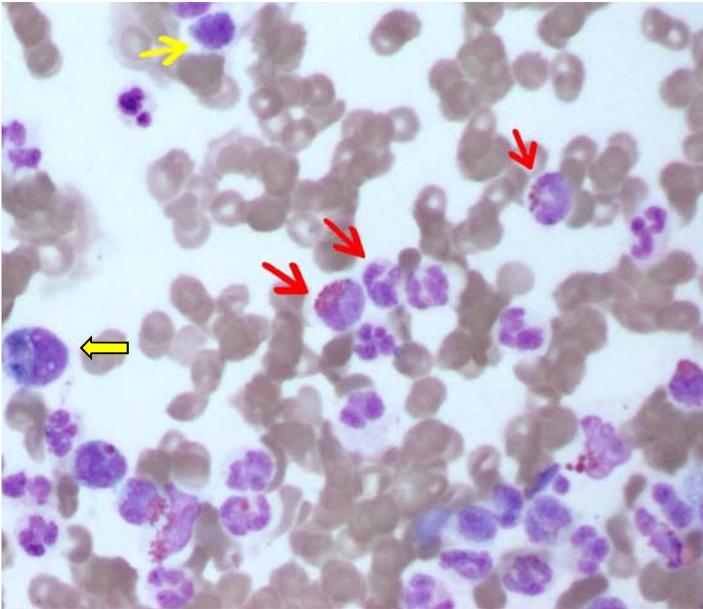
Feature Article continued

Figure 2: Eosinophilic granuloma, cat. These aspirates or impression smears often reveal numerous macrophages (yellow arrows) and eosinophils (red arrows) with occasional neutrophils.

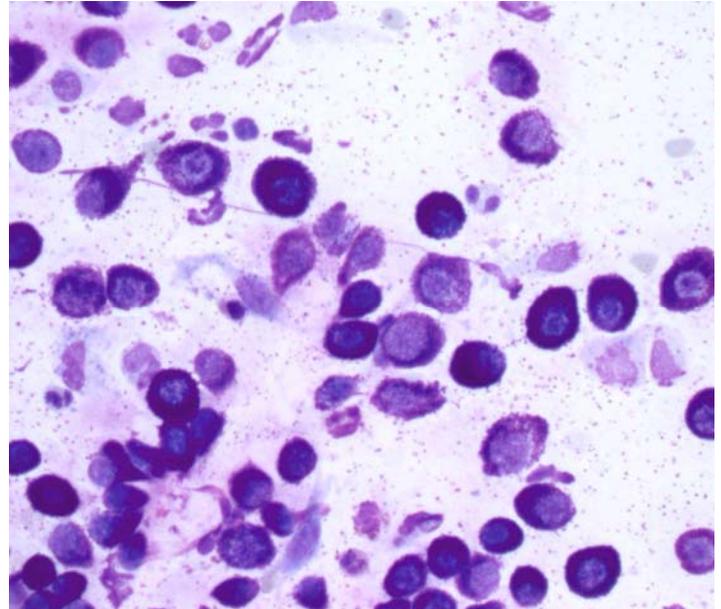


Figure 3: Mast cell tumor, dog. Round cells such as Mast cells (lymphocytes, plasma cells, and histiocytes) exfoliate quite easily and often in high numbers. Note the basophilic metachromatic granules in the Mast cell cytoplasm.

Dermal and Subcutaneous Aspirates

1. One or two unstained slides per site is preferable; generally, more slides does not increase the quality of the sample.
2. Use fine bore needles (23 – 25 gauge) to collect the cells; larger needles tend to cause more bleeding and may not increase cell yield.
3. Make squash preparations of samples with thick aspirated material. This helps to spread cells into a thin layer and reduce sections that are too thick to examine with the least disturbance of the cell membranes.
4. Mesenchymal tumors (such as lipomas) rarely exfoliate on aspiration.

Lymph Node Aspirates

1. One or two unstained slides per site is preferable; generally, more slides does not increase the quality of the sample.
2. Small bore needles (23-25 guage) should be used and generally only light aspiration is needed since the cells exfoliate readily and often will be found in the needle without aspiration when placed in the node.
3. The major limitation is that only a small section of the lymph node is being examined; therefore, a

Feature Article continued

lymphosarcoma that has not diffusely affected the node may be missed and interpreted as a reactive node. Conversely, if a hyperplastic nodule is aspirated it will look like lymphosarcoma on cytology.

4. In cases of generalized lymphadenopathy (including potential lymphosarcoma), we recommend surgical removal of one or two lymph nodes to include the entire node (no wedge or punch biopsies). The entire node conveys the whole picture and architecture is not lost.

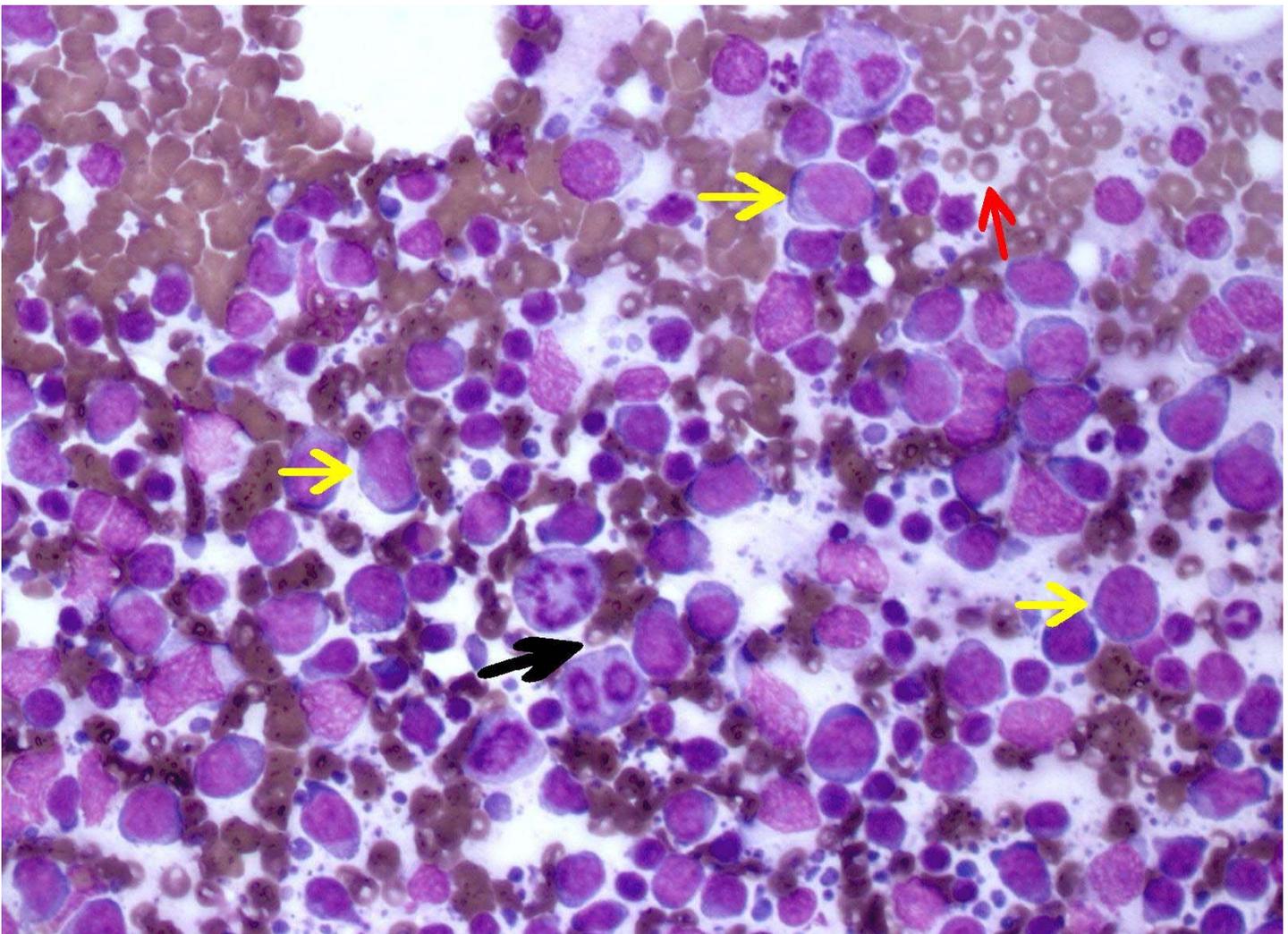
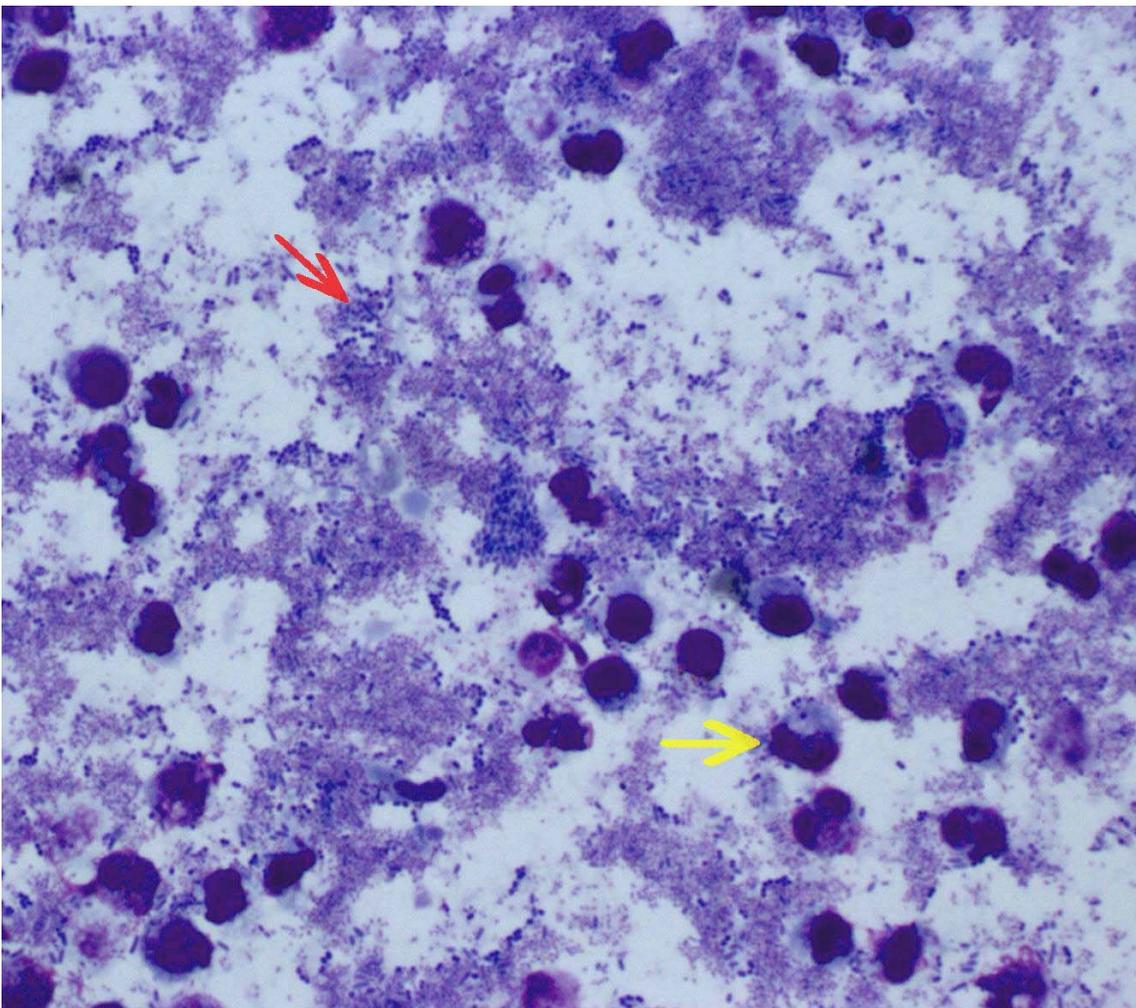


Figure 4: Lymphosarcoma, dog. Aspirates of lymph nodes often exfoliate numerous cells when aspirated. Note the large lymphoblastic cells (yellow arrows) and their large nuclear size compared to normal erythrocytes (red arrows). Normal, well differentiated small lymphocyte nuclei are more hyperchromatic and smaller than an erythrocyte.

Note the high mitotic rate (two mitotic figures on either side of black arrow and one below the base of the black arrow).

Feature Article continued**Urine and Body Cavity Fluids (Thoracic and Abdominal)**

1. Examination of *urine, pleural, peritoneal, and pericardial fluids* can help to define the nature of the disease process causing the effusion (true transudate, modified transudate, or exudate). In some cases, analysis of the cellular make up of these fluids will allow a specific diagnosis to be made (pyothorax, malignant neoplasia, bile duct rupture, etc).
2. Minimize blood contamination and trauma of the sample by using fine gauge needles or butterfly catheters to collect samples.
3. **Please send the fluid instead of sending just slides for cytology.**
4. If bacterial culture is requested place 1-2ml of the fluid in a sterile red top tube.
5. For cytology, collect and submit the sample in EDTA (purple top) blood tubes. EDTA helps to prevent clotting and assists cell preservation.
6. The fluid should be refrigerated and shipped immediately with frozen gel packs to avoid cellular degeneration.

**Figure 5:**

Septic Peritonitis, cat. Fluids from abdominal or thoracic cavity should be evaluated on their protein count and cellular count. This exudate fluid (increased protein and cell count) contains numerous nucleated degenerate neutrophils (yellow arrow) with myriad small bacilli (red arrow). This is a classic example of septic bacterial peritonitis.

Feature Article continued**Lavage (Nasal, Transtracheal, or Prostatic)**

1. Submit the fluid samples in EDTA (purple top) tubes.
2. Make smears from the catheter tips after washing and then submit. Often the smear from the material adherent to the catheter is of greater diagnostic value than the fluid itself.
3. If the washing fluid contains flocculent material, use a sterile hypodermic needle, pick out some of this material, and make slides (smears) for submission.
4. Where bacterial infection is suspected, submit part of lavage material in a sterile red top tube for culture and indicate the request on the submittal form.

In conclusion, cytology can provide an effective, non-invasive diagnostic tool for many dermal and subcutaneous masses, lymph node aspirates, body cavity fluids, lavages, and urine specimens. The main limiting factor in reaching a cytological diagnosis is the number of nucleated cells that are present on the slides submitted.

Please call the veterinary pathologists at the Rollins Laboratory if you have any questions or concerns about possible aspiration or fluid submissions so we can ensure submission of a high-yield (diagnostic) cytological specimen.

Short Cuts

COMPANION ANIMAL

Canine

A 7 year old, intact male, moderately overweight Pointer Cross canine was presented to the veterinary hospital with a primary problem of ataxia and a cold right hind limb with necrosis of the foot. The patient died with cardiac arrest approximately 20 minutes after the initiation of fluid and antibiotic therapy.

On necropsy examination, the right hind limb was swollen with circumferential sloughing of hair from the metatarsus, leaving a sleeve of green-brown necrotic skin. Extensive hemorrhagic necrosis of the soft tissue at and below the stifle was striking. Coronary arteries were pale yellow to white, prominent, thick-walled, and markedly less pliable than normal. Arterial cross sections revealed the projection of yellow-brown nodules into the lumen. Similar changes were observed in various other small muscular arteries throughout the body. Longitudinally opened carotid arteries had numerous, confluent, small yellow flat elevations along the intimal surface. The distal aorta was thick walled and eccentrically enlarged

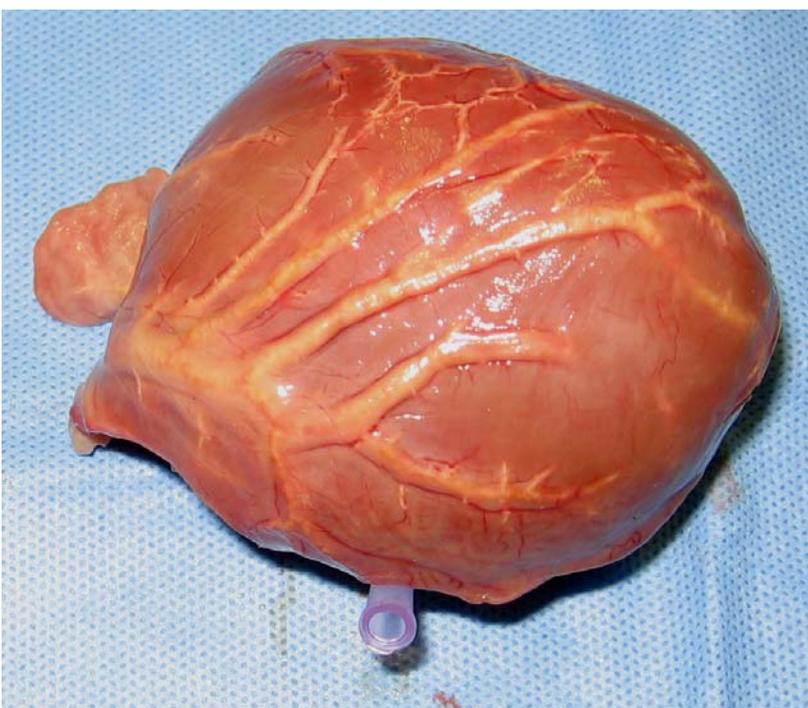
COMPANION ANIMAL, CONTINUED

Figure 1:

Distal aorta, fixed. Note the plaque accumulation occluding the lumen.



from the deposition of plaques and clumps of yellowish gray material within the internal lamina. The lumen of the distal aorta as well as the right external pudendal artery was almost completely occluded.

**Figure 2:** Note the distended coronary arteries.

Widespread hemorrhage and ischemic necrosis of the right hind limb distal to the stifle resulted from impingement on the lumina of the femoral artery and its branches. The thyroid glands were small, measuring 1 cm by 5 mm each. Histopathology confirmed moderate to severe thyrofollicular atrophy and severe, multifocal atherosclerosis of coronary as well as peripheral arteries

In domestic animals the deposition of cholesterol and other lipids in arteries in more than trace amounts occurs only in dogs. This is almost invariably associated with hypothyroidism or diabetes mellitus. Atherosclerosis of the degree encountered in this dog is rare and clinical consequences of severe atherosclerosis are infrequent in dogs regardless of the fact that the vessels most severely involved are those of the heart.

Dr. Richard Oliver

COMPANION ANIMAL, CONTINUED

Feline

A three-year old, domestic shorthaired, spayed female cat was presented for necropsy following a one week history of dyspnea with a pronounced abdominal, expiratory press. Muffled heart sounds were also noted on thoracic auscultation.

The cat was current on vaccinations. According to the owner, a female sibling died at approximately one year of age during a routine ovariohysterectomy. The veterinarian reported that the cat had several developmental defects, such as the presence of an extra ureter, that were found on postmortem examination.

On gross examination, this cat was in good body condition with a body weight of approximately 4 kg, pink mucous membranes, and normal hydration. When examined internally, the only viscera present in the abdominal cavity were the liver, kidneys, and distal portion of the colon. The stomach, spleen, and small and large intestines were displaced cranially into the left thoracic cavity through a defect in the diaphragm. The stomach was filled with dried food kibble.

The left lung lobes were generally hypoplastic, but the tissue was pink. On histopathologic examination, this lung tissue was atelectatic. The right lungs were normal in appearance.

It is likely that this condition was chronic since the defect in the diaphragm had smooth margins, the left lung lobes were hypoplastic, and there was no evidence of recent trauma. It is also likely that over time the abdominal viscera may have gradually become displaced into the thoracic cavity. The acute, terminal clinical signs resulted from the cat's inability to continue to compensate to the abnormal physical placement of the abdominal viscera.

Dr. Kim Townsend

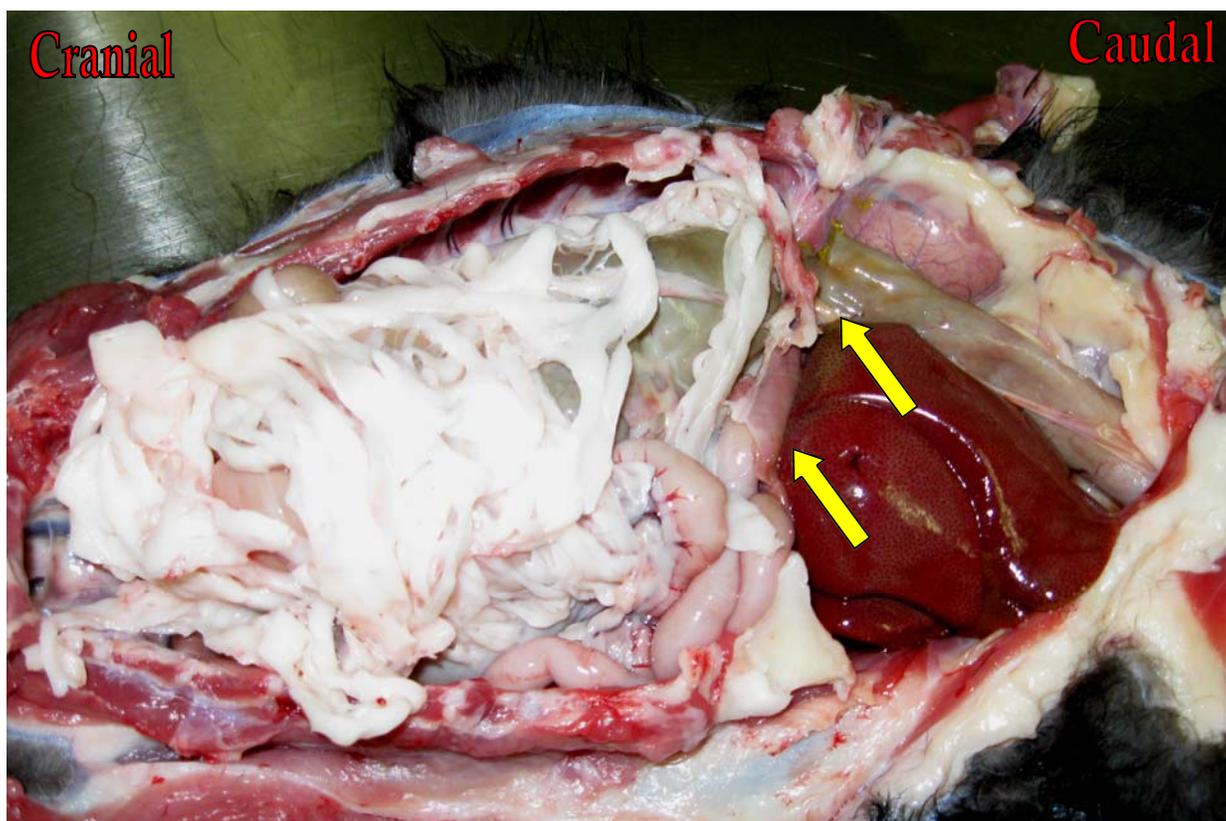


Figure 3: The exposed thoracic and abdominal cavities of a 3 year old cat. The arrows indicate the diaphragm.

COMPANION ANIMAL, CONTINUED

Exotics

Dystocia is defined as abnormal labor or childbirth. It is a term that most practitioners would associate with mammalian species. This diagnostician was recently exposed to a broader application of the term.

Western NC was subjected to abnormally low temperatures from late December 2009 through early January 2010. Over a two week period, concurrent with the onset of sub-freezing ambient temperatures, an owner reported losing around 30 adult Common Carp. The fish were swimming at the pond's surface usually while lying on their sides. The fish were able to swim away when approached. This particular pond had a very high stocking density (about 50,000 pounds of fish in approximately 3 surface acres) and had an iced over surface covered with snow during most, if not all, of this episode.

One 12 kg female carp was submitted for laboratory evaluation. She had a good amount of flesh with respect to skeletal muscle mass yet there was virtually no visceral fat present. The gastrointestinal tract was devoid of content and the majority of the body cavity was dominated by an expanded air bladder along with a vast amount of ovarian tissue which showed some indication of deterioration (reddish-brown, firm areas throughout). Mucus scrapes and gill preps indicated a low parasite burden with scant numbers of *Trichodina* sp. And *Gyrodactylus* sp. found respectively. Histopathology confirmed widespread, chronic ovarian degeneration. The diagnosis was Dystocia.

In spawning fish dystocia is the lack of spawn coupled with the non resorption of eggs in the body cavity. Ovarian swelling induces impingement on the pneumocystic duct and the fish's ability to pneumoregulate is impaired. (The air bladder swells and can't be properly deflated – you try diving with a life preserver!) Increased pressure in the body cavity also leads to a decrease in feed consumption and, ultimately, liver and kidney malfunction. The condition is greatly exacerbated by extreme cold with water temperatures below 37 degrees F being the critical point.

Special thanks to Jeffrey M. Hinshaw, Ph.D. Associate Professor and Extension Specialist at the Mountain Horticulture and Crops Research Station for his assistance on this case.

Dr. Richard Oliver

LIVESTOCK

Swine

Three pigs were examined recently from a single house of approximately 400, 35 day old pigs. The history indicated a sudden increase in mortality to approximately 7 pigs per day over a 3 day period. Affected pigs were primarily found dead and swelling of tissue around the eyes had been noted in some of the dead pigs. Coughing or diarrhea had not been observed. Periocular edema was present in all 3 pigs. Edema of the mesocolon (see photo) was noted in 1 pig and edema of the gastric mucosa was present in 2 of the pigs. Beta-hemolytic, *Escherichia coli* was isolated from multiple tissues. Molecular diagnostic genotyping by multiplex PCR assay was performed on a sample of the *Escherichia coli* from the intestine which was positive for Stx2e and F18 by PCR analysis. SLT II/Stx2e (Shiga-like toxin) is the edema disease factor. F18 is a pilus which mediates adhesion to enterocytes. The porcine *E. coli* genotyping panel at

LIVESTOCK, CONTINUED



Figure 4: Note the severe edema present in the colonic mesentery.

the Rollins Laboratory includes the following virulence factors: F18, K88, LT, Sta, Stb, 987P, K99, F41 and Stx2e/SLT-2. Edema Disease was concluded in these pigs, which is a disease of rapid onset in post weaning pigs caused by enterotoxigenic strains of *Escherichia coli*. Occurrence is most common in pigs usually within 1 to 4 weeks after weaning. Treatment is often difficult due to the rapid onset of disease.

Dr. Reg Ridenhour

POULTRY

The Rose Hill laboratory was presented with two Quail with a flock history of increased mortality with signs of anorexia, lethargy and holding their heads down. On necropsy examination adhesions were

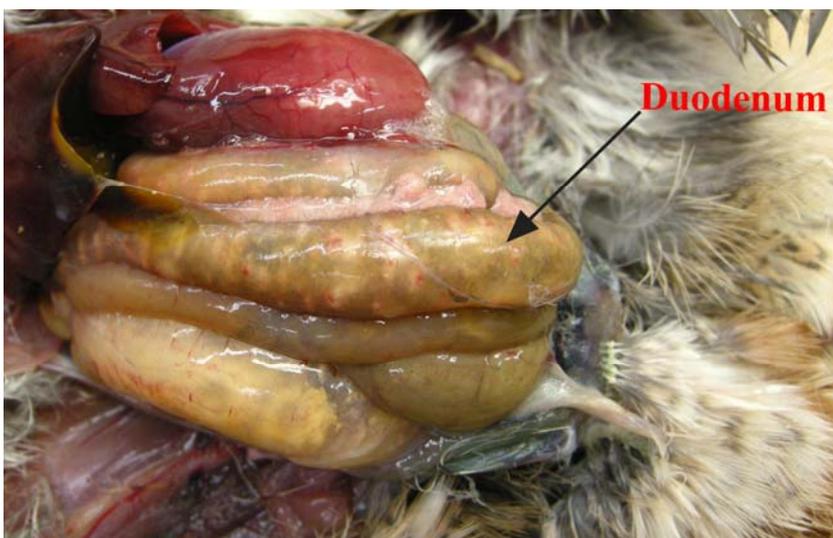


Figure 5: Ulcerative enteritis, 17-week-old quail. Multifocal, necrotizing and ulcerative lesion in the mucosal surface is visible through the serosal surface of the duodenum.

present between adjacent loops of intestine. Segments of the intestines contained multifocal well defined white circular lesions measuring up to 5 mm in diameter. Histopathology revealed a severe acute necrotizing and ulcerative enteritis with abundant intra-lesional rod shaped bacteria. On bacterial culture of the intestines, *Clostridium perfringens* was isolated. Ulcerative enteritis is a contagious disease of chicken and quail characterized by ulcers in the intestines and cecum. Typically there is a rapid spike in mortality, which can be more severe in quail than other spe-

POULTRY, CONTINUED

cies. The causative agent is *Clostridium colinum*, an anaerobic, rod shaped, gram positive bacteria which is transmitted orally, usually from contaminated feces. It is typically introduced through infected carriers or from flies.

Dr. Aziz and Dr. McComb

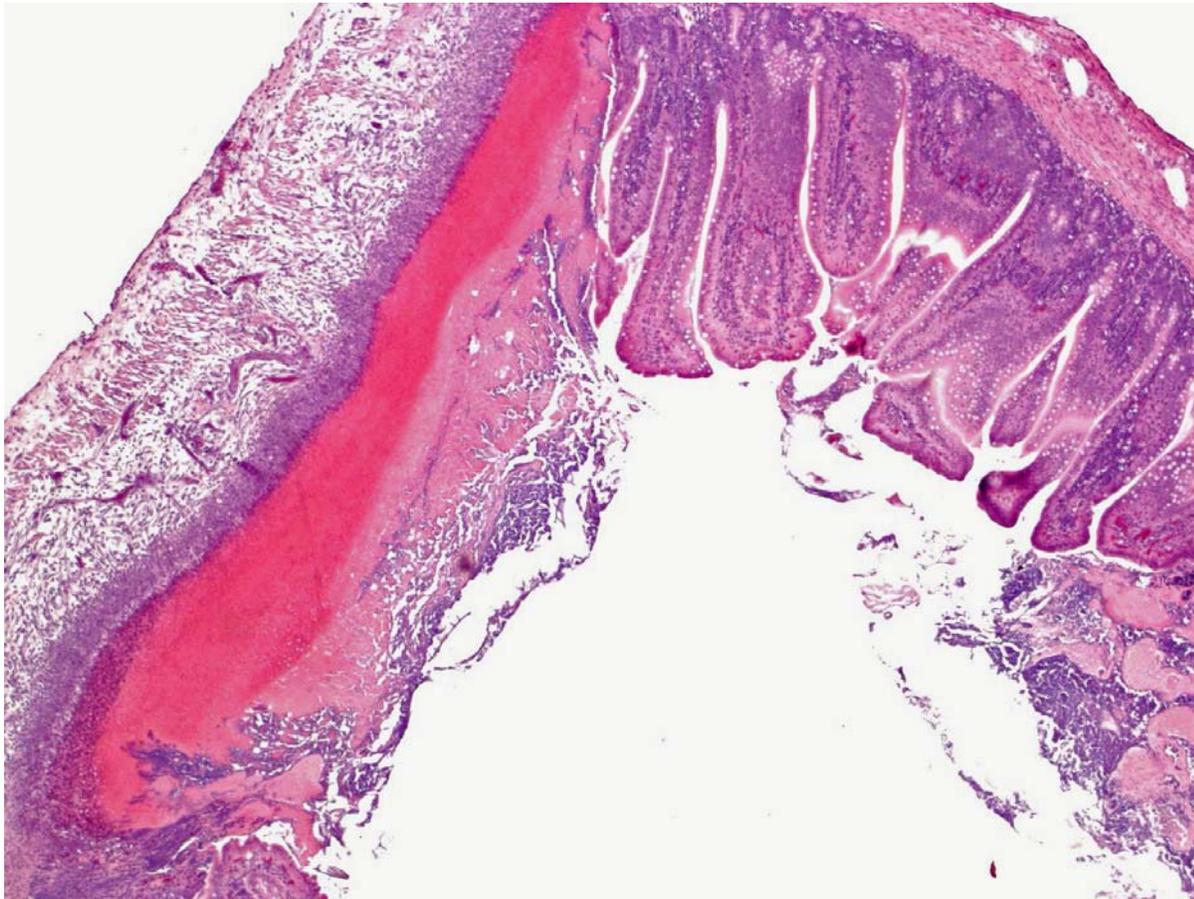


Figure 6: Small intestine, ulcerative enteritis, 16-week-old quail. This micorphotograph shows a focal area of ulcerative necrosis that involves the entire depth of the mucosa.. The ulcer is filled with acellular debris admixed with numerous bacillus-shaped bacteria. There is also destruction of the other intestinal wall layers. The base of the ulcer consists of a basophilic-stained band of necrotic tissue and inflammatory cells, followed by a thick layer of fibroblastic granulation tissue

DEPARTMENTAL NEWS

ROLLINS LABORATORY

Bacteriology recently welcomed Kim Settle, an ASCP certified Medical Laboratory Technician who will be responsible for specimen processing.

MONROE LABORATORY

Carrie Rowell was recently welcomed as Medical Laboratory Technician II

CE ATTENDANCE

Sandra Murphy, Bacteriology Supervisor, attended a 4-day, NPIP Salmonella Isolation and Identification Workshop held in Athens, Georgia in September of 2009. All labs performing NPIP testing are required to have a technologist attend the workshop.

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) held their annual meeting in San Diego, CA in October of 2009. In attendance from the NCVDLs were Drs. Post, Mock, Erickson, Oliver, Rouse, and Rushton. Also in attendance were Beverly Wood, Molecular Diagnostics Supervisor; Ghazala Jawad, Quality Manager; and Tom Miller, IT Manager.

Dr. Kim Hagans attended the Mid-America Veterinary Conference in October 2009

The North Carolina Veterinary Conference (NCVC) was held in Raleigh, NC in November 2009. Attending from the NCVDLs were Drs. Robinson and McComb.

The NC Poultry Health Meeting was held in association with the NCVC in November. Drs. Aziz and Ridenhour attended.

Dr. Ridenhour attended the NC Extension Poultry Advisory Committee meeting in November.

Dr. Drum attended the 23rd annual South Carolina Large Animal Short Course in November.

The 4th annual NCVDLs Inter-laboratory Work Conference was held at the Rollins Laboratory in November 2009. Attending were Drs. Marshall, Post, Erickson, Aziz, Tucker, Moisan, Rushton, Haugland, Robinson, Drum, Swanson, Townsend, Rector, Hagans, Ridenhour, and McComb.

Dr. Tucker attended the American College of Veterinary Pathologists (ACVP) Annual meeting in December 2009, held in Monterey, CA. She presented a case of uterine adenoma with stromal decidual alteration at the Female Reproduction Mystery Slide Symposium.

The 7th annual One Medicine Symposium was held in December in Durham, NC. Attending from the NCVDLs were Drs. Marshall, Erickson, Aziz, and Robinson. Dr. Erickson spoke on the influenza virus, and Dr. Marshall spoke on cooperative efforts between the NCDA&CS and NCDHHS.

Elizabeth Ortiz, Bacteriology Technician, attended CEM Training at NVSL in January.

Dr. Erickson presented on Novel Influenza to the Virginia Pork Producers Association in January.

Directory

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[Jennifer Pruitt—Serology](#)

[Beverly Wood—Molecular Diagnostics](#)

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