January 2012

Volume 7 Issue 1

The NCVDLS REPORT

Accredited by the American Association of Veterinary Laboratory Diagnosticians

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 6, 2012

May 28, 2012

July 4, 2012

Our laboratories will be closed on the above listed days.

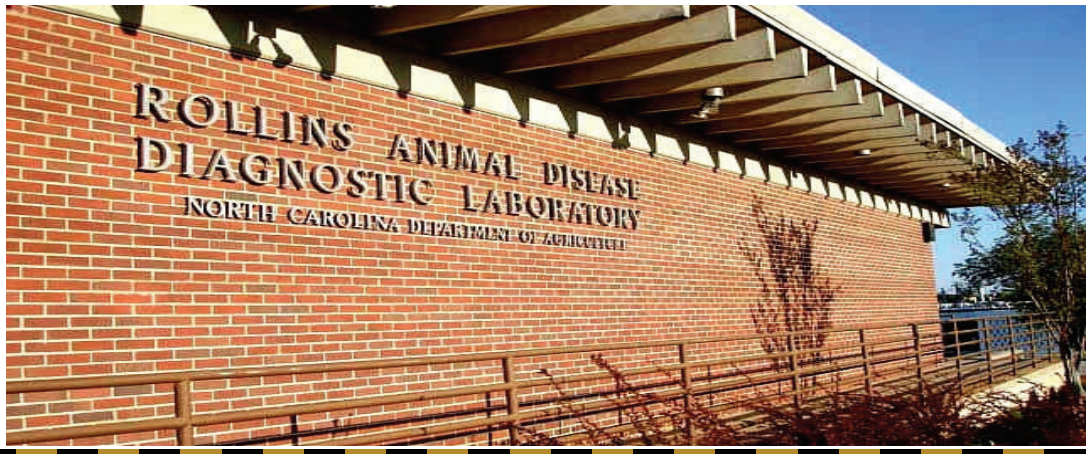
Please e-mail NCVDL@ncmail.net with any comments and/or suggestions concerning The NCVDLS Report
Editor - Dr. David Drum

Message from the Director

Since our last newsletter was published, we have been very busy at the NCVDLS.

We are beginning preparations for our accreditation site visit by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The purpose of AAVLD accreditation is to accredit public veterinary diagnostic laboratories in North America relative to technical and operational competence compatible with appropriate standards (ISO 17025) and to provide an administrative assessment. An accredited laboratory is one that is competent and capable of providing a full range of diagnostic services which must include the following disciplines: pathology, bacteriology, mycology, virology, parasitology, serology, and toxicology. Site visits are generally a 3-4 day evaluation by trained auditors that include a review of the laboratory's quality-assurance system, facilities and personnel. Our current AAVLD accreditation expires on December 31, 2012.

As you are aware, we upgraded our Laboratory Information Management System (LIMS) in July, 2011. As a result of this upgrade, test results for accessions submitted prior to July 18th, 2011 will no longer be available through our website after February 15th, 2012. If you need to access these older submissions on-line, please do so before mid-February. Please also be aware that we are continuing to work with our LIMS vendor to make enhancements to the client web portal that will result in it becoming more "user friendly". We appreciate your feedback in this endeavor.



Please see our Client Corner section for details pertaining to new test services being offered: the Rapid-Chek SELECT *Salmonella* Enteritidis test for our poultry clients at the Hoyle C. Griffin Laboratory; USDA's Veterinary Services Process Streamlining for electronic submission and reporting of Coggins test results at the Rollins Laboratory; and bacterial cultures for the diagnosis of Contagious Equine Metritis, also available at the Rollins Laboratory.

Happy New Year!

Karen W. Post DVM, MS

Client Corner

For our poultry clients, the Hoyle C. Griffin Laboratory currently offers conventional NPIP *Salmonella* environmental cultures that will detect all groups of salmonellae from a sample, including Group D1 strains (e.g. Enteritidis), for a cost of $10 per sample with final results taking as long as 10 working days. A new rapid method for testing environmental samples that screens specifically for Group D1 salmonellae was recently validated in-house and is now being offered as a service to our clients: the SDIX Rapid-Chek SELECT *Salmonella* Enteritidis test. The initial cost of the test will be $15 per sample; however, this price may decrease based upon increased testing volumes. This assay, which has NPIP approval, can deliver negative or presumptive results within 3 working days of sample receipt, significantly faster than the conventional culture method. Please contact either Dr. Kimberly Hagans or Dr. Reginald Ridenhour at the Griffin Lab for further information (704)-289-6448.

The Rollins Laboratory has offered clients electronic Coggins reporting with Global Vet Link for well over a year. Now we have another certificate system for you to choose from. The USDA has developed a web-based system known as the Veterinary Services Process Streamlining (VSPS) system. Veterinarians can apply for accreditation online, validate/update their contact information, and create electronic certificates of veterinary inspection (eCVI) which allows them to attach official test charts for Equine Infectious Anemia (EIA) and automatically submit the documents to state officials or laboratories. To access VSPS, accredited veterinarians must apply for USDA eAuthentication (to prove identity), and create a VSPS profile. To get started in the VSPS system, you may simply apply for e-authentication at https://vsps.aphis.usda.gov/vsps/.

The Rollins Laboratory was recently approved by the USDA to conduct bacterial cultures for the diagnosis of Contagious Equine Metritis. This was a result of having sent a technologist to a week-long training course at the National Veterinary Services Laboratory in Ames, Iowa and the subsequent successful completion of a proficiency test. For this test, a specific set of swab specimens must be collected and submitted by an accredited veterinarian along with a completed USDA/APHIS VS1-form. Refer to our electronic UserGuide which is available on our website: http://www.ncagr.gov/vet/ncvdl/.

Feature Article

By Dr. Peter Moisan

Oak toxicity in cattle

This past fall, as in other years, the NCVDL has accessioned a number of cattle that have experienced oak (acorn) toxicity. From 2008-2011, we received a total of 21 submissions (3-8 per year) from herds that experienced losses of one or multiple cattle due to oak toxicity.

The typical history is from a herd that has overgrazed the pasture or perhaps changed to a pasture in which there are stands of oak trees and little other available grazing. Though the cattle are initially reluctant to eat acorns and oak leaves, some seem to develop a fondness for the acorns and will consume them in abundance when they are available and alternative sources of nourishment are in short supply. Acorns are more plentiful in some years and in some pastures, so the number of affected herds and cases at our laboratory can vary.

Cattle are the most affected ruminant animals when oak toxicity is considered. Sheep are less susceptible, then goats, and then deer and other wild ruminants. Deer and other wild ruminants are reasonably resistant to intoxication from tannins because they have large quantities of proline-rich proteins in the saliva. These proteins are constitutive in the saliva of wild ruminants, and to a much lesser extent in saliva of domestic animals. In addition to being resistant to the effects of tannins, in conditions of increased dietary tannins (such as during the fall), additional inductive proline-rich salivary mucins are produced by deer. Monogastric animals are variably susceptible to the astringent effects of tannins. Pigs and chickens are somewhat susceptible. The taste of oak sprouts and acorns seems to be offensive to horses, and thus horses are at lower risk of oak intoxication. However, if acorns are ingested in abundance by a horse, the astringent effects can cause enteritis and typhlocolitis, secondary to thrombosis of small blood vessels in the intestines.

The clinical signs in an adult cow are those of progressive renal failure. After consuming the tannin-laden forage, the animal becomes anorexic and dehydrated. Feces are dark, firm, scant, and often covered with mucous and occasionally flecks of fresh blood. Though this type of fecal consistency is common in any cattle that have anorexia, it is especially common during renal insufficiency. A gaunt appearance develops and the cow is oliguric. The course is usually 7-14 days. Necropsy reveals a dehydrated carcass with obvious vascular congestion and hemoconcentration. Variable amounts of blood-tinged edema fluid surround the kidneys. A uriniferous odor is present. The gastrointestinal tract contains far less than the usual amount of ingesta, digesta, and feces. Abomasal ulcers are occasionally seen as well. Acorns are usually present in the rumen and intestines but may not be abundant in all cases. Microscopic abnormalities are most pronounced in the kidneys. There are granular casts in the proximal tubules and in the more loosely compacted casts, it is apparent that the casts are comprised of necrotic tubular epithelial cells from the necrotic upstream tubules. Neutrophils in small numbers are also found in affected tubules. The medullary tubules contain brightly eosinophilic proteinaceous urine, which indicates that the resorptive capabilities of the proximal tubules have been compromised by the tannin molecules. Nephrocalcinosis and metastatic mineralization of pulmonary alveoli and systemic vascular structures is often seen.

Feature Article continued

All parts of the oak plant are toxic to animals, but acorns seem especially abundant and available.

The toxic principle in oak is tannin, which is actually a group of chemicals in the polyphenol family. Tannins are widely distributed in plants and particularly in trees. In acorns, in which the tannins are concentrated, they are located in the outer portions of the seed and have bactericidal and allelopathic qualities that affect the survival of the germinal tissue of the seed. The toxic attributes of tannins come from the ability to precipitate proteins. Tannins are complex organic molecules and astringents that are used in the tanning process of leather and it is the astringent quality that causes precipitation of proteins. These compounds have the chemical structure of polyphenolic molecules, and when of sufficiently high molecular weight, polyphenols will complex with proteins.

Though we are concentrating on the toxicity of oak and the tannin molecules, they have favorable qualities for ruminants as well. By binding in protein complexes, tannins prevent the early rumen-based digestion of the proteins by bacteria and protozoa and comprise part of the bypass portion of the dietary protein. This preserves urea nitrogen for incorporation into proteins in the more caudal aspects of the digestive tract. In excess, tannins can cauterize, by astringent action, the mucosa of the abomasum. Salivary enzymes contain mucins and these are precipitated by the tannins. When there is more tannin in the diet than salivary mucins to complex them, absorption into the bloodstream can occur. Filtering of the tannic acid byproducts in the proximal renal tubules presents individual epithelial cells with such metabolic byproduct compounds as gallic acid and pyrogallols that are toxic to the epithelial cells. The necrotic epithelial cells form the casts that are so familiar in cases of oak poisoning. Figures 1-5 illustrate microscopic findings from recent cases of toxic tubular necrosis caused by acorns and oak forage indiscretion. Colitis arises from the astringent qualities of the tannins but also from vascular damage due to the azotemia and hypercalcemia of renal failure.

Useful information about the biochemistry and biological effects of tannins can be obtained from the Tannin Page web site reference below.

References:

1. Maxie MG and Newman SJ. Urinary system. In: Pathology of Domestic Animals, vol. 2. p. 473. 2007.
2. The Tannin Web Page at Cornell University College of Agriculture Web Site.

Feature Article continued

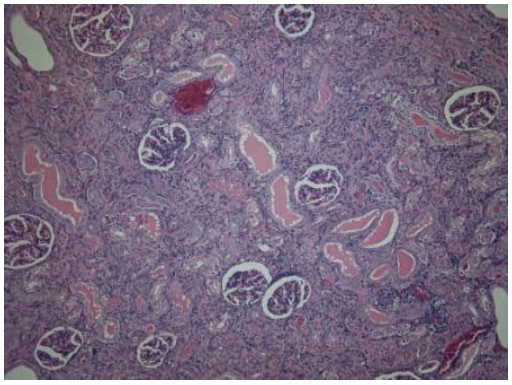


Figure 1. Bovine kidney. Oak toxicity. Tubular necrosis with granular casts and proteinaceous urine.

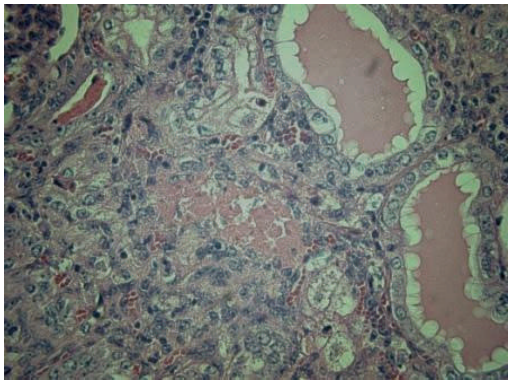


Figure 2. Bovine kidney. Oak toxicity. Tubular necrosis with cellular casts and proteinuria.

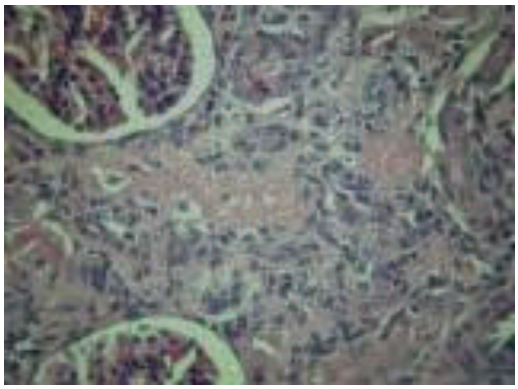


Figure 3. Bovine kidney. Oak toxicity. Tubular necrosis and cellular casts.

Feature Article continued

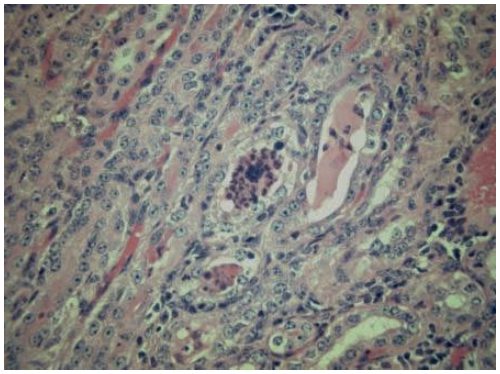


Figure 4. Bovine kidney. Oak toxicity. Tubular necrosis and cellular casts with intratubular neutrophils.

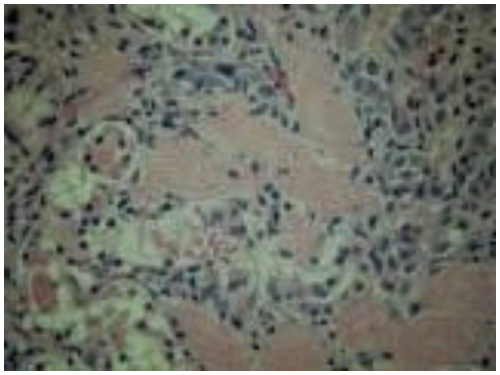


Figure 5. Bovine kidney. Oak toxicity. Tubular necrosis and cellular casts.

Short Cuts

Companion Animal

Canine

***Clostridium piliforme* (Tyzzer's disease) infection in a puppy**

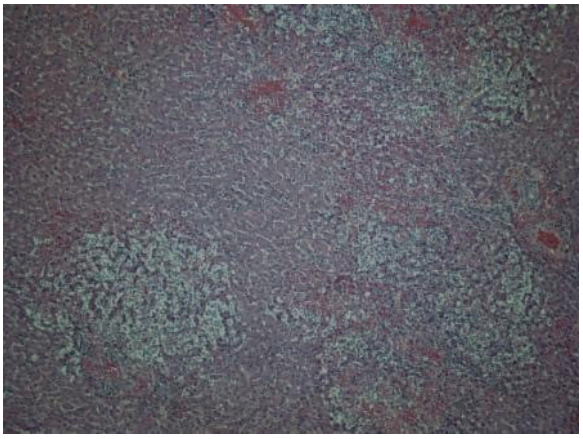
In one unusual case presented to the Rollins Laboratory, a cross breed terrier-type puppy (age 5 weeks) was presented with a history of diarrhea that became bloody. Coccidiosis was diagnosed and was treated accordingly with some positive effects. The puppy was active and playing, but with diminished appetite during the 4 days after the initiation of treatment. It was somnolent on the day of death.

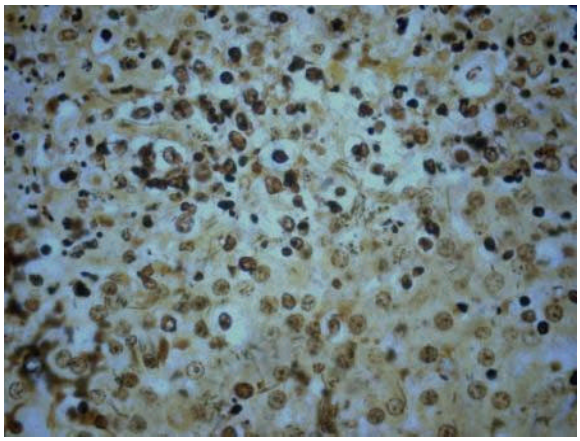
The findings at necropsy included pinpoint hemorrhages in the capsular surface and parenchyma of the liver, which had a tan appearance. There was reddening of the small intestinal and colonic mucosa and scant feces were present. A fecal examination at the time of necropsy confirmed the earlier diagnosis of coccidiosis as coccidial oocysts were identified within a fecal smear.

Companion Animal, continued

In sections of liver, multifocal, random, necrosuppurative hepatitis was associated with slender argyrophilic bacilli that were present within foci of necrosis but primarily within the cytoplasm of hepatocytes at the margins of the lesion (Figures 1 and 2). The random foci of necrosis contained necrotic hepatocytes and moderate accumulations of degenerate neutrophils within abundant cellular debris. Mild, segmental enteritis was associated with occasional coccidian protozoa, of which multiple life stages were located within the cytoplasm of affected villous tip enterocytes.

Young animals, most notably foals and hamsters, are most often affected by *Clostridium piliforme*, and at this laboratory we have confirmed the lesions in foals, kittens, and puppies. The hepatic lesion is similar in all mammals that succumb to this agent. *Clostridium piliforme* is a soil-dwelling, strictly anaerobic bacterium that is responsible for hepatitis in many species, similar to the dog in this case. Small characteristic "haystack" colonies develop in the liver, presumably producing toxins, and cause the small foci of necrosis and suppuration that were seen in this patient. Along with lesions in the liver, *C. piliforme* causes myocarditis as well as a typhlocolitis that is most likely associated with the invasion process. Though infection with *C. piliforme* has a low morbidity rate, it has a very high mortality rate once infection is established. Entry to the hepatic circulation is afforded by a concurrent lesion within the gastrointestinal tract. In dogs and cats, it seems that enterocolitis from a viral, bacterial, or protozoal (this patient) agent may be required for invasion across a damaged mucosal barrier. The disease has also been associated with canine distemper, which is caused by a morbillivirus that causes immune suppression.





Figures 1 and 2: Sections of canine liver with *Clostridium piliforme*

References:

1. Quinn PJ et al. Veterinary Microbiology and Microbial Disease. 2002. Iowa State University Press. pp 94-95.

2. Whittaker D. Tyzzer's disease in puppies. Vet Record. 1988. 26; 122: p 310.

Drs. Jennifer Haugland and Peter Moisan

Companion Animal, continued

Aspergillosis in dogs

During spring and summer 2011, the NCVDL system encountered 2 cases of systemic aspergillosis in canine patients. The disease, mostly limited to German shepherd dogs and caused by *Aspergillus terreus*, is fairly rare in our diagnostic accessions. The organisms are ubiquitous, residing in the soil and infections can occur from this and other species of *Aspergillus*.

Aspergillosis is considered initially a local infection, often occurring at the site of a compound fractured bone. From the bone, dissemination occurs and extends to kidney, intervertebral discs, lung, and other sites. Discospondylitis is a common (possibly universal) feature of the disseminated disease. An IgA deficiency is reported to be the defining immunosuppressive finding in affected individuals, hence the large number of German shepherd dogs in case reports of systemic aspergillosis (as well as several other severe infectious diseases) in the literature. Disseminated *A. terreus* infections are described in immune deficient humans as well, being well-documented in the human medical literature.

Aspergillus terreus grows rapidly on fungal media and has a variable colonial appearance and color with light to heavily sporulating colonies. Microscopically, in culture, the regularly septate mycelia are parallel-walled with walls 4-5um apart. The conidiophores are long and thick-walled. These end in short branches called phialdes or sterigmata. The phialdes produce chains of spherical conidia. In addition, unique to *A. terreus*, accessory conidia are produced by mycelia in vitro and in vivo. These accessory conidia help with identification of the agent and enable distinction from other potentially systemic opportunistic *Aspergillus* species. Also, the accessory conidia produce higher levels of surface B-glucan than the phialidic conidia. This increased B-glucan induces greater levels of fibrin and cellular inflammation in affected tissues.

The first case from June 2011 involved a 5-year-old neutered female German shepherd dog that was reported to have a 2-3 month history of back pain and progressive hind limb weakness and ataxia. Radiographs revealed lysis and bony proliferation of the middle thoracic vertebra. Euthanasia was performed and the animal was presented for necropsy to the Arden Laboratory. Relevant findings included irregularly shaped kidneys and severe discospondylitis and bony lysis at T4-5 and T6-7. Histological findings included vasculitis with nephritis, vertebral discospondylitis and osteomyelitis, pyogranulomatous myocarditis, splenic necrosis and infarcts, and degenerative change within the white matter of the spinal cord. Fungal hyphae were present in all affected tissues and fungemia due to *A. terreus* was confirmed by mycological culture. The second case from August 2011 and involved a 4-year-old neutered female German Shepherd dog with lethargy and lameness of several months duration. Bone biopsy of an area of limb (unspecified bone) long bone revealed proliferation of the cortical osteoblasts with new bone production. Further diagnostics performed over the next several weeks indicated that the patient had generalized cortical proliferation of the long bones of all limbs with masses within the thoracic cavity, consistent with a clinical diagnosis of hypertrophic pulmonary osteopathy. Euthanasia was performed and necropsy conducted at the presenting veterinary clinic. Two masses of unknown tissue type were sampled from the thoracic cavity and submitted in 10% formalin. No material was submitted fresh for culture. These tissues were examined histologically and confirmed to be lymph nodes that were nearly totally effaced by fungal hyphae consistent with *Aspergillus terreus*

Companion Animal, continued

Figures:

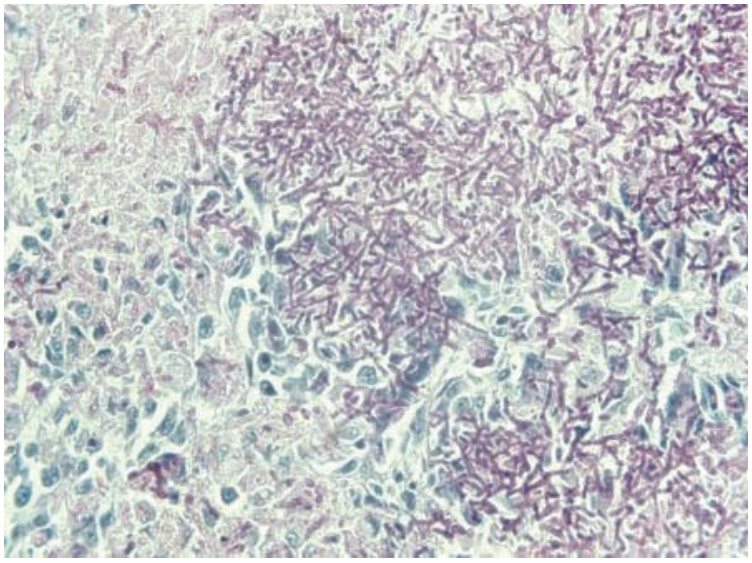


Figure 1: Canine Lymph Node, *Aspergillus terreus*

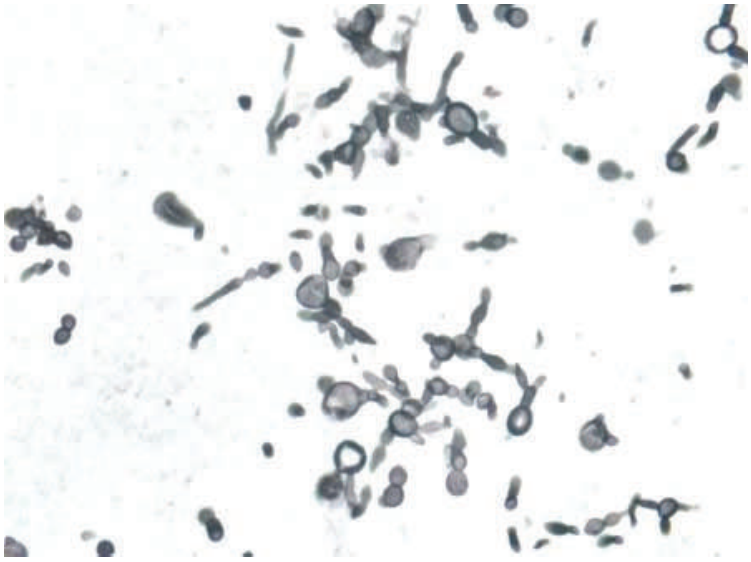


Figure 2: Canine Liver, *Aspergillus terreus*

Companion Animal, continued

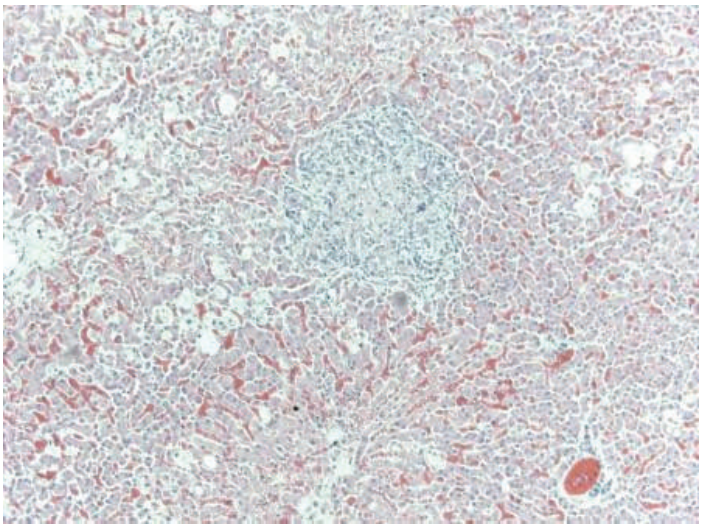


Figure 3: Canine Liver, *Aspergillus terreus*

References:

1. Balajee SA. Aspergillus terreus complex. Med Mycol. 47 Suppl 1:S42-46:2009
2. Deak E et al. Aspergillus terreus accessory conidia are multinucleated, hyperpolarizing structures that display differential dectin staining and can induce heightened inflammatory responses in a pulmonary model of aspergillosis. Virulence. 3:200-207. 2011.

Kabay MJ et al. The pathology of disseminated Aspergillus terreus infection in dogs. Vet Pathol. 22:540-547. 1985.

Moisan PG, Oliver RC, Rushton SD

Feline

A 3-year-old male neutered DSH feline was presented to the emergency hospital with a 1 week history of decreased appetite, lethargy, abdominal distension and possible vomiting. This cat was previously healthy, current on vaccines and tested negative for feline leukemia and feline immunodeficiency virus. Physical examination revealed abdominal distension, painful abdomen and mild tachypnea. The complete blood count revealed marked leukocytosis, neutrophilia and elevated total bilirubin and blood urea nitrogen. On ultrasound, an abdominal effusion was identified and an abdominocentesis yielded white/cloudy fluid. Cytology of the aspirate was extremely high in neutrophils with rods and cocci. An abdominal exploratory was performed and all the organs were covered in a fibrinous exudate with no obvious source for the peritonitis.

On necropsy, the abdominal cavity contained approximately 50 ml of exudate resembling tomato-soup. The abdominal viscera, body wall, caudal diaphragm and omentum were diffusely covered by up to 2 mm thick fibrinous exudate.

Companion Animal, continued

Histopathologic examination indicated pyogranulomatous and fibrinous peritonitis of the liver, spleen, pancreas, small intestine and omentum with intralesional bacteria and Splendore-Hoeppli material and a pyogranulomatous interstitial nephritis. *Actinomyces* sp. was isolated via aerobic culture.

Initial review of this case was consistent with a bacterial infection; however based on the wide degree of pyogranulomatous inflammation, feline infectious peritonitis (FIP) could not be ruled out. Immunohistochemical analysis of the small intestine, kidney and lymph node tested positive for coronavirus.

This was an interesting case because the peritonitis was due to both a bacterial (Actinomyces *sp.*) and viral (FIP) infection. Slendore-Hoeppli are eosinophilic amorphous proteinaceous aggregates that often surround the pathogenic organisms as a result of a local antigen-antibody (see photographs). Gram-positive bacteria consistent with *Actinomyces* sp. were identified along the periphery of the Splendore-Hoeppli material. In this cat, the bacterial infection was secondary due to immunosuppression by the virus.

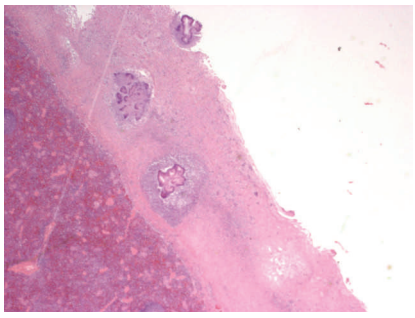


Fig 1. Spleen. H&E. Splendore-Hoeppli material

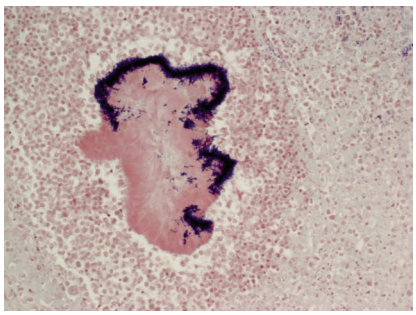


Fig 2. Spleen. Gram. Splendore-Hoeppli material with intralesional bacilli

Mahogany Caesar

Companion Animal, continued

An 8-year-old DLH cat was euthanized due to progressive weight loss of two months duration, vomiting and an oculonasal discharge for one week. Ophthalmic examination revealed no demonstrable vision or dazzle reflexes from either eye, absent pupillary light response in both eyes with dilated pupils at rest, and optic nerve edema with protrusion of the optic nerve head in the vitreal cavity of the left eye. Optic neuritis of both eyes and trigeminal neuropathy of the left eye were diagnosed.

Necropsy examination revealed moderate thickening of both optic nerves; the left optic nerve was firm and measured 1.5 times thicker than the right optic nerve. Exiting the foramen of the skull, the left trigeminal nerve was markedly thickened, particularly the maxillary nerve branch that merged into the infraorbital nerve. The left maxillary branch was firm, tortuous and measured 5 mm in diameter. The right maxillary nerve branch appeared normal and measured 2 mm in diameter. The frontal sinus was completely filled with brown mucous.

A metastatic epithelial neoplasm and diffuse neuropathy with digestion chambers and spheroids were diagnosed in the trigeminal and optic nerves. The gross and histopathologic findings explained the neurologic deficits identified in this cat. The primary site of the tumor was not known or identified; however nasal or periadnexal locations were differentials. Immunohistochemistry was recommended to further characterize this neoplasm.

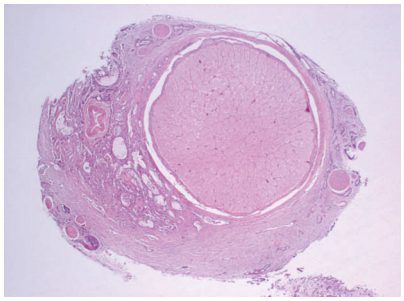


Figure 1

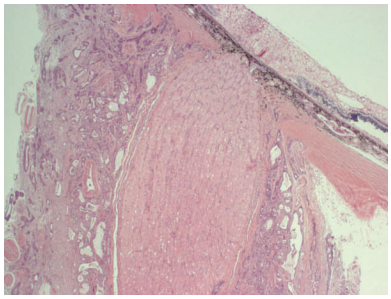


Figure 2

Expansion of the dura mater of the trigeminal nerve (Figure 1) and optic nerve (Figure 2).

Mahogany Caesar

Exotics

Unusual Disease in Koi Fish

During the month of February, the Rollins Animal Disease Diagnostic Laboratory received tissues from a field necropsy of an outdoor koi fish. There had apparently been increased death losses in the pond and the owner requested an investigation by the local Veterinary Exotics Practitioner. Sections of gill, kidney, hepatopancreas, stomach, intestine, spleen, swim bladder and ovary were examined. Lesions of pathological significance were limited to the gills, in which sporangia and spores of the organisms were lodged between secondary lamellae among large numbers of macrophages and smaller numbers of neutrophils. The morphological diagnosis was granulomatous and neutrophilic branchitis that was moderate to severe and chronic, with intralesional protozoa identified as *Dermocystidium koi*.

Companion Animal, continued

Special stains were applied to the affected tissue sections and it was revealed that PAS stain was most effective in demonstrating the organisms, though the routine H&E stains were also very demonstrative of the protozoa in sections (Figures 1,2,3).

Bacteriology results from the hepatopancreas revealed growth of *Aeromonas* species and *Shewanella putrefaciens*, which are each opportunistic pathogens of freshwater fish and other vertebrates.

Dermocystidium koi has been referred to as a protozoal organism, but it has recently been reclassified as a fungus, and is similar in its life cycle and structural characteristics to *Rhinosporidium seeberi*, a pathogen of mammals. Sporangia fill with spores to form nodules in the gills and skin of koi and other fish. (In other species of fish, different species of *Dermocystidium* can cause systemic infection, affecting visceral organs as well.) Thousands of spores are released by single sporangia to perpetuate the infectious process. The organisms are present in pond water and affected ponds can have yearly outbreaks. Spring is the most common time of the year for this condition. Though lesions can be substantial when the sporangia rupture, mortality is low and recovery is usually uneventful. Formalin fixed tissue sections usually demonstrate the spores, but occasionally hyphal structures are also present, though none were seen in our case.

This condition develops in the spring primarily, as with most infections that occur due to poor water quality or overcrowding of the fish. With warming of the water in outdoor lakes and ponds, spring is the time when most water quality problems occur and thus the time when the diagnostic laboratory is most occupied with fish pathology. Differentials for *Dermocystidium* infection include many bacterial, protozoal, and fungal.

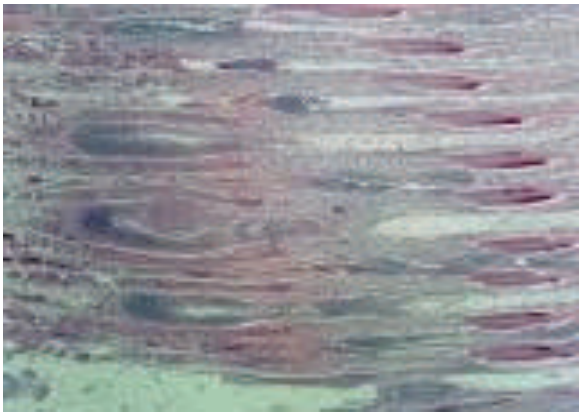


Figure 1

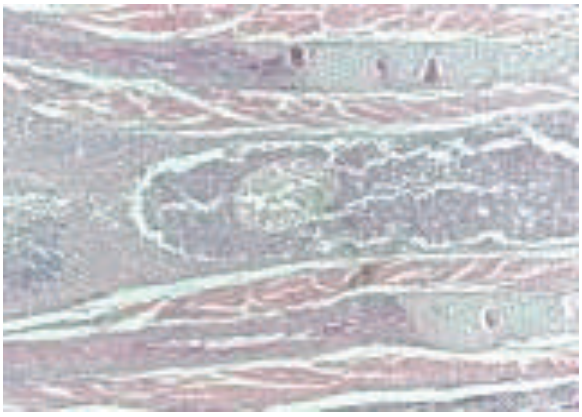


Figure 2

Figure 1: Koi gill: Granulomatous inflammation associated with sporangium of Dermocystidium koi. HE. Low magnification.

Figure 2: Koi gill: Granulomatous inflammation associated with sporangium of Dermocystidium koi. HE. Medium magnification.

Reference:

1. Wildgoose WH. *Dermocystidium koi* found in skin lesions in koi carp (*Cyprinus carpio*). Vet Rec. 1995 137:317-318

Surgical Biopsy

Cytology vs. Biopsy

Cytology is an often used procedure that offers a definitive or close presumptive diagnosis with minimal invasiveness and often no anesthesia. Unfortunately the quality of the cytology sample is most dependent on the number of nucleated cells on the slide. The drawbacks of cytology include not only a small nucleated cell population but the lack of tissue orientation and are the cells present representative of the entire mass.

Biopsy sections often provide the definitive diagnosis more often than cytology giving tissue orientation, arrangement and margins. However, the invasiveness of the biopsy procedure and that some diseases can be diagnosed via cytology makes cytology a more viable ancillary test in some cases, especially when dealing with animals that are anesthetic risks.

Lymph Nodes: Lymphadenopathy, whether focal or generalized, is a common reason for needle aspiration and biopsy removal submissions. Aspiration of nodes often yield large number of cells and can provide a much more diagnostic sample than most of the other cytologies seen at the NCVDL.

Because needle aspirates only sample a small section of the node, an early lymphosarcoma that affects only a part of the node can be missed and conversely if hyperplastic nodules are aspirated the sample can falsely look like Lymphosarcoma.

For that reason aspiration of multiple enlarged nodes is recommended. For animals in which chemotherapy will likely be used and/or aspirates that are not definitive, removal of an entire single or multiple lymph nodes for histologic examination and immunohistochemistry for differentiation of B or T cell lymphosarcoma is recommended.

If other nodes can be aspirated or removed, please try and avoid the mandibular nodes. These nodes are nearly always hyperplastic (reactive) and in subtle cases of lymphosarcoma or other processes, these reactive changes can make it difficult to provide a definitive diagnosis compared to the prescapular, axillary, inguinal and popliteal nodes.

Bottom line is that aspirates of lymph nodes can be a very useful method for a presumptive diagnosis, however, definitive diagnosis and further phenotypical differentiation would be needed with biopsy sections.

Skin Masses: Skin masses are the most common biopsy and cytology submissions at the NCVDL. Neoplasms of the skin and subcutis generally fall into the category of epithelial, round and spindle cell tumors.

Epithelial tumors such as sebaceous adenoma, squamous cell carcinoma and hepatoid adenomas often exfoliate well on aspiration. These masses often present as small to large clusters of cells that often display cytologic changes that can differentiate benign vs malignant. Cytology can provide the practitioner with a presumptive diagnosis, however, biopsy sections will be needed for confirmation, margins and whether any signs of metastasis are present.

Round cell tumors such as Mast cell tumors, Histiocytomas and Plasma cells tumors can be diagnosed via cytology because they exfoliate quite well. These masses present as individual round cells. Mast cell tumors often show their metachromatic granules on cytology, however, as with other tumors, round cell tumors require biopsy examination to confirm the diagnosis as well as determine margins and possible grade or tumor. Histiocytomas exfoliate on aspiration, however, if the mass is ulcerated impression

SURGICAL BIOPSY, CONTINUED

smears often will exfoliate well on slides. **Again, grading for Mast cell tumors is only performed on biopsy sections. Grading is not possible on cytology.**

Spindle cell tumors such as fibrosarcoma, hemangiopericytoma, fibromas and hemangiosarcomas exfoliate poorly which can cause many samples to be non-diagnostic. When they do exfoliate it is very difficult to tell the difference between the masses and sometimes difficult to tell the difference between neoplastic fibroblasts (Fibrosarcoma) and reactive fibroblasts (granulation tissue). **Biopsy submissions are almost always needed for any diagnosis on these types of tumors**.

In conclusion, cytology offers a quick, non-invasive process to determine an occasional definitive and often presumptive diagnosis. Biopsy specimens, although more invasive and expensive, provide a far more definitive diagnostic specimen in most cases and can provide further identification of the mass via immunohistochemistry and special stains. The use of both of these tests can provide a thorough examination of a mass or infiltrate. If you have any questions regarding cytology or biopsy specimens please feel free to call any of the pathologists at the NCVDL in Raleigh.

Steve Rushton

LIVESTOCK

Porcine

Porcine Toxoplasmosis

A back-yard pig farm, producing meat for an extended family, had two litters of 8 piglets total that were sustaining high losses at 4 to 6 weeks after farrowing. Six of 8 piglets had died by the time of this submission; the sows and other pigs on the premises remain healthy with no losses. The sows are not related. The pigs are kept in outdoor pens with a pond and a fresh water source. Vaccinations are not administered.

At necropsy, the 5 week old pig was is fair nutritional condition. The tonsils have a thick surface plaque of fibrin and debris. The spleen is enlarged and the surface has fibrin deposition. Thoracic and abdominal lymph nodes are enlarged. The lungs are mottled red and tan and were firm. The liver contains numerous small tan foci.

Histopathologic examination of the tissues reveals necrotizing tonsillitis, pneumonia, lymphadenitis, splenitis and hepatitis all with lymphoid depletion. Numerous individual protozoal organisms (zoites and tissue cysts) are detected within the tonsil, lungs, lymph nodes, spleen and liver. *Toxoplasma gondii* is confirmed by immunohistochemistry. In addition, Porcine Circovirus II is detected by immunohistochemistry.

LIVESTOCK, CONTINUED

Toxoplasma gondii is a protozoal organism associated with disease in a wide range of species. Porcine toxoplasmosis is generally asymptomatic and the organism is found in tissue cysts in muscle and elsewhere. However, abortion or stillbirth, neonatal disease and disease at weaning can be seen. Findings in piglets can include encephalitis, myocarditis, pneumonia, hepatitis, lymphoid necrosis and placentitis. Transmission of *Toxoplasma gondii* can be transplacental or by ingestion of either oocysts from food or water contaminated by cat feces or by ingestion of tissue cysts from consumption of rodents, dead pigs, goat whey or uncooked or undercooked garbage.

Dubey (2009) reports seroconversion of 23% of market pigs and 42% of sows in the USA. One study reports seroconversion of <1% of commercial market pigs. Feral pig seroprevalence in GA was found to be 18% and in SC 34%. With increasing numbers of small farms and outdoor pens for raising pigs, the incidence of Toxoplasmosis is expected to increase. Toxoplasmosis is a zoonotic disease and pork is a major source of toxoplasmosis in humans worldwide. Freezing the meat, or cooking to a temperature greater than 160 F, will kill the organism. Care in handling raw meat is recommended. For additional information on toxoplasmosis in humans, please consult your physician or other healthcare professional.

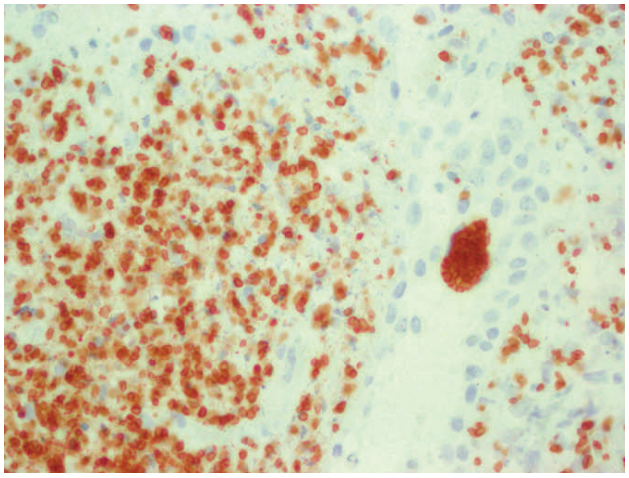


Figure: Photomicrograph of immunohistochemical assay for toxoplasmosis in the lung. The organisms stain red and there are numerous individual organisms associated with necrotic tissue. One large tissue cyst is also present.

References:

Dubey, JP, Toxoplasmosis in pigs-the last 20 year, Veterinary Parasitology 164(2009) 889-103

Dubey, JP, Swine Toxoplasmosis, www.http:/ncagr.gov/vet/FactSheets/Toxomplasmosis.htm

Tucker, Alison, Toxoplasma gondii, The NCVDLS Report, 4(3) 2009.

Allison Tucker

LIVESTOCK, CONTINUED

The bodies of a 5 month old neutered male and 7 month old female mixed breed pig were presented to the laboratory for post mortem examination. The provided history stated one pig was found dead the day before, and then these two pigs were found dead that morning. They added a new batch of pigs about two weeks ago. The pigs live outside, on pasture and are dewormed every three months. There were no signs of illness noticed among the pigs.

Notable post mortem lesions in both pigs included a uniform, checkerboard pattern of firm purple colored lung tissue. The bronchiolar lymph nodes were pale in color and markedly enlarged. The female pig also had focal areas of mucosal ulceration in the area of the ileocecal valve / papilla. Histopathology showed bronchiolitis, lymphoproliferative, moderate, multifocal with focally extensive neutrophilic bronchopneumonia.

Pasteurella multocida was isolated from the lungs of both animals. *Arcanobacterium pyogenes* was also isolated from the lungs of the male pig. There was no growth after 48 hours on aerobic culture of bronchiolar lymph nodes of both animals.

Pooled samples of lung tissue from both animals were submitted for Molecular diagnostics. The samples were negative for were **positive** for Porcine Reproductive & Respiratory Syndrome Virus European strain - PCR.

The cause of pneumonia was attributed to Porcine Reproductive & Respiratory Syndrome (PRRS) virus and a secondary bacterial pneumonia. It was interesting that two years prior, the North American strain of PRRS was diagnosed on this same farm. The isolate from this current outbreak (European strain) likely came in with the new batch of added pigs.

While PRRS has been a disease of concern on the commercial pork production industry for years, the diagnosis of PRRS for the new or small independent producer can come a surprise. The differences in production methodologies between commercial and independent producers (mixing of different age groups, lack of "all in-all out" production, purchase of replacement animals from multiple sources) can cause problems in both introduction and re-introduction of the disease. These production Porcine Reproductive & Respiratory Syndrome Virus North American strain - PCR and Swine Influenza Virus - PCR, but methods also frustrate the problem of controlling spread of the disease once introduction of the virus onto the farm occurs.

Reference:

Focus on... Porcine reproductive and respiratory syndrome, Prepared by FAO EMPRES, Issue No 2 - 2007

David Drum

Departmental News

CE attendance

Dr. Reggie A. Ridenhour, DVM 2011 North Carolina Veterinary Conference and North Carolina Poultry Health Meeting in Raleigh, NC during November 2011.

Dr. Mahogany Wade-Caesar attended the North Carolina Veterinary Conference in Raleigh, NC on November 3-4, 2011.

Dr. Allison Tucker attended the CL Davis Symposium Respiratory Pathology on September 30, 2011 and the AAVLD 54th Annual Conference on October1-2, 2011 in Buffalo, NY and was co-chair for the 2011 AAVLD Diagnostic Pathology Slide Seminar.

Dr. David Drum attended the East Tennessee Veterinary Medical Association Meeting on October 1-2, 2011 in Gatlinburg, TN, and attended the Seventh Annual Equine Encore, Equine Medicine Conference on October 20-21, 2011 in Athens, GA.

Dr. Gene Erickson officially retired 2/1/12 after 20+ years serving as Head of Microbiological Services. Congratulations on a long and happy retirement.

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