

# The NCVDLS REPORT



Accredited by the American  
Association of Veterinary  
Laboratory Diagnosticians

## Veterinary News and Information From North Carolina's Diagnostic Laboratories

### From The Director by David T. Marshall, DVM

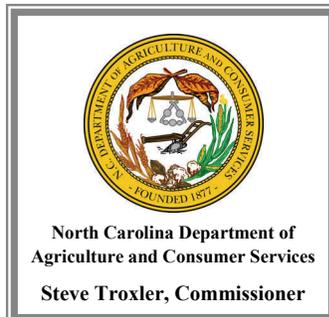
Greetings from the staff of the veterinary diagnostic laboratory system. I hope everyone's holiday season was joyous and relaxing, as the coming year presents significant opportunity and challenges.

Since our last newsletter, several significant developments have occurred which will shape our efforts this coming year. We are still awaiting final confirmation of the dates of our AAVLD accreditation site visit. Several factors, including site review team members' busy schedules, have pushed back the timing of the review from last fall to this coming February or March. We have provided the team leader with several potential available weeks during those months, and look forward to hosting them in the immediate future. I feel that the feedback that we receive will be beneficial in charting our course for the future.

The laboratory system will be participating in two Foreign Animal Disease (FAD) planning exercises this Spring and Summer. One will be a one day tabletop scenario utilizing a National Animal Health Laboratory Network (NAHLN) tool developed and facilitated by an independent third party contractor. The other, scheduled for April, will be an extensive North Carolina developed and focused field exercise designed internally around a highly pathogenic Avian Influenza outbreak. We will be partnering with our Veterinary Division Field Forces section, our state Public Health and Wildlife agencies, USDA-APHIS, industry, and Emergency Management to identify gaps in our plan, and challenge the laboratory's surge capacity for testing in the event of an outbreak. Both events should be valuable in furthering our FAD preparedness and response capabilities for the future.

North Carolina has been experiencing a significant outbreak of Infectious Laryngotracheitis (ILT) in our broiler industry this fall and winter, as have many other southeastern states. The viral disease appears to involve a vaccinal revertant strain that is losing its attenuation and developing a higher level of virulence. The laboratory system has been integral to responding to this disease, through confirmatory diagnosis through histopathology, ILT PCR testing on initial cases within new counties, and subsequent virus referral to the University of Georgia for sequencing. Various vaccination and biosecurity strategies have been employed with varying degrees of success. Thanks to Dr. Tahseen Aziz for his diligent avian pathology work on the high numbers of ILT cases we are processing. We will continue to provide the diagnostic support until this disease event is brought to a successful conclusion.

Our Quality Assurance/Quality Control program continues to gain momentum. We have appointed Ghazala Jawad as our new QA manager, and have completed official audits of all sections within the Rollins laboratory as well as the four branch



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

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any comments and/or sug-  
gestions concerning The  
NCVDLS Report

### Holiday Closings...

January 21, 2008

March 21, 2008

Our laboratories will be closed on the  
above listed days.

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**From the Director** (Continued)

laboratories. This is a major accomplishment and has prepared us well for our upcoming AAVLD accreditation site visit.

Congratulations to Herman Honeycutt, our Business and Technology Applications Analyst, and Dr. Erickson for their leadership in helping Rollins Laboratory achieve one of only two perfect scores in the NAHLN IT messaging and readiness evaluation, as reported by the NAHLN Steering Committee IT report. This messaging capability is a requirement for NAHLN participation and allows seamless, real time transfer of testing results to the USDA for foreign animal and other NAHLN identified diseases of high consequence. For more information on the NAHLN, go to : [http://www.aphis.usda.gov/animal\\_health/nahln/](http://www.aphis.usda.gov/animal_health/nahln/). Rollins Laboratory is one of 12 core laboratories within the network, with testing capability for Avian Influenza, Exotic Newcastle Disease, and Classical Swine Fever, among others.

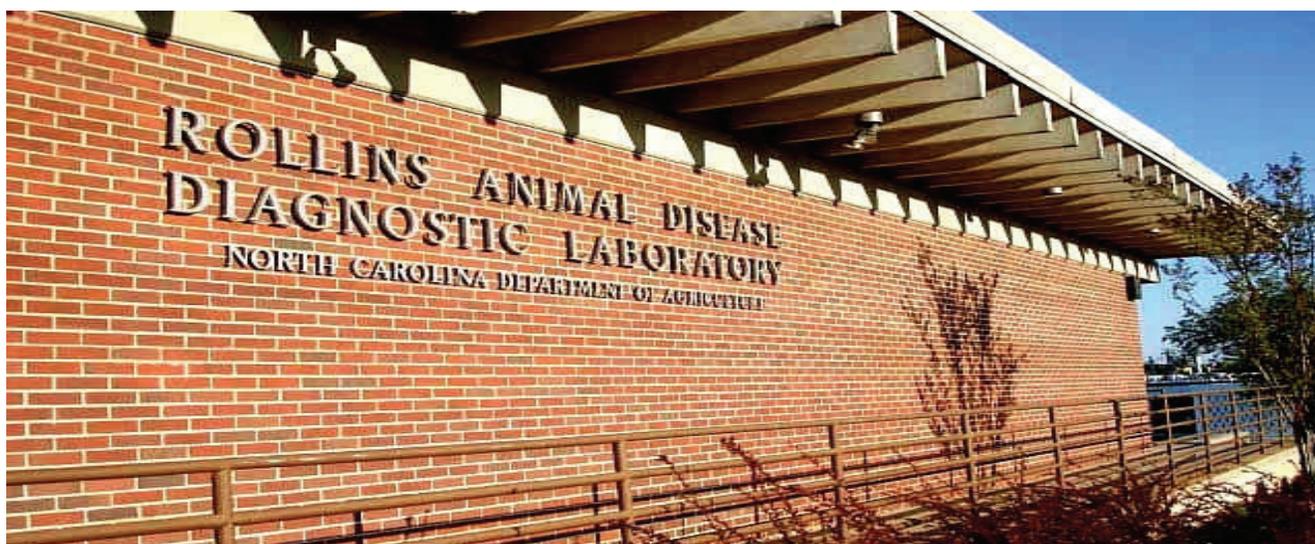
Lastly, on the physical facility front, I am pleased that a bid was awarded on January 10<sup>th</sup> for the \$492,903 Western Laboratory renovation and repair project in Arden. This project has been struggling through the approval process for over a year, but appears to finally be a “go” with work expected to begin next month.

Thanks for your support and, as always, feel free to contact me with any comments or concerns.

Regards,

A handwritten signature in blue ink that reads "David T. Marshall, DVM".

David Marshall, D.V.M.  
Director





### Diagnostic Laboratory Advisory Committee

Dr. Jim Floyd	NCSU- College of Veterinary Medicine
Mr. Larry Wooten	N.C. Farm Bureau
Dr. Richard Kirkman	Private Veterinary Practitioner
Dr. Gene Erickson	NCDA&CS Veterinary Diagnostic Laboratory System
Dr. Rick Sharpton	Perdue, Inc
Dr. Shannon Jennings	Carroll's Foods
Dr. Leslie Wolf	DHHS- State Public Health Laboratory
Dr. Karen Post	NCDA&CS Veterinary Diagnostic Laboratory System
Dr. Eric Gonder	Goldsboro Milling
Dr. Mary Ann McBride	NCDA&CS Veterinary Division
Mr. Jeff Turner	Murphy Brown, L.L.C.
Dr. Randy Jones	Livestock Veterinary Services
Dr. Jennifer Haugland	NCDA&CS Veterinary Diagnostic Laboratory System
Dr. Gene Nemechek	GIS-Hog Slats
Dr. David Marshall	NCDA&CS Veterinary Division
Dr. Betsy Sigmon	Creature Comforts Animal Hospital

## Client Corner

### 2008 Holiday Schedule by Jeremi Clark

Holiday	Observance Date	Day of the Week
New Year's Day	January 1, 2008	Tuesday
Martin Luther King Jr's Birthday	January 21, 2008	Monday
Good Friday	March 21, 2008	Friday
Memorial Day	May 26, 2008	Monday
Independence Day	July 4, 2008	Friday
Labor Day	September 1, 2008	Monday
Veteran's Day	November 11, 2008	Tuesday
Thanksgiving	November 27 & 28, 2008	Thursday & Friday
Christmas	December 24-26, 2008	Wednesday-Friday

**Methicillin Resistant *Staphylococcus aureus*** by Dr. Karen Post

Methicillin resistant *Staphylococcus aureus* (MRSA) is a multi-drug resistant Gram positive bacterium that is a critically important human pathogen having traditionally been associated with hospital-acquired infections. During the last 10 years, community-acquired (CA-MRSA) infections have been reported. These are contracted by persons who have not been recently hospitalized or have had a medical procedure. CA-MRSA infections are usually manifested as pimples or boils and occur in otherwise healthy people. Because MRSA is a pathogen of growing importance and receiving much media attention, veterinary practitioners may be faced not only with questions from clients but also with treating these infections in their patients.

Methicillin resistance is mediated by the production of an altered penicillin binding protein which confers resistance to all beta-lactam antimicrobial agents. It is encoded by the *mecA* gene, located on a mobile genetic element called the staphylococcal chromosomal cassette. Frequently, MRSA strains are multi-drug resistant to a wide range of antimicrobials like clindamycin, tetracycline, fluoroquinolones, and erythromycin. This makes infections difficult to manage and results in increased morbidity, mortality and treatment costs when compared to infections due to methicillin-susceptible strains of *Staphylococcus aureus*.

MRSA is an emerging pathogen in companion animals, especially dogs, cats and horses, where it has been shown to cause numerous disease conditions ranging from soft-tissue infections, post-surgical wound infections, arthritis, pneumonia, sepsis or even death. Several case reports suggest that MRSA infections can be transferred between humans and animals. As in humans, MRSA can colonize skin, oral and nasal mucosae, and other body sites in healthy animals. Colonization involves survival of the bacterium without any adverse effects. Colonized animals could serve as a source of infection for other animals and humans.

The majority of reports of MRSA in pets have dealt with clinical cases or outbreaks. Much less is known about colonization rates in healthy pets in the community. A study of dogs and cats presented to a Canadian primary care veterinary clinic did not identify the organism in 188 dogs or 39 cats. Another study conducted in dogs that visit human hospitals also failed to identify MRSA. A survey of dogs presented to the Ontario Veterinary College isolated MRSA from only 1 of 193 animals. A recently published German paper reported the isolation of MRSA from 18 dogs and 4 cats when 869 clinical specimens were examined. This low prevalence of MRSA in the pet population may be due to the fact that *Staphylococcus aureus* is not the predominant coagulase positive staphylococcal species that colonizes the skin of dogs and cats.

Outbreaks of MRSA in horses and humans in Canada have researchers concerned that MRSA infections in horses may be emerging as a serious zoonotic and veterinary nosocomial disease. In horses, there have been increasing reports of both MRSA colonization and infection. Results of a survey of MRSA colonization in Canadian and New York horses confirm a reservoir of colonized horses on a variety of farms and provide evidence that 1 MRSA strain is predominantly involved in MRSA colonization in horses and humans that work with horses. MRSA outbreaks have occurred in large veterinary teaching hospitals and there is evidence that MRSA was transmitted between horses and humans in those situations.

MRSA has been identified in pigs and people that work with them. There are concerns over the roles these animals may play as reservoirs for human infection. MRSA colonization in pigs was first reported in the Netherlands in 2003. A new MRSA strain was identified in patients who were associated with pig farming. These strains could not be genotyped by conventional methods (pulse-field gel electrophoresis). A subsequent study concluded that pig farming was a risk factor for increased nasal carriage of MRSA in humans. In 2006, screening of 540 grow-finish pigs in Danish slaughterhouses showed that 39% harbored MRSA. The use of antimicrobial drugs as group medication was associated with finding MRSA colonized pigs in yet another report.

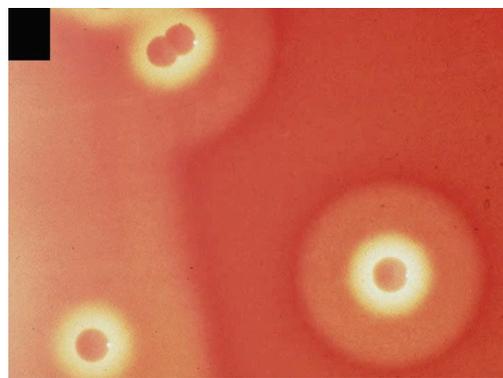
MRSA diagnosis involves simply isolating the organism (**Figure 1**) and performing antimicrobial susceptibility testing. In horses, nasal swabs are used for screening. For screening other companion animals, no



### **Methicillin Resistant *Staphylococcus aureus*** (Continued)

standardized methods have been developed but it is recommended to submit a combination of nasal, rectal and perineal swabs for culture.

For the practitioner faced with managing an infection, some form of treatment is required. Generally, topical therapy is recommended, if possible. Because all MRSA isolates are not multi-drug resistant, many commonly used antimicrobials can be highly effective in treating systemic infections. Contact with affected animals should be limited because the primary mode of transmission is through direct contact with the organism. Risk factors for developing CA-MRSA infection would include close skin contact with infected individuals, coming in contact with contaminated items or environmental surfaces, poor hygiene and crowded living conditions. The affected animal should be placed in isolation with restricted access. Barrier contact precautions should be practiced. The isolation environment should be disinfected; most common commercial disinfectants have a good spectrum of activity against MRSA. Any in contact inanimate objects must also be disinfected. Thorough handwashing techniques must be exercised. Animal owners should be informed of a potential zoonotic threat.



**Figure 1:** *S. aureus* colonies on blood agar plate.

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## Disease Trends

### **Toxicity Diseases in Beef Cattle** by Dr. Peter Moisan

The recent drought and the present Fall-Winter feeding season have combined to keep North Carolina Beef Practitioners and the Pathologists and Diagnosticians at the NCV DLS busy. During the fall we routinely see a variety of seasonal diseases in beef cattle that are not common during the other seasons. These include toxicoses from oak (*Quercus* species), redroot pigweed (*Amaranthus retroflexus*), cocklebur (*Xanthium* species), moldy sweet potatoes, and nitrates. Also, we are on alert for such fall and winter diseases as anaplasmosis (*Anaplasma marginale*), abomasal impaction, and pregnancy toxemia. As of December 2007, oak toxicity and anaplasmosis have been identified from a number of locales in North Carolina, and have been seen at several of the NCDA Laboratories. Nitrate intoxication has been diagnosed in at least 2 instances during this time, as well.

The dangers of toxicoses are highlighted during a drought as cattle hungrily consume forages and weeds they ordinarily would not eat. The problem is compounded when the toxic products are made available to livestock in underutilized pastures or woodlands. In this article, I will review a few important clinical and pathological features of some toxicoses relevant to North Carolina producers.

**Oak poisoning** is common in North Carolina. Several species of oak are native to North Carolina and each contains tannins and gallotannins, the toxic principles, in varying quantities. As a result, toxicity is dependent on the species of oak consumed as well as dose of the products. Oak poisoning has been referred to as acorn toxicity; however, the shoots, bark, and mature leaves also all contain the toxic principles. The shells of green acorns are apparently more toxic than those from mature acorns. Though some parts of the plants are apparently more palatable than others, consumption of oak products may be due to the ubiquity of the plants during poor pasture conditions rather than any preferential eating of the plants. Acorns may be consumed by choice, however, and can cause mechanical impaction of the rumen in a few instances.

The tannins and gallotannins of oak plants are metabolized in the rumen to toxic by-products that include pyrogallols, gallic acid, and digallic acid. These products are nephrotoxic, producing proximal tubular epithelial necrosis in the renal cortex, with subsequent acute renal failure. Tannins and the metabolic by-products cause cellular damage to the tubular epithelial cells since they are concentrated in the proximal tubules. Incidentally, tannins and tannic acid are used in the leather tanning industry due to their cauterizing effects on leather.

Clinical signs of oak toxicity are related to the renal and gastrointestinal systems. Consumption of the products is followed in 2-3 days by signs of renal failure that include oliguria and subsequent polyuria and dehydration. Anorexia follows, and affected cows show features of constipation, with arching of the back while straining to defecate, pelleted fecal material, and abundant mucous and possibly blood in the feces. Diarrhea then develops, with black, tarry stools, indicative of hematochezia, following the constipation. Death follows in 4-10 days after initial intoxication in untreated cases.

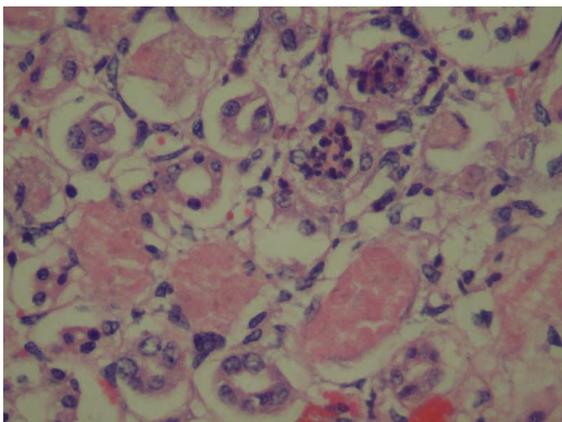
The history in these toxic cases usually indicates exposure to oak products, however, as oak is nearly universally present in rural settings in North Carolina, clinical pathological findings and necropsy lesions are necessary for a definitive diagnosis. The BUN and creatinine values are greatly elevated in fatal cases. Calcium and phosphorous values are also elevated. Hemoconcentration is noteworthy due to dehydration. Proteinuria is often marked, indicating severe renal disease, close to the time of death.

Necropsy findings in some cases reveal the offending leaves in the rumen or acorn shells in the rumen and intestine, but the amount may vary due to the amount of time between the consumption of the products and time of death. The material may have time to pass through the digestive tract and a quantitative estimate of the amount consumed cannot be made by gross assessment. Perirenal edema with swollen kidneys and



### **Toxicity Diseases in Beef Cattle** (Continued)

occasional renal cortical hemorrhages are most often seen (**Figure 1**). In addition, intestinal ulcers and tarry feces are common in fatal cases. Impaction of the rumen with acorns may not necessarily be accompanied by renal failure. Histopathological assessment of the kidney, the diagnostic test of choice, reveals a non-specific coagulation necrosis of the proximal tubules (**Figure 2**). Necrosis is accompanied by granular casts and proteinuria. Metastatic calcification, a feature of renal failure that occurs due to hypercalcemia, is also identified in many cases. Mineralization of the tongue and abomasum is an important feature seen in bovine renal failure. Ulcers of the gastrointestinal tissues may be a consequence of the uremia and mucosal mineralization.



**Figure 2:** Oak Toxicity. Non-specific coagulation necrosis of the proximal tubules.



**Figure 1:** Oak Toxicity. Bovine swollen kidney and renal cortical hemorrhages.

**Pigweed** (*Amaranthus* species) intoxication results in lesions similar to those of oak intoxication. Indeed, the history of poor pastures, gross lesions, and histological features are often totally indistinguishable. Possibly distinguishing pigweed intoxication from oak poisoning is the fact that pigweed intoxication does not seem to result in perirenal edema. The toxic principle causing the renal lesions of pigweed intoxication has not yet been identified. Additional toxic principles that have been identified in pigweed include elevated nitrate levels and oxalates, making this a truly toxic plant. Nitrate and oxalate intoxication from these plants is rare in comparison to the toxicity nephrotoxin. Fortunately, cattle will not routinely consume *Amaranthus* species unless no other plants are available in the pasture.

**Cocklebur** (*Xanthium* species) produces a glycoside known as carboxyatractyloside. This product inhibits oxidative phosphorylation and effectively blocks ATP from entering mitochondria of the liver and other tissues. The resulting intoxication causes hepatic necrosis. With inhibition of respiration, the periaccinar hepatocytes are most susceptible to the toxin.

The clinical signs of cocklebur intoxication are reasonably non-specific and primarily reflect hepatic and gastrointestinal disease, and possibly hepatic encephalopathy. Abdominal pain and tenderness with lethargy and depression are described. Hepatic encephalopathy, a syndrome commonly seen in ruminants, ensues in some animals that survive a few days. Adult cattle that consume the product are often simply found dead. Icterus is seen in some cattle that survive a few days. The history of animals intoxicated by cocklebur can indicate the consumption of the early growth of *Xanthium* after a rain following a drought. Pigs are usually intoxicated by consuming the emerging shoots of these plants. Cattle tend to be intoxicated when the mature plants are inadvertently incorporated into hay during baling. *Xanthium* plants are hardy and grow during drought conditions that stress most forage grasses. As a result, some hayfields may contain abundant weeds (*Xanthium* species and others as well). Curing of the hay product does not cause diminishment of the carboxyatractyloside content. Though cocklebur plants (especially when mature) are apparently unpalatable, cattle seem to be unable to separate these from the plants in the hay and are intoxicated as a result.

Gross lesions with cocklebur poisoning are referable to the liver and gastrointestinal tract. Liver tissue is



### **Toxicity Diseases in Beef Cattle** (Continued)

pale and possibly hemorrhagic with a nutmeg pattern on the cut surfaces. There may be erosion or ulceration of the intestine. The gastrointestinal contents may contain mature cockleburs. With neurological signs, there may be abrasions of the skin of the face and limbs secondary to seizures, opisthotonus, and paddling before death. As an aside, when dealing with bovine diseases with neurological signs, submission of brain and liver is recommended in these cases in order to rule out syndromes involving neurological diseases as well as those with hepatic encephalopathy.

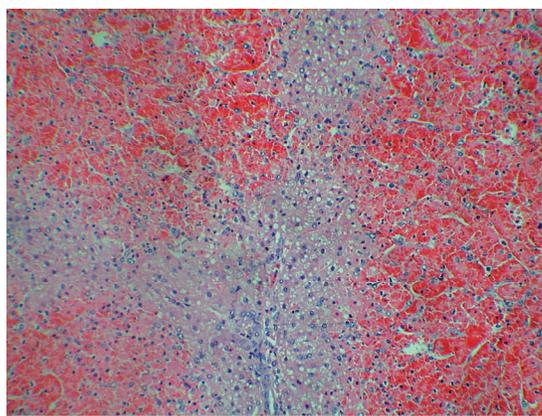
Histological features in cocklebur toxicity are primarily seen in the liver, in which periacinar to occasionally midzonal and submassive hepatocellular necrosis is seen (**Figure 3**). Minimal inflammation is present and the necrotic hepatocytes are rounded, hypereosinophilic, and pyknotic. Hemorrhage is occasionally seen in the sections. In some animals, proximal tubular necrosis of the kidney tissue is also prominent. This occurs because the carboxyatractyloside is secondarily excreted by the kidney, resulting in injury to the tubular epithelium of the nephrons.

**Moldy sweet potato** (*Ipomoea batatas*) intoxication is due to fungal growth on the potatoes with subsequent production of 4-ipomeanol, a pneumotoxic principle produced by the fungi. Pneumotoxicity is the result of transformation of the 4-ipomeanol into toxic intermediate products by the mixed function oxidases that are found in the Clara cells of the terminal airways. The toxic intermediate products from metabolism of 4-ipomeanol cause necrosis of the nearby endothelial cells of the alveolar capillaries. As the endothelial cells are damaged, the alveolar capillaries leak fibrin and other serum products. Damage to the Type I respiratory epithelial cells follows damage to the endothelium, and necrosis of each results in proliferation of the Type II epithelium as a damage repair mechanism.

The history with sweet potato poisoning usually includes feeding of spoiled by-products from sweet potato processing plants. These potatoes are palatable and an excellent feed for cattle with nutritive value and economic savings. Problems develop when the sweet potato products spoil after being exposed to rain. In most instances, covering with tarps or storage under a shed will prevent mold growth. Most intoxicated animals have a history of having been fed from a truck that has been parked in the open weather for a few wet days.



**Figure 4:** Moldy sweet potato toxicosis: Interlobular pulmonary edema and emphysema.



**Figure 3:** Cocklebur toxicosis: Hepatocellular necrosis.

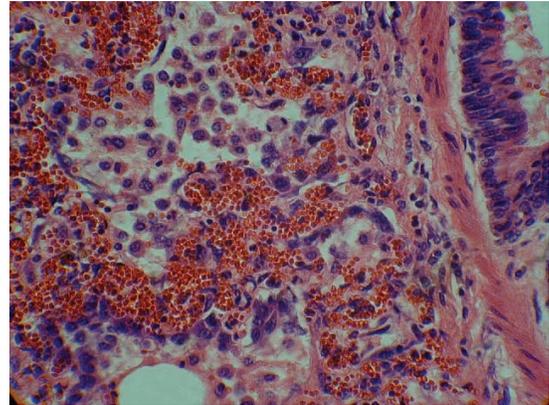
Affected cattle are usually adults. A few hours after ingestion of the sweet potato products, respiratory distress develops. Typical signs include open mouth breathing with protrusion of the tongue. Anxiety develops; the elbows are abducted; and there is rapid death if the cattle are forced to move to another site for examination. The gross lesions are confined to the lungs (**Figure 4**). Interlobular septa are widely distended by edema fluid and emphysema. Emphysematous bullae are often seen after coalescence of the interlobular air.

Microscopic lesions of sweet potato poisoning are also limited to the lung tissue. In addition to the microscopic appearance of the interlobular air and edema fluid, damage to the alveoli is pronounced and diffuse. Alveoli are flooded with abundant eosinophilic edema fluid. Suspended within the



### **Toxicity Diseases in Beef Cattle** (Continued)

fluid are accumulations of fibrin, alveolar macrophages, and scant necrotic debris (**Figure 5**). Some accumulations of organized fibrin form hyaline membranes within the denuded alveolar walls. In most alveoli, hyperplasia of the Type II pneumocytes form a “cobblestone” appearance of large cells, also partly lining the alveolar walls. An important differential diagnosis with moldy sweet potato intoxication is bovine respiratory syncytial virus (BRSV) infection, another very common lung disease that is also seen in adult cattle. This viral disease usually causes illness and death in only individual cattle, whereas moldy sweet potatoes cause disease in groups in most instances. Histologically, BRSV causes bronchiolitis and is associated with syncytial cells, which are features absent with moldy sweet potato poisoning.



**Figure 5:** Moldy sweet potato intoxication,. Necrotizing interstitial pneumonia.

**Nitrate** intoxication in North Carolina is nearly always from the consumption of toxic hay, though damaged crops and some nitrate-accumulating plants can also be toxic. Nitrates, present in all plants as part of the nitrogen cycle, are converted to nitrites ordinarily by anaerobic bacteria in the rumen. The nitrite is absorbed readily into the bloodstream and oxidizes the ferrous ion of hemoglobin to the ferric ion, forming methemoglobin. Methemoglobin is incapable carrying oxygen, thus an animal becomes hypoxic. Nitrates are more concentrated in damaged plants, since nitrate continues to be absorbed by a damaged plant. However, when drought or frost damage occurs, damage to the plant prevents conversion of the accumulated nitrate to nontoxic nitrogen during the nitrogen fixation process.

The clinical signs of hypoxia from nitrate/nitrite intoxication occur within 2-3 hours of ingestion of the toxic plants, as nitrite is absorbed rapidly from the rumen. Respiratory distress is prominent, but most animals are discovered dead, usually in groups. The history usually indicates that a new batch of untested hay has been given to the cattle. Pockets of high-nitrate hay are usually found within the toxic hay, rather than a uniformly elevated nitrate content, and some bales may not cause intoxication whereas others in the same batch may be lethal.

Gross lesions in cattle that have died from nitrate intoxication are not seen in all cattle, particularly those



**Figure 6:** Bovine blood. Methemoglobinemia from nitrate toxicosis.

dead for a few hours. The characteristic finding is “chocolate-colored” blood (**Figure 6**). Blood oozing from the cut surfaces of necropsy specimens is often a brown or chocolate color, but our findings in affected animals are that this is not always reliable on animals that have been dead for several hours. The most consistent finding is the presence of nitrates within the aqueous humor of the eye. The fluid in a positive case causes a nitrate dipstick to turn pink within 30 seconds after immersion. These dipsticks are available from pet stores and are used to test for nitrate levels in aquarium water. They provide an excellent presumptive test for nitrate intoxication in field situations, particularly when decisions about the feed or treatment of the cattle must be made. There is a low level of nitrates and nitrites within the aqueous humor of adults and calves that can be considered less than toxic. The practitioner is encouraged to contact the NCVDL about these numbers if in

doubt, as we have conversion tables for calculating the levels of nitrates consumed. In addition, in positive cases, a small amount of aqueous humor from a suspect animal can be frozen in a red top serum tube for submission to a reference laboratory and more precise measurement.



### **Toxicity Diseases in Beef Cattle** (Continued)

Testing for nitrate levels in hay is always recommended due to the frequency with which toxic forage is seen in North Carolina. This can be accomplished by submission of samples from all feed sources to a feed testing laboratory, such as the Constable Laboratory. Consultation with a County Extension Agent will also be helpful in determining methods for sampling for nitrates.

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Plumlee KH: Clinical Veterinary Toxicology. 2004; 477 pages. Elsevier Press, Philadelphia.

### **Review of Bovine Abortion/Stillbirth Cases Submitted in 2007** by Dr. Tim McComb

Submissions to the NCVDLS between Dec. 1, 2006 and Dec. 1, 2007 for bovine abortion evaluation were reviewed. Twenty nine finalized cases met the following criteria: an intact fetus was submitted in a condition conducive to ancillary testing OR formalin fixed and fresh tissues were submitted from an intact fetus accompanied by a detailed report of the gross findings. Case diagnoses were classified as either 1- definitive, meaning a specific etiology was isolated, 2- morphologic, meaning only gross or histologic lesions or patterns are determined, or 3- inconclusive, meaning no abnormalities were determined. A definitive diagnosis was reached in four submissions (14%) and a morphologic diagnosis was reached in 7 cases (28%). Bovine Viral Diarrhea Virus (BVD) was detected in two separate cases, and *Salmonella* sp and *Campylobacter jejuni* were cultured from tissue pools in another two separate cases.

Routine herd vaccination was either not practiced or not provided in the history for the two BVD diagnoses. Salmonellosis is a frequent cause of bovine abortion, however *C. jejuni* is more commonly cited for abortion in small ruminants. Although *Salmonella typhimurium* and *S. dublin* are considered common isolates in cattle, serotyping from this case identified *S. pomona*, which has limited information associated with cattle. For cases where a definitive diagnosis could not be made, placentitis or placental vasculitis was the most common morphologic diagnosis.

Of 18 cases with an inconclusive diagnosis, 12 were defined by the history as a herd problem (having multiple animals affected in the same season); however, only three of these submitted multiple cases.

In ten cases, placenta or amnion was included with the submission. Of these ten, four had a morphologic diagnosis based on placental lesions alone, and in one case the only tissue to yield growth on bacterial culture was the placenta.

It is interesting to note the gestational age of submissions, where only 14% of Holstein submissions were in the third trimester, while 86% of beef breed submissions (Angus, Hereford, and crosses) were in the third trimester. Some of this may be explained by the subjective nature of differentiating an abortion from a stillbirth. Most of these third trimester submissions were full term or near full term and cases received as stillbirths were not included in this review, which may contribute to the discrepancy.

Unfortunately, arriving at a definitive diagnosis is not a common result when evaluating abortion cases. Some estimate that a definitive diagnosis is reached in only 20-40% of all abortions. While this may seem discouraging, it emphasizes the importance of submitting cases early in a herd problem (sometimes before it is even recognized as a herd problem), and making multiple submissions as the problem progresses. One challenge facing a successful diagnostic abortion work-up is autolysis. The fetus autolyzes very rapidly and



### **Review of Bovine Abortion/Stillbirth Cases Submitted in 2007** (Continued)

typically has been dead for a period of time prior to being expelled from the uterus, allowing the body temperature of the dam to enhance the autolytic process before the fetus is discovered. Even moderate autolysis will hinder the ability to find histopathologic lesions and limit the ability to isolate viral pathogens. Another challenge is receiving fetal membranes, or placenta. Gross and microscopic lesions of the placenta can be useful in narrowing down the causes of abortion. This tissue also decomposes very readily making thorough evaluation prior to shipment helpful as well. Also for this reason, submitting a large portion of placenta is encouraged in order to characterize the gross pattern of any apparent lesions.

When doing a field necropsy for abortion evaluation it is important to harvest tissues of importance. As mentioned above, placenta is very valuable in working up a case. One of the most often overlooked samples is abomasal content. The fluid in the abomasum is representative of the intrauterine environment and has the best chance of remaining sterile. Finally, sampling affected and non affected animals in the herd for serological comparisons is also an often overlooked resource when investigating herd abortion problems. Below is a table of tissues that are utilized for each test.

<b>Tissues</b>	<b>Tests</b>
Placenta	Culture, Histology
Abomasal Fluid	Culture (Aerobic, C. fetus, C. jejuni, listeria)
Lung	Culture (aerobic, C. fetus, C. jejuni, listeria), Viral Isolation (BVD, IBR), IHC (BVD), Histology
Thymus	Viral Isolation (BVD, IBR), IHC (BVD), Histology
Spleen	Viral Isolation (BVD, IBR), Histology
Kidney	Viral Isolation (BVD, IBR), Fluorescent Antibody Test (Leptospirosis), Histology
Liver	Culture, Viral Isolation (BVD, IBR), Histology
Tonsil	Viral Isolation (BVD, IBR), IHC (BVD)
Brain	Histology, IHC (Neospora)
Heart	Histology, IHC (Neospora)
Ear Notch	IHC (BVD)

## **Interesting Cases**

### **Red Maple Toxicity in Horses** by Dr. Peter Moisan

Red maple leaves are toxic to horses. *Acer rubrum*, the red maple, is a common tree in North Carolina, easily recognizable in the fall due to the brilliant red leaves that shed in abundance as they dry. Horses occasionally eat these dried leaves and may actually preferentially consume the leaves. Though the autumn is the primary time that our diagnostic laboratories are presented with these cases, the dried leaves are toxic to horses throughout the year. As a result, leaves encountered from damaged branches and storm-felled trees are



### **Red Maple Toxicity in Horses** (Continued)

also a danger. Grazing animals other than horses that consume the dried maple leaves do not seem to be affected by the toxic principle.

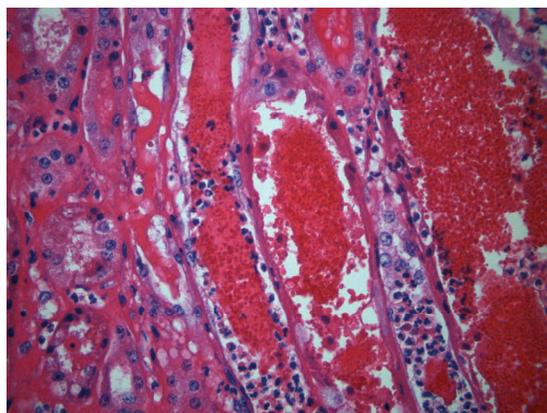
The pathophysiology of red maple toxicity is well understood, though the toxic principle in the dry leaves is yet to be determined. The product is absorbed following oral consumption and induces methemoglobinemia in erythrocytes. Heinz body anemia is the result. Heinz bodies are condensed accumulations of denatured hemoglobin that form 1-2µm spherical bodies in the erythrocytes. Affected erythrocytes are recognized and removed from the circulation by the macrophages of the spleen and liver. The loss of normal erythrocytes results in a severely depressed hematocrit with anemia. Icterus also develops from the liberation of the hemoglobin into the circulation. Excess hemoglobin, metabolized by the liver under normal conditions, cannot be removed from the circulation by the macrophages and is excreted via the kidneys. Accumulations of the hemoglobin product, filtered by the glomeruli of the kidney, develop in the tubular lumens of nephrons. Hemoglobin casts form in the tubules and necrosis of the tubular epithelium develops. The necrosis of these tubular epithelial cells is considered to be from the accumulation of iron liberated from the hemoglobin rather than from the hemoglobin itself. Iron is a catalyst of oxidative metabolism. When in excess, membrane damage and other toxic oxidative changes occur, such as those seen in damaged renal tubules.

Clinical signs in an intoxicated horse reflect the damage to the erythron and concurrent anemia. Renal signs are also seen. Damage to a significant number of erythrocytes results in anemia. Hemoglobinemia results in profound icterus with hemoglobinuria. This is followed by acute renal failure with oliguria or anuria. Weakness is primarily due to the anemia. Death follows in 2-5 days after consumption of the leaves. A dose of 1.5 grams/kg body weight (approximately 1.5 pounds for an average-size adult horse) constitutes a fatal dose. Anemia, resulting from severe depression of the hematocrit, can cause necrosis of the liver. In some affected horses, loss of liver function will cause the signs of hepatic encephalopathy.

The gross lesions with red maple toxicity reflect damage to the erythron and kidney. Generalized icterus is the result of marked hemoglobinemia that occurs with rupture of the affected erythrocytes. Hemoglobinuria is the result of the massive amount of hemoglobin presented to the glomeruli of the kidneys. Red urine is described in most cases. Darkening of the renal parenchyma (the so-called “gunmetal” kidney) has been seen in most of our cases. In some instances, swelling of the liver is also noted, reflecting necrosis of hepatic tissue from hypoxia. Traumatic injuries may be the result of encephalopathy in a few animals.

The microscopic lesions in red maple toxicity, taken together with the history and gross findings, are pathognomonic for erythrocyte destruction and hemoglobinuric nephrosis. Cholestasis of bile in the bile ducts and canaliculi of the liver reflects the erythrophagocytosis and liberation of hemoglobin. Finely granular hemoglobin casts form in the renal tubules and are identified concurrently with necrosis of the epithelial cells (**Figure 1**). In horses that survive a few days, bile staining of epithelial cells of the renal tubules can also be identified. An additional liver lesion, reflecting hypoxia from erythrocyte loss and anemia, is necrosis of the peri-acinar and midzonal hepatocytes (**Figure 2**).

Differential diagnoses for cases of equine hemoglobinuric nephrosis secondary to Heinz body anemia include onion and phenylbutazone toxicity. These products will also cause similar clinical signs and history must be carefully considered for diagnostic purposes.



**Figure 1:** Equine kidney. Hemoglobinuric nephrosis. Tubular hemoglobin casts are granular and eosinophilic and are associated with necrosis of the renal tubular epithelium.

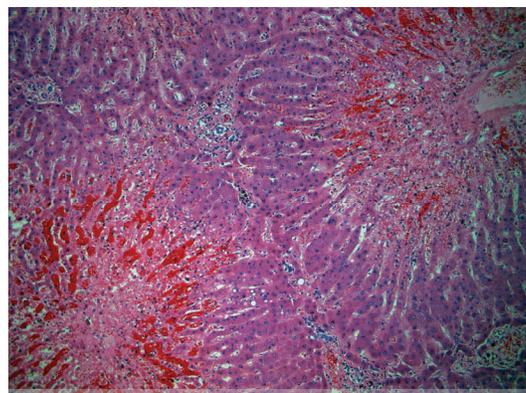


### **Red Maple Toxicity in Horses** (Continued)

Prevention of red maple toxicosis in horses involves exclusion of horses from pastures in which there are red maple trees. The wilted leaves are toxic and toxic events are most often seen in the fall. Removal of the trees is the best preventive step, but proper pasture management will avoid toxicity as well. Down limbs and trees can occur at any time of the year.

Our most recent herd exposure and losses with red maple toxicosis was reported by the Elkin Laboratory. A group of horses from West Jefferson, North Carolina were affected. The third animal to die was a 6-year-old Tennessee walking horse mare. This animal was presented to the Elkin Laboratory for necropsy. The gross examination revealed that the 340kg mare was in normal states of body condition and hydration. Lesions of pathological significance were not identified grossly.

Histological evaluation of the tissues revealed submassive degeneration of the liver consistent with hypoxic change. Hemoglobinuric nephrosis was identified in sections from the kidney. The necrotic tubules of the renal epithelium contained large granular casts consistent with hemoglobin. Further historical information indicated that a red maple tree had fallen in the pasture and that the affected horses were seen grazing on the wilted leaves a few days before clinical signs and death occurred.



**Figure 2:** Equine liver. Periacinar and midzonal necrosis. Necrosis of the hepatocytes of the periacinar and midzonal regions is associated with anemia from loss of hepatocytes.

### **References**

Merola V, Volmer PA: Red Maple, in Plumlee KH, editor: Clinical Veterinary Toxicology. St. Louis, Missouri, 2004, Mosby Press

Rooney JR, Robertson JL: Hemolymphatic System, in Equine Pathology. Ames, Iowa, 1996, Iowa State University Press.

### **Blastomycosis in a Cat** by Dr. Stacy Robinson

A 6 year old male, domestic shorthair, predominantly outdoor cat had a history of having multiple abscesses. Material from the abscesses was described by the referring veterinarian as thick and resembling “grits” with little to no odor. The abscesses were lanced, drained and flushed. The cat was treated with Baytril and Clavamox. The cat did not respond to the antibiotics. The cat tested negative for both FeLV and FIV. The cat died during the evening.

The 4.0-4.5 kg cat was presented to the Rollins Diagnostic Laboratory for necropsy. The cat was in good body condition and mild dehydration status. There was diffuse white stippling on the cut surface and pleural surface of the lungs. Nodules were palpated within the pulmonary parenchyma. Multifocal, pinpoint, stippling was within the pale tan cortex of the cut surface of the kidney. A cavitation in the skin that was associated with necrosis of subcutaneous tissue and adjacent skeletal muscle was located 2 to 3 cm from the base of the tail. Another similar wound was located over the left hock and extended above and below the joint. Bilaterally the popliteal lymph nodes were enlarged.

#### Histological findings:

- Lung; pneumonia severe, multifocal and coalescent, chronic active, pyogranulomatous with broad-base budding yeast.
- Popliteal lymph node; Lymphadenitis, severe, diffuse, chronic, active, pyogranulomatous, with broad-base budding yeast.
- Haired skin, subcutis; dermatitis and panniculitis, severe regionally extensive, chronic active, pyogranulomatous with broad-base budding yeast.



### **Blastomycosis in a Cat** (Continued)

#### Direct Examination of the Popliteal Lymph Node with Diff Quick Stain:

- Revealed yeast resembling *Blastomyces*.

#### Fungal cultures:

- *Blastomyces dermatitidis* was isolated from the left hind leg subcutaneous tissues, popliteal lymph node and lung.

#### Additional information that was obtained after the diagnosis of blastomycosis was made:

- This cat lived in a wooded area with a nearby creek. A dog from the same neighborhood was diagnosed with blastomycosis a month before the diagnosis was made in this cat.

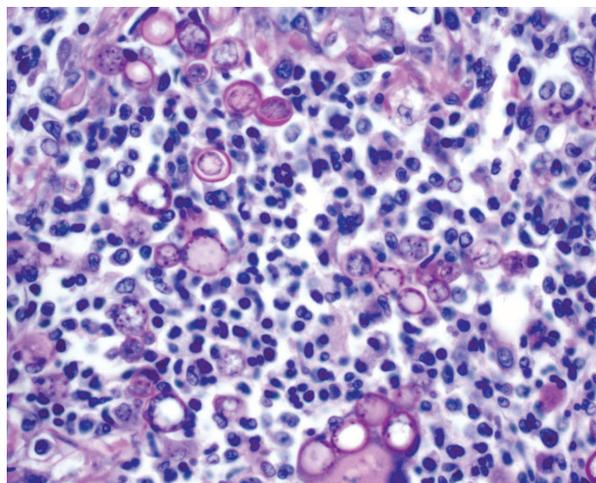
Blastomycosis is a systemic fungal infection caused by *Blastomyces dermatitidis*. The soil is believed to be the reservoir. *Blastomyces* grows best in acidic, sandy soil, high in organic matter that is located near water. Proximity to water and exposure to excavation sites increase the risk for infection with this organism. Other risk factors include residence or travel to the Midwestern, south-central and southeastern areas of the United States.

Blastomycosis is most commonly diagnosed in people and dogs but other animals such as cats, horses, and ferrets have been infected with the organism. The most common signalment of dogs infected with this organism are large breed male dogs that are 2 to 4 years of age and one of the sporting or hound breeds. Blastomycosis is rarely diagnosed in cats. Cases of blastomycosis in both indoor/outdoor cats and indoor only cats have been reported in the veterinary literature.

Dogs and cats acquire the infection usually through inhalation of the infective spores from the environment. The spores enter the terminal airways and a primary infection develops within the lungs. The yeast can then be disseminated to other organs through the blood or lymphatics. In dogs, the organism most often disseminates to the skin, eyes, bones, lymph nodes, subcutaneous tissues, external nares, testes and brain but can disseminate to other organs as well. In the few reported cases of blastomycosis in cats, the organism has been found in the lung, liver, heart, trachea, spleen, kidney, eye, central nervous system, lymph node, gastrointestinal tract, pleura and peritoneum.

Clinical signs in dogs include weight loss, anorexia, dyspnea and coughing, ocular disease such as uveitis and glaucoma, and skin lesions. Lethargy, weight loss, dyspnea, ocular disease, and draining skin lesions seem to be the most common clinical signs in cats. In many of the reported cases in cats, there appears to be no evidence of immunosuppression at the time of diagnosis. Many cats are negative for FeLV, FIV and other immunosuppressive conditions.

A diagnosis of blastomycosis is best obtained antemortem via cytology from draining skin lesions transtracheal washes, prostatic fluid and aspirates from enlarged lymph nodes. The yeasts are between 5-20 micrometers in diameter, nonencapsulated, broad-based and have a thick refractile, double cell wall. Culture in private practices is not recommended because of risk of inhalation of the infective spores by employees. Antifungal medications such as itraconazole and amphotericin B have been used successfully to treat this disease.



**Figure 1:** Blastomycosis cytology.



### **Blastomycosis in a Cat** (Continued)

*Blastomyces dermatitidis* is a zoonotic agent. However, *Blastomyces* is not transmitted directly from animals to people via aerosols. People are at risk of acquiring the infection from the same environment from which their pets have acquired it. People have developed the disease from penetrating wounds caused by objects such as knives and needles, contaminated with the organisms as well as from bites from an infected dog. Veterinarians have developed the disease after performing a necropsy on an infected animal.

### **References**

Gilgor, C, Graves, TK, Barger, AM, and O'dell-Anderson, K. 2006. Clinical aspects of natural infection with *Blastomyces dermatitidis* in cats: 8 cases (1991-2005). JAVMA 229: 96-99.

Greene, CE., 2006. Infectious Diseases of the Dog and Cat, 4<sup>th</sup> ed., St. Louis, MO pp.569-575.

Songer, JG and Post, KW., 2005. Veterinary Microbiology Bacterial and Fungal Agents of Animal Disease, St. Louis Missouri pp. 377-380.

## Departmental News

### **ROLLINS LABORATORY**

**Histopathology...** Dr. Marti Hanes has resigned her position as Veterinary Pathologist effective October 31, 2007 to assume a position in Laboratory Animal Pathology at University of Texas Health Science Center in San Antonio.

**Bacteriology...** Medical Laboratory Technician, Catherine Tancrelle, resigned in November to pursue other interests. Rae Murphy has recently been hired to fill this vacancy. She comes to us from the Animal Health Laboratory at the University of Guelph and has an extensive background in Veterinary Microbiology.

**Quality Assurance...** Ghazala Jawad, MS has been selected as our Quality Assurance Manager filling a vacancy of several months duration. She has an extensive background in both veterinary diagnostics and quality control/assurance and will be an asset to our laboratory quality program.

**Receiving...** Welcome to Paul Locicero who is the newest addition to the receiving section as a veterinary laboratory assistant.

### **ROSE HILL LABORATORY**

Welcomes Dr. Tim McComb, who recently joined the staff as Veterinary Diagnostician. He received his DVM from Cornell University in 2006 and was employed as dairy practitioner in Florida for 1.5 years prior to moving to North Carolina.

### **MONROE LABORATORY**

Medical Laboratory Assistant II, Catherine Bennett, resigned after one year to pursue other interests.

Maggie Nelson, Veterinary Laboratory Assistant I, is a volunteer for the Union County Literacy Council. For her efforts, she has received an award of recognition and was featured in a local newspaper article. During a Carolina Panthers football game, she was given the Community Quarterback Award for her commitment to helping others.



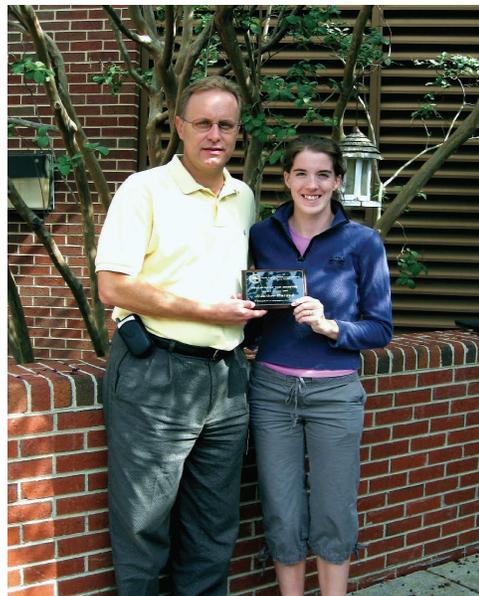
Dr. Tim McComb



## Employees of the Quarters

*Congratulations Heather!*

NCVDLS recognized Heather Durand, Medical Laboratory Technologist I in the Bacteriology Laboratory, as its Fall 2007 Employee of the Quarter. She was nominated for her efforts in the extensive revision and organization of section laboratory quality control documents. This was in addition to her regular job duties. Heather is an extremely conscientious employee and considerate of her co-workers. In her spare time, she enjoys sports and playing the piano.



*Congratulations Denise!*

Congratulations are in order for M. Denise Blanchard our Winter employee of the quarter. Denise has been employed at Rollins Laboratory since 2003 as a Processing Assistant IV where she has the huge responsibility of placing orders for all the NCV DLS facilities. Her diligence and attention to detail with items ordered have resulted in monetary savings for our system. She was nominated by Dr. Aziz for her outstanding efforts to reorganize the NCV DLS stockroom to make it more efficient and for implementing a computer-based inventory system. In her spare time she enjoys reading, doing crafts with her two children, and working in her flower garden.



## Veterinary Staff

### Rollins Laboratory (919) 733-3986

**Director**

Dr. David Marshall

**Assistant Director**

Dr. Karen Post

**Veterinary Diagnosticians**

Dr. Jennifer Haugland

Dr. Stacy Robinson

Dr. Mahogany Wade

**Veterinary Pathologists**

Dr. Tahseen Abdul-Aziz

Dr. Peter Moisan

Dr. Steven Rushton

**Veterinary Microbiologist**

Dr. Gene Erickson

### Arden Laboratory (828) 684-8188

**Director**

Dr. Richard Oliver

**Veterinary Diagnostician**

Dr. David Drum

### Elkin Laboratory (336) 526-2499

**Director**

Dr. Darrell Rector

**Veterinary Diagnostician**

Vacant

### Monroe Laboratory (704) 289-6448

**Director**

Dr. Kim Hagans

**Veterinary Diagnostician**

Dr. Reg Ridenhour

### Rose Hill Laboratory (910) 289-2635

**Director**

Dr. Carlton Rouse

**Veterinary Diagnostician**

Dr. Tim McComb