

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

Is it possible that another quarter has elapsed since our last newsletter? Each week seems to bring a myriad of challenges and new issues that the NCVDLS is looked to for support and expertise. The past three months have been no different, with Avian Influenza and a major pet food contamination event compounding the traditional increase in case load that we see in the fall and winter of each year.



The expanding H5N1 Avian Influenza occurrence in Asia has brought the entire issue of bird influenzas into the eye of the media and the general public. The NCVDLS has been the primary resource for animal influenza surveillance for years, conducting over 285,000 avian flu serology tests in 2004 for both the commercial industry and backyard bird owners, in addition to numerous necropsy, viral isolation, and PCR procedures. The recent media attention has only increased the demand for these services. This, coupled with the recent National Chicken Council's announced program to test a representative sample of each meat type broiler flock prior to slaughter, will keep the system at the forefront of the surveillance effort. We take pride in being depended on for our expertise in the effort to protect our poultry industry and the health of the general public.

December 2005 brought us the news of the [Diamond Pet Food](#) recall, as 19 different varieties of dog and cat food produced in South Carolina, some with elevated aflatoxin levels from moldy corn raw ingredients, were suspected of being distributed to North Carolina and 21 other eastern states. As of this writing, our laboratory system has evaluated over thirty animals suspected of liver failure secondary to eating contaminated food, and classified twelve deaths as "highly suggestive" of acute or chronic aflatoxicosis. Coupled with feed analysis by our Food and Drug Protection division and epidemiological questionnaires distributed to practicing veterinarians, North Carolina has been a lead state in investigating this matter. Currently, the event is ongoing with anticipated additional cases to analyze before coming to a conclusion.

On January 23, 2006, Canada announced the confirmation of their fourth case of Bovine Spongiform Encephalopathy (BSE) in a six year old animal from the province of Alberta. This finding emphasizes the importance of the laboratory system's role in surveillance for this disease of public health significance. Since June 1, 2004, we have submitted samples from over 225 bovines meeting the "high risk" definition that have been presented to the laboratory system, all of which have tested negative. We will continue cooperating with the USDA in this enhanced surveillance capacity until epidemiological data supports a reduction in the testing level.

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Points of Interest...

- Aflatoxicosis
- Bovine Rabies
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- Equine Neuritis
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- Sudden Death
- Work Conference

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Holiday Closings...

- New Year's Day-January 1
- MLK's Birthday-Jan. 16
- Good Friday-April 14

Our laboratories will be closed on the above listed days.

**From the Director** (continued)

A Request For Proposal (RFP) in early January for studying the consolidation of the NCDA&CS Veterinary Laboratory System with the State Laboratory of Public Health and the SBI Crime Laboratory was distributed. The bid to conduct the study is expected to be awarded by February 20, with a final report to the General Assembly by May 22, 2006. We have serious concerns regarding this misguided study and welcome any input that you may have on the issue. We will keep you posted as this study evolves through routine postings on our web site: www.ncvdl.com.

Congratulations to Brandon Brown, our Fall Employee of the Quarter. Brandon is a Medical Technologist II in our Serology section, and was recognized for his dedication to service, cooperative positive attitude, and willingness to help coworkers with any task needing attention. Brandon is a true public servant, and an example of the many dedicated employees that can be relied upon to accomplish our mission and provide service to the citizens of North Carolina.

We are pleased to announce that Dr. Martha Hanes has been selected to fill our final Veterinary Pathologist vacancy, and will begin work on March 15, 2006. Marti comes to us with a wide array of skills and experience, and is triple boarded as a diplomate of the American College of Veterinary Pathologists (ACVP), the American Board of Toxicology (ABT), and the American College of Laboratory Animal Medicine (ACLAM). We are pleased to welcome Marti to our staff, and are confident that she will prove to be a valuable adjunct to the services we can offer to you, our clients.

In other employee news, we held an all day work conference in late November for all Rollins and branch lab veterinary pathologists and diagnosticians. Agenda items included coordination of case investigations and write ups, QA/QC and SOP development and implementation, as well as a discussion of which testing procedures should be added or eliminated from our menu of services. In addition, a program was initiated in January to rotate satellite laboratory veterinarians through the Rollins facility for one week assignments in order to train with our staff pathologists and increase their exposure to protocols here at the central facility. We are making significant strides in better incorporating satellite laboratory activities into overall system protocols.

In early November, Dr. Post and Lou Ann Risser, QA/QC Supervisor, attended an all day workshop and training session in conjunction with the American Association of Veterinary Laboratory Diagnosticians' (AAVLD) meeting in Hershey, Pennsylvania. Information attained at that session is helping to further advance our Quality Assurance program implementation.

Please feel free to contact me regarding any laboratory system issue that you feel needs discussing. Our goal is to provide you, our veterinarian and citizen stakeholders, with the best available services to accommodate your animal health needs.

Regards,

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
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Dr. Gene Nemecek	GIS-Hog Slats
Dr. David Marshall	NCDA&CS Veterinary Division

Client Corner

Canine Aflatoxicosis and Tainted Dog Food by **Dr. Peter Moisan**

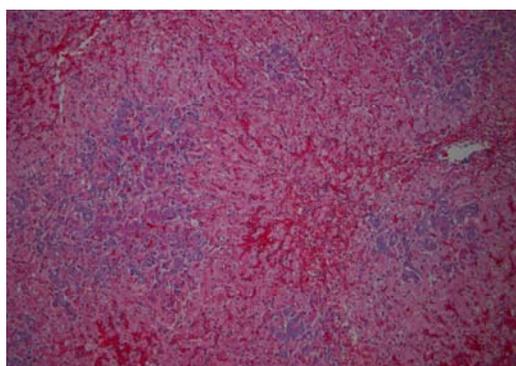


Figure 1. Hepatocellular necrosis and degeneration.

Following reports of contamination of commercially available dog food in North Carolina, the North Carolina Veterinary Diagnostic Laboratory System (NCVDLS) received numerous inquiries and necropsy submissions of dogs from concerned owners. It was reported by company press release that 19 proprietary brands of Diamond Dog and Cat Food products were contaminated by aflatoxins as a result of mold growth in the cereal ingredients of their food products. As of January 27, 2006, there have been 12 histologically confirmed cases of canine toxic hepatopathy most likely due to aflatoxins. Most cases have been in hunting dogs fed bulk dog food, as well as in a few pet dogs. These cases have been seen at the Arden, Elkin, Monroe, and Rollins Laboratories. Confirmed feline cases have not yet been seen in this state. In addition to the

necropsies performed on dogs with suspected aflatoxicosis, feed samples have been submitted to the laboratories and to the Constable Laboratory for aflatoxin quantification and many have been positive for aflatoxins. A number of additional dogs have also been submitted to the laboratory for necropsy with a suspicion that they have died from aflatoxin consumption, only to find that they have died from a variety of non-toxic or less exotic causes.



Canine Aflatoxicosis and Tainted Dog Food (continued)

Clinical histories for affected patients include features of acute liver failure. Hepatic enzyme levels are highly elevated and liver function tests indicate severe hepatic impairment. The course of the disease, with supportive care, is usually 3 to 5 days, which is fairly typical of any cause of acute liver failure. Clinical signs reported by clinicians and owners include gastrointestinal hemorrhage and varying levels of icterus. Post mortem findings from our laboratories indicate that gastrointestinal hemorrhage and icterus are fairly constant findings. Petechiae are commonly seen in the subcutis and internal viscera, and are probably secondary to disseminated intravascular coagulation. Some of the livers have exhibited a nutmeg pattern and a few have been slightly shrunken, however, marked gross liver changes have not been seen in any of these cases.

The typical histological features were consistent with acute to subacute hepatopathy without inflammation. Periportal necrosis of hepatocytes is common and there is submassive vacuolar degeneration of the remaining hepatic lobular tissue. Occasional karyomegaly and binucleated cells are seen in the periportal areas of hepatic lobules. Bile duct proliferation is generally marked and is often bridging in these cases. Early fibrosis is usually identified, beginning at the portal triads and dissecting into the sinusoids of the lobules. Plump, immature fibroblasts are associated with thin tendrils of collagen in the fibroblastic response. Inflammatory cells are normally few in number and not a consistent feature in these cases.

The amounts of aflatoxin have varied markedly between the dog food samples submitted for analysis; however, many have been positive for aflatoxin. All of the samples submitted with the hepatopathy dogs showing histological lesions of suspected aflatoxicosis have contained measurable levels of aflatoxin, varying from 15 to 400 ppb. These levels correlate closely with aflatoxin levels that were reported in a Texas episode of canine aflatoxicosis that occurred in 1998¹. In our sampling, the B1, B2, G1, and G2 subtypes of aflatoxins have been demonstrated.

Aflatoxins are potent mycotoxins produced by strains of *Aspergillus flavus* and *Aspergillus niger*. Like many other fungal toxins, they are metabolites that are produced by the fungi during growth in spoiled or damaged grain products².

The substances are potent mitotic inhibitors that bind to chromosomal DNA and prevent the transcriptional activity of RNA polymerase. In other words, the binding of the aflatoxins to the DNA molecules prevents the production of new RNA from the DNA template in the chromosome of hepatocytes, precluding subsequent cellular replication. In this instance, there was reportedly contamination of a single large batch of corn by the organisms with subsequent growth and toxin production. Though these fungal species (and their metabolites) are ubiquitous, the growth of the fungi with subsequent aflatoxin production only occurs in certain circumstances when damage to the grain coincides with elevated moisture conditions.

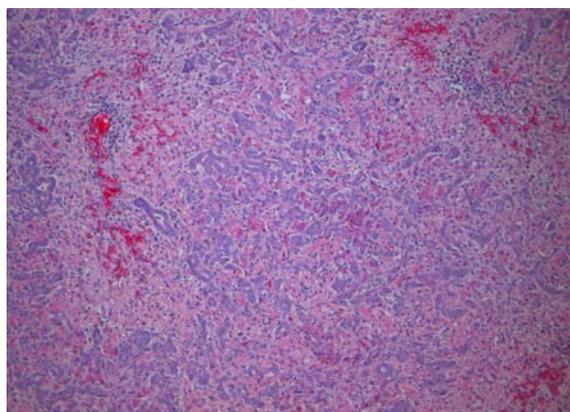


Figure 2. Bile duct hyperplasia.

1. Bingham AK, Huebner HJ, Phillips TD, Bauer JE. Identification and reduction of urinary aflatoxin metabolites in dogs. *Food Chem Toxicol.* 2004, 42:1851-1858.
2. Yu FL, Dowe RJ, Geronimo IH, Bender W. Evidence for an indirect mechanism of aflatoxin B1 inhibition of rat liver nuclear RNA polymerase II activity in vivo. *Carcinogenesis.* 1986; 7:253-257.



Employee of the Quarter

Congratulations, Brandon Brown!

The North Carolina Veterinary Diagnostic Laboratory System has awarded its Fall 2005 Employee of the Quarter to Brandon Brown. Brandon, who is a member of the Serology Department at Rollins Laboratory, is being recognized for his exemplary work ethic and steadfast dependability. Brandon began employment in 2000 as a Veterinary Laboratory Assistant and was later promoted to a Medical Laboratory Technologist in Serology. Whether aiding in the Histology or Receiving Departments, Brandon is appreciated for being conscientious and taking the extra step to ensure that all work is completed. Brandon is an excellent employee; he is dependable, gets the work done in a timely and efficient manner, and is always projecting a smile with an optimistic attitude. Rollins Laboratory regards Brandon Brown as an asset and looks forward to his continued success.

Award presented by Agriculture Commissioner Steve Troxler



Photo taken by Charlotte Dover

Departmental News

ROLLINS LABORATORY:

Holiday Festivities...Agriculture Commissioner **Steve Troxler** attended the annual holiday party at Rollins Laboratory. Festivities included a large holiday dinner, games and prizes, as well as the awarding of the Employee of the Quarter to **Brandon Brown**. The laboratory also came together to participate in the Boys and Girls Club of Wake County's Angel Tree Program. The Boys & Girls Club consists of five clubs dispersed throughout Wake County. Each participating employee chose one of the twenty-eight Angels from our holiday tree and purchased gifts for their selected child. All the gifts were placed under the tree during the party and later picked up by a representative from the club. The Angel Tree was a huge success with many toys and clothes going to very deserving children. We look forward to continuing this wonderful holiday tradition.

Quality Assurance/Safety...Rollins' safety officer, **Kathy Schmidt**, attended the Annual OSHA Update at the Friday Center in Chapel Hill. Kathy was also certified in CPR and AED after completing training downtown.

Reception...Rollins would like to welcome our new phone receptionist **Cindy Orlosky**, who officially started her position on December 1.

Receiving...We are also welcoming **Judy Liverman** back to the laboratory. Judy is returning to Rollins as a Laboratory Assistant; she previously worked in that capacity from June 1995 until February 1999.



Photo taken by Charlotte Dover

**ROLLINS LABORATORY (continued):**

Molecular...In November, **Chad Menard** attended the NPIP Avian Influenza Diagnostic Workshop in Athens, Georgia.

Bacteriology... **Rob L'Hereux** accepted employment as a Medical Laboratory Technician in November. He has a bachelor's degree in Biological Sciences and laboratory experience in food microbiology and quality control. **Carol Crabtree** attended a Dr. Science Update in Clinical Microbiology CE seminar held at the William F. Andrews Center.

Histology...We would like to congratulate **Allison Heatherly** on her graduation from [NC State](#) with a bachelor's in Animal Science in December.

Veterinarians... Rollins would like to welcome our newest Veterinary Pathologist **Martha Hanes DVM**, Diplomate American College of Veterinary Pathologists; she also has board certification with the American College of Laboratory Animal Medicine and the American Board of Toxicology. Dr. Hanes comes to us from a private toxicology research facility and brings with her a wide range of skills. We look forward to her starting with us on March 15, 2006.

The first Inter-laboratory Work Conference for NCVDLs Veterinarians was held at the Rollins Laboratory on November 19. Topics of discussion included diagnostic test updates, quality assurance, and report standardization. Our goal is to have this meeting on an annual basis in hopes of providing improved diagnostic services for our clients.



The following NCVDLs employees attended the November annual meeting of the American Association of Veterinary Laboratory Diagnosticians in Hershey, Pennsylvania: **Drs. David Marshall, Gene Erickson, Jennifer Haugland, Stacy Robinson, David Drum, Karen Post** and **Ms. Beverly Wood** (Rollins Molecular Diagnostics Lab Supervisor) and **Lou Ann Risser** (Quality Manager). In addition, **Drs. Robinson and Haugland** participated in an eight-hour continuing education symposium entitled "Diagnostic Toxicologic Pathology" as part of the CL Davis Symposium. Specific topics that were covered included organ specific toxicity of the liver, heart, skeletal muscle, lungs and kidney, as well as, poisonous plants and diagnostic toxicology. **Drs. Erickson, Marshall and Post** and **Ms. Beverly Wood** and **Lou Ann Risser** attended the NAHLN/AAVLD QA Symposium. Document control, proficiency testing, assay approval, and new technologies were items of discussion.

Dr. Peter Moisan, Veterinary Pathologist, has been re-certified as a Beef Cattle Specialist with the American Board of Veterinary Practitioners to update his original 1996 certification as a Beef Cattle Specialist. The specialty requires re-certification every 10 years. **Dr. Moisan** joins 10 similarly qualified Beef Specialists in the United States. In addition to the Beef Cattle Specialty, **Dr. Moisan** is also a specialist in Food Animal Practice with the American Board of Veterinary Practitioners. With these specialty qualifications, we remind you that in addition to his regular duties as Pathologist in the North Carolina Veterinary Diagnostic Laboratory System, **Dr. Moisan** is also available for beef, swine, dairy, and small ruminant herd visits and consultations at the request of private practitioners in the state of North Carolina. Please contact him at the Rollins Laboratory for further details.

**ARDEN LABORATORY:**

Congratulations and many thanks go to **Paul Rector**, a Medical Laboratory Technician, on 30 years of service this past November; he has been a tremendous help to the laboratory. **Dr. Richard Oliver** attended the National Multi-hazard Symposium: "One-Medicine" Approach to Food Defense in Durham this past December; also, in January, **Dr. Oliver** attended the annual meeting for the Association of North Carolina Boards of Health.

MONROE LABORATORY:

Cathleen Harvey was hired as a Medical Laboratory Assistant in November of 2005.

ROSE HILL LABORATORY:

The Virginia Sheep Producer's Association elected **Dr. Hope Lucas** as its North Carolina board representative on January 7.

Disease Trends

Investigation of Multiple Stillbirths and Agalactia in a Beef Herd by **Dr. Jennifer Haugland**

Three stillborn Angus calves were presented to the Rollins Laboratory in October from an Angus herd in Yadkinville, North Carolina. According to the history provided, there were 20 stillbirths out of 250 calvings this past fall. These calves were full term, full size, and were minimally autolyzed. No dystocia or retained placentas were experienced in the affected dams. First-calf heifers and older cows (6 to 9 years) were affected. The live calves were strong and active, although there were a few calves that died within a few days. It was later discovered these neonatal deaths were due to starvation because the dams were experiencing agalactia. There were many cows, usually older, that had agalactia in the herd. Many of these calves were grafted onto cows that delivered dead calves. The cows had been grazing tall fescue pasture since February. Supplemental protein was fed to the first calf heifers prior to calving and the feed was increased during early lactation. A similar problem of stillbirths occurred 2 to 3 years ago. At that time, some calves and cows were tested for BVD PI by IHC analysis of ear notches and all were negative. Titers against *Leptospira* and *Campylobacter* organisms were also checked a few years ago and they were negative. The herd is vaccinated once a year with a killed vaccine, while the bulls are vaccinated against *Campylobacter*. The body condition scores of the cows were 6 to 8/9. Calves were weaning at good weights.

Extensive testing was done on samples from these 3 stillborn calves and from the sera of 8 cows. No significant histological lesions were found in brain, heart, lung, liver, kidney, thymus, spleen, ear notch, or small intestine tissues from all 3 calves. Cultures for aerobic bacteria from lung, liver, and stomach contents were negative. Special cultures for *Campylobacter jejuni*, *Campylobacter fetus*, and *Listeria* spp. were also negative. Kidneys were negative for *Leptospira* spp. by PCR analysis. Viral isolation of pooled tissues for IBR and BVD viruses were also negative from all 3 calves. IHC analysis on lymphoid tissues for BVDV was negative in the two calves tested. Thoracic fluid collected from one calf had no antibodies to *Leptospira* serovars of *pomona*, *canicola*, *icterohaemorrhagiae*, *grippotyphosa*, and *hardjo*. The thoracic fluid also had no antibodies to IBRV, BVDV, and *Neospora caninum*. Cow sera had non-diagnostic titers to *Leptospira* spp. and IBR virus, and there were negative titers to *Neospora caninum*. There were titers to BVDV type I virus that ranged from 1:32 to 1:512 and there were titers to BVDV type 2 that ranged from 1:8 to 1:32. BVDV titers greater than 1:64 could indicate exposure to the virus or could be a response to recent vaccination. These same sera were negative for BVDV antigen by antigen capture ELISA, therefore indicating the titers were not likely due to viral infection. After this thorough workup, we concluded there were no infectious causes of the stillbirths.

Since infectious agents were not considered a cause of the stillbirths, we started looking at other factors. The history of agalactia and the consumption of tall fescue were key historical factors. Reproductive problems and



Investigation of Multiple Stillbirths and Agalactia in a Beef Herd (continued)

agalactia due to the ingestion of endophyte infected tall fescue are generally considered to be rare problems. Recently, there have been several reports of similar problems occurring in cattle that were eating endophyte infected fescue in the last trimester of pregnancy. Well written reports from the University of Tennessee Extension Office and the University of Missouri Extension Office discuss fescue toxicity in cattle. The UT report¹ describes the agalactia syndrome as a problem that generally occurs in mature, fall-calving cows, often affecting 10 to 15 percent of the herd. The [UM report](#) describes the reproductive problems associated with fescue toxicity as late term abortions, stillbirths, and thickened placentas. The herd under investigation had also expressed other typical signs of fescue toxicity; including salivating, standing in water, and panting from hyperthermia. In 2002, their extension agent reported that 50 to 70 percent of the fescue grass tested was infected with endophyte; this is a very high concentration of endophyte infected fescue pasture. This farm added clover to dilute the concentration of the endophyte, and also Tasco™, which has been reported to help reduce the endophyte's adverse effects on the immune function, was added to the minerals. However, the cows reportedly stopped eating the mineral mix containing Tasco™ in August. The reduced consumption of Tasco™ or, perhaps, year to year differences in endophyte concentrations may have contributed to developing clinical signs of fescue toxicity.

Fescue toxicity as the cause of stillbirths and agalactia is not a diagnosis that can be made with any one test or with a history of stillbirths and agalactia. Cows should also be exhibiting other signs of fescue toxicity, and infectious agents should be ruled out. Testing the pasture for endophyte levels is a great way to wrap up the case. The NC Agriculture Extension Service and NCDA&CS Plant Industry Division offer a fescue endophyte testing service. You can call 919-733-3930 or your county extension office for more information.

1. "Occurrence, prevention and treatment of fescue-related agalactia in fall-calving cows", The University of Tennessee Extension. Info Series: AS-B 297.

Pythiosis in Dogs by Dr. Peter Moisan

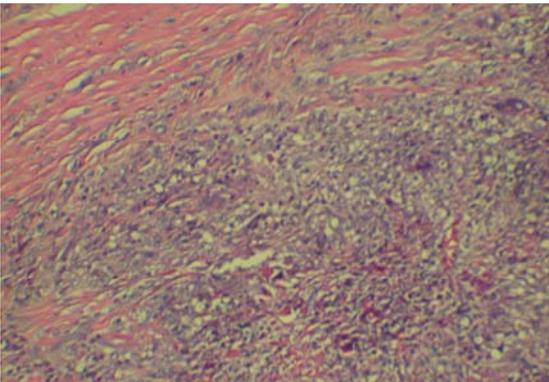


Figure 1. Granulomatous and eosinophilic inflammation resulting from *Pythium insidiosum* invasion of small intestine.

Pythiosis is a fairly uncommon disease of animals that is usually described only in the southeastern United States, though a few cases have been seen in Kansas and elsewhere. The agent, *Pythium insidiosum*, is an aquatic fungal-like pathogen that is a member of the new kingdom Stramenopila, existing somewhere taxonomically between plant and animal. The disease can occur in the skin of dogs and horses, but the gastrointestinal form of dogs is far more common in our diagnostic submissions. We receive material containing the agent eight to ten or more times per year.

Pythiosis is primarily a disease of large breed dogs that are between 6 and 24 months of age. Most have frequent exposure to swamp or stagnant pond water, which is the habitat of the etiological agent. Symptoms are vague and usually involve weight loss and anorexia with occasional vomiting and anorexia over a period of several weeks. Surgical intervention or gross necropsy results indicate tumor-like masses or granulomas of the stomach or duodenum, usually. However, pythiosis can occur in any level of the gastrointestinal tract, including the esophagus and rectum, and usually involves the mesenteric lymph nodes and adjacent mesentery. Euthanasia is frequently elected at the time of surgery due to the inoperable nature of the masses and the poor response to anti-fungal therapy once such massive visceral involvement is recognized.

Histological features of the disease include granulomatous and eosinophilic gastrointestinal inflammation



Pythiosis in Dogs (continued)

(Figure 1.) associated with rarely septate hyphae with non-parallel walls that are 15 to 25mm in diameter. The organisms are usually visible only with silver stains (Figure 2.). The inflammation extends from the sub-mucosa of the intestine to the mesentery and lymph nodes.

We receive formalin-fixed biopsy and necropsy material containing these specimens frequently, but fresh material is seldom submitted. *Pythium insidiosum* can be cultured. Fresh material should be shipped to the laboratory in water at room temperature to arrive as soon as possible; shipment on ice may decrease the chance of recovery. In the history, a differential diagnosis that includes pythiosis is also very helpful.

Pythiosis is **not** contagious to humans or animals from infected dogs or horses.

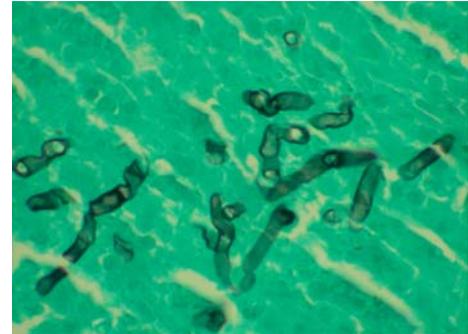


Figure 2. High magnification of silver stain showing large irregular hyphae within intestinal tissue.

The Dilemma of Sudden Death by Drs. Bob Glock and Carlos Reggiardo (Arizona Veterinary Diagnostic Laboratory)

The following article was reprinted from the [AZVDL Newsletter](#) (Volume 4, Issue 3; September 1999) with permission from Drs. Bob Glock and Carlos Reggiardo, as well as the AZVDL Newsletter.

To reach a diagnosis of the cause of death of an animal a joint effort by the owner, attending veterinarian, and diagnostician is usually required. A careful observation by the owner of any changes in an animal's environment and behavior, as well as a good medical history and a detailed clinical evaluation by the veterinarian, often generate invaluable diagnostic information, without which a necropsy and laboratory testing may not be sufficient to fully characterize the cause of death. It is not uncommon for us to receive submissions of animals where most information is missing. The primary history (or most often the **only** history) is "sudden death". In a number of these cases the "sudden" death is the result of a progressive clinical disease, which was simply not observed by the owner, but significant lesions were observed on necropsy. Cats and birds seem to be less likely to let their owners know how distressed they are. A good example is a case where there was a severe chylothorax but the cat was "found dead". In some cases, a true acute or peracute condition such as trauma, poisonings with substances physically identifiable in the ingesta, or a gastric torsion can be easily diagnosed on necropsy. But it is not uncommon to face cases where we can only eliminate known identifiable causes of death, and offer, at best, our educated but uncertain diagnosis, which is a situation particularly frustrating for owners. Some specific comments regarding various species follow.

Canine: Dogs of various ages are often presented with the history of sudden death. In the warmer months in Arizona, these are often related to some type of heat stress or water deprivation. There are no specific lesions of these conditions and we usually eliminate other likely causes and then depend on what history is available to try to conclude the possibility of heat exhaustion. Unfortunately, these animals are frequently found in the sun during hot weather and the specimens may be quite autolyzed making our search more difficult. There is another type of sudden death in dogs that are in apparent good health and are turned out in the backyard for exercise. Then a few minutes to a few hours later they are found dead. Sudden death can also occur as a very distressing sequela to anesthesia or even post anesthetic recovery. In these rare but distressing deaths we sometimes suspect heart failure, but the diagnosis is only solidly positive when we can find lesions such as actual cardiomyopathies. The dramatic myocardial infarctions typical of human "heart attacks" are extremely rare in animals. It is not possible at this time to do a very good job of evaluating anesthetic technique or dosages in the postmortem situation. Often the most important thing we can do is to try to help the people involved to understand that this is a rare event and appears to be basically unavoidable. The anesthetic death is a good example of the need to warn clients that there is always some risk associated with a procedure.

Horses: The occasional sudden death in a horse may actually be a severe colic that became fulminating with-



The Dilemma of Sudden Death (continued)

out observation. Sometimes the history or the animal's surroundings can help with that diagnosis. Electrocutation may also be a concern and there have been situations where malicious administration of drugs have been involved (some of which are difficult to trace). We recently had a case of a horse that was heavily exercised and became stiff progressing to death within a few minutes. The only finding was somewhat elevated potassium levels in the anterior chamber of the eye. However, clinicians tell us that it is not definitive evidence of hyperkalemia. The final decision was that this was probably representative of a poorly defined syndrome known as "exhausted horse syndrome". This may be a combination of disturbances in electrolyte balances, acid base balances, and other physiologic parameters. Horses do not commonly die of "heart attacks".

Bovine: We must consider some of the common causes such as bloat, acidosis, and lightning strike. Lightning strike is a particularly interesting situation because there frequently are no burn marks and the presumptive diagnosis is often a matter of eliminating other possible causes of death. We recently had a situation where some adult cows were hauled for a period of about two hours and seven out of 60 died within the next several hours. Here again the practitioner involved was unable to find any specific lesions and we did not find anything in the laboratory. The assumption is that there was probably some metabolic problem, perhaps related to relatively warm temperatures. The diagnosis of most of these sudden deaths will most likely remain elusive because of the nature of the insult, economic or technical limitations on how much testing is possible, or simple autolysis of the cadaver. But, as in the observation of "frogs in the yard" in the case of bufotenin intoxication described below, the submission of a medical history when available and a careful description of the animal's environment can sometimes greatly facilitate the diagnosis.

Bovine Pneumonia with *Mannheimia haemolytica* by Dr. Peter Moisan

Mannheimia (Pasteurella) haemolytica is considered the lynchpin of bacterial pneumonia in weaned beef calves, particularly in large groups at auction or in the early stocker and feedlot periods. The agent is a gram negative coccobacillary bacterium that produces a potent leukotoxin and a vasoactive endotoxin that is similar to other gram negative organisms. *Mannheimia haemolytica* Type A, is the usual organism isolated from bovine cases of fatal bronchopneumonia, and is a normal inhabitant of the upper respiratory tract of cattle.

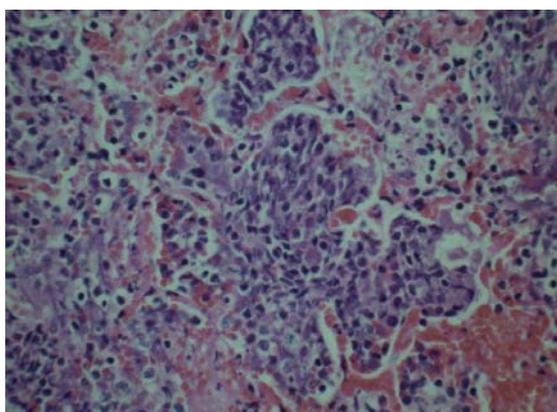


Figure 1. Sequestrum of necrotic tissue within the bovine pulmonary lobule.

The pathogenesis of bacterial bronchopneumonia of cattle is complex but usually involves colonization and damage to the lower respiratory tract following injury to primary protective mechanisms of the upper respiratory tract. Viruses, such as IBR or BRS viruses, most notably, cause damage to the ciliated respiratory epithelium of the airways. This damage destroys the integrity of the mucociliary escalator of the bronchi and trachea. As a result, pathogens, dust, and cellular debris that ordinarily would be elevated to the oral cavity and swallowed are retained in the alveoli and bronchioles. *Mannheimia haemolytica* produces a leukotoxin, a protective protein that is toxic to neutrophils. The damaged neutrophils are destroyed and degranulated. Toxic neutrophilic enzymes are released into the alveoli, causing damage to the surrounding pulmonary tissues. (In an interesting experiment to prove the mechanism of action of leukotoxin¹, cattle experimentally deprived of neutrophils

did not suffer serious damage to the lung from leukotoxin. This lack of deterioration is because there are no toxic neutrophilic enzymes to cause the lung damage after the release of the leukotoxins by the bacteria.)

An examination of lung affected by *M. haemolytica* exhibits fairly characteristic lesions even when the or-



Bovine Pneumonia with *Mannheimia haemolytica* (continued)

organisms are not isolated by bacteriological culture. The leukotoxin causes the characteristic destruction of the neutrophils, as described. In addition, the potent endotoxin causes thrombosis by activation of local clotting mechanisms in the lung. Characteristic sequestra form in the lung sections as a result (Figure 1.). These lesions are quite typical. After a few days with the primary *M. haemolytica* infection, the organisms are overtaken by, in most cases, *Pasteurella multocida*. Eventually, if the animal survives long enough, bronchiectasis of the small airways develop into abscesses that are populated by *Arcanobacterium pyogenes* and various species of anaerobes. The failure to isolate *M. haemolytica* does not necessarily indicate the absence of this ubiquitous agent. Similarly, a negative culture of lung taken late in the disease process does not indicate a failure of bacteriological methods – it most likely indicates that the organisms have been superseded by *P. multocida* or *A. pyogenes*. Viruses involved in the initiation of bacterial pneumonia include IBR, BRS, PI3 (rare), and BVD. The BVD virus is considered to be a comorbid infection with many of the cases of *Mannheimia haemolytica* that are unresponsive to treatment.

Post mortem changes in cases of *M. haemolytica* broncho-pneumonia of cattle are also fairly typical. As with most bacterial pneumonia, the distribution of consolidated lung is in the cranioventral direction. There is a “meaty” texture to the affected lung. Fibrin is most often present in thin layers to thick clumps over the pleural surfaces. There is a clear line of demarcation of the border between affected and unaffected lung tissue. Samples taken for bacteriology, virology, and histopathology should be taken from this area of demarcation, as this is the region that is the most recently affected and most likely to contain the offending bacteria and viruses, as well as the classic histological features.

Culture results are most accurate when taken from cattle that are early in the disease process and have not yet been treated with antibiotics. When *M. haemolytica* is suspected in a pneumonia outbreak, nasal swabs, if collected properly, may be useful in disease diagnosis. Transtracheal washes are preferable to nasal swabs however, because they are usually less contaminated with normal upper respiratory flora. If there are questions about sampling of live or dead cattle, please contact Dr. Moisan at the Rollins Laboratory.

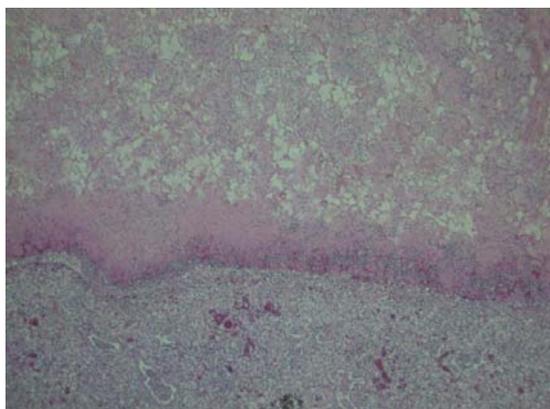


Figure 2. Streaming neutrophils within the bovine alveolus.

1. Stevens PK, Czuprynski CJ. *Pasteurella haemolytica* leukotoxin induces bovine leukocytes to undergo morphologic changes consistent with apoptosis in vitro. *Infect Immun* 1996, 64:2687-2694.

Interesting Cases

Unusual Presentation of Rabies in a Beef Heifer by Dr. Darrell Rector (Elkin Laboratory)

A nine-month-old Angus heifer was submitted for necropsy to the Elkin Laboratory in July. The owner reported that the heifer initially had lameness of the right rear foot but over the ensuing week, lameness had progressed to rear limb paresis and paralysis. The owner euthanized the animal immediately prior to laboratory submission. The animal had been vaccinated for Blackleg, IBR, *Pasteurella*, and Type 2 BVD. It had also been treated for internal parasites. Diet consisted of grass forage, a commercial grain ration, and a high magnesium mineral mix. Other calves in the same pasture with this heifer did not show similar clinical signs at the time of presentation.

At necropsy, the 360 kg heifer was dehydrated and slightly emaciated. There was no evidence of neuromus-

**Unusual Presentation of Rabies in a Beef Heifer** (continued)

cular trauma. In addition, gross lesions were not identified in any of the other body tissues or internal organs. Differential diagnoses at the time of necropsy included spinal cord injury, parasite migration in spinal cord, and possibly, rabies.

Test results following necropsy:

1. Magnesium (ocular fluid): 1.9 mg% (normal \geq 2.0 mg%)
2. Nitrates (ocular fluid): Negative.
3. Histopathological exam: encephalomyelitis with Negri bodies.
4. Fluorescent antibody test was positive for the rabies virus at the North Carolina Department of Public Health Laboratory.

Diagnosis: Rabies

Rabies is an acute viral infection of the nervous system that is often difficult to diagnose solely by clinical signs. The first sign of infection is usually depression, a vague sign seen in many illnesses. Affected animals usually stop eating early in the course of the disease, but as the disease progresses, they may exhibit a depraved appetite and chew on or attempt to eat wood, stones, or other objects. Paralysis of the muscles of the throat is usually pronounced, so there may be ptyalism (drooling) and grinding of the teeth. In many cases, cattle bellow incessantly in an altered, characteristically low-pitched voice. Some animals attack other animals, humans, or inanimate objects. Weakness of the hind-quarters, giving a swaying gait or knuckling over of the fetlock joint may occasionally be observed early in the disease or as it progresses. In one report¹, 97 cattle with rabies were surveyed. Twenty-two percent of the animals developed the furious form, in which charging and attacks were reported; while, 38 percent developed the paralytic form with neck/throat or hind-quarter involvement. Forty percent had clinical signs that could not be clearly classified as either form. The clinical course of the disease in cattle is 4 to 7 days.

1. Schnurrenberger, P. R., Martin, R. J., and Meerdink, C. L.: Rabies in Illinois Farm Animals. J. Am. Vet. Med. Assoc. 156:1455, 1970.

Cauda Equina Neuritis by Dr. Stacy Robinson

An 18 year old Tennessee Walking Horse mare was presented to the Rollins Diagnostic Laboratory with an approximately 3 week history of progressive neurological disease. Initially, the horse had signs of right-sided facial nerve paralysis. These paralyzes included the loss of the blink reflex of the right eye, as well as drooping of the lower lip and right ear. A week later, the horse lost the ability to move her tail. Within another week, the mare was no longer able to urinate or defecate without rolling on the ground. Finally, the horse became ataxic in the rear legs and developed a wide-based rear limb stance. The animal continued to lose motor function on the left side. Euthanasia was performed and a necropsy was requested.

No abnormalities were detected in the nervous tissue during the gross examination. The urinary bladder contained approximately 50 ml of thick purulent material.

Microscopic examination of the right and left trigeminal nerves and ganglia revealed subacute to chronic, severe right trigeminal ganglioneuritis and mild left trigeminal ganglioneuritis. Lymphohistiocytic ganglioneuritis and meningomyelitis were identified in the cauda equina of the spinal cord. Severe ulcerative urocystitis was also identified in sections from the urinary bladder.

Flourescent antibody testing was negative for rabies virus. Polymerase chain reaction testing was negative



Cauda Equina Neuritis (continued)

for eastern equine encephalitis and West Nile viruses. Finally, the animal was negative for rhinopneumonitis virus (equine herpes virus-1) by virus isolation techniques.

Cauda equina neuritis^{2,3} is a condition of horses in which features of polyneuritis are predominated by clinical signs of neuritis of the cauda equina. Damage to the nerves in the cauda equina results in paresis and paralysis in the tissues of the tail, urinary bladder, rectum, anus, and perineum. Despite the name, the trigeminal nerves, other cranial nerves, and even the autonomic nervous system may be affected. Involvement of the vestibular nerves can result in severe ataxia. The etiology of cauda equina neuritis is not known. In one study², an adenovirus was associated with the condition. In reports from foreign countries², the lesion has been associated with strangles and respiratory disease outbreaks. One report¹ has associated cauda equina neuritis with *Halicephalobus gingivalis* infection and migration. The lesion may also be due to some form of autoimmune disease³. The etiology of the sporadic cases seen in the United States has not been determined.

1. Johnson, JS, Hibler, CP, Tillotson, KM, and Mason, GL.: Radiculomeningomyelitis due to *Halicephalobus gingivalis* in a Horse.
2. Rooney, JR and Robertson, JL. Equine Pathology. 1st ed. Ames, 1996, Iowa State University Press.
3. Summers, BA, Cummings, JF, and de Lahunta. Veterinary Neuropathology. 1st ed. St Louis, 1995, Mosby.

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