



Accredited by the American
Association of Veterinary
Laboratory Diagnosticians

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

I write this article having recently returned from Minneapolis, Minnesota and the annual national meeting of the American Association of Veterinary Laboratory Diagnosticians (AAVLD), our accrediting organization. Several NCVDLS employees attended and contributed to a strong agenda and information exchange. The assemblage sent us home well armed with knowledge to further develop our Quality Assurance, Emergency Response, and continuity of operations plans, all essential requirements for the upcoming reaccreditation review this coming spring. To me, these meetings reinforce the continued advancement and sophistication within the veterinary diagnostic field as new technologies are developed.



The AAVLD accreditation review team has indicated a Spring 2007 time frame for visiting and reviewing our system. Unlike the past, they will physically visit and audit all facilities, including branch laboratories. We must submit to them, by December 31 of this year, a comprehensive application for reaccreditation, including copies of our Quality Manual, Tier II SOP documents, and examples of test procedure SOPs. The completion of this documentation will be a significant task for the next two months, but I feel comfortable that we will be able to successfully comply with the request. Laboratory accredited status is important to satisfy international trading partners as to our state's animal and food product health status, as well as a requirement for American Animal Hospital Association veterinary facility certification.

An important component of a functional diagnostic laboratory system is a functional emergency response plan. In the event of a major disease event requiring extensive diagnostic testing, a plan must be in place and exercised to provide surge testing capability while continuing to provide routine services. Our system's planning activities continue, with the development of a draft response plan and the formation of an incident command structure. In addition, sixteen employees have recently received formal Incident Command System (ICS) training to the 100 or 200 level. ICS is the industry standard structure through which activities such as operations, logistics, planning, finance, and safety are consolidated into a unified emergency command and response.

We were fortunate this session to have received from the General Assembly approximately \$400,000 of funding to remodel an underutilized section in our Rollins chemistry laboratory into BSL 2 molecular diagnostic space. A contract for study and design was awarded in early November, with the goal of a late Summer 2007 completion date. This enhancement will allow further expansion of our rapid molecular PCR testing capability for foreign and domestic diseases. In addition, we

In This Issue...

Client Corner.....3
Employee of the Quarter..12
Departmental News.....13
Disease Trends.....6
Interesting Cases.....9
Veterinary Staff.....14

Points of Interest...

- Avian Botulism
- Avian Submissions
- Camelid BVDV Update
- Cytology Submissions
- Equine IAHD
- Feline Phaeohyphomycosis
- Porcine Circovirus-2

NCVDLS-Rollins Lab
1031 Mail Service Center
Raleigh, NC 27699-1031
Phone: (919) 733-3986
Fax: (919) 733-0454
Web site: www.ncvdl.com

Please e-mail
NCVDL@ncmail.net with
any comments and/or sug-
gestions concerning The
NCVDLS Report

Holiday Closings...

Veteran's Day-November 10
Thanksgiving-Nov. 23 & 24

Our laboratories will be closed on the
above listed days.

**From the Director** (continued)

were fortunate to receive an additional \$421,000 in Repair and Renovation funds to address several critical needs in our facilities, including a new chiller and roof for the Rollins laboratory.

Congratulations to our Summer 2006 Employee of the Quarter, Ms. Lia Collman, a Medical Laboratory Technician II at the Arden, North Carolina facility. Lia is the first employee from a branch laboratory to be so recognized, and is quite deserving. Lia approaches all of her duties with a positive, "can do" attitude. She is responsible for sample receipt and processing, mailing of samples to the Rollins facility for further diagnostics, rabies sample submission, support receptionist duties, and serological testing. She has an infectious personality, lots of initiative, and is respected by her coworkers and clients. Congratulations, Lia!

We recently completed a busy two days participating in the North Carolina Veterinary Conference at the Research Triangle Park in the Raleigh-Durham area on November 2 and 3. Over 700 veterinarians and veterinary technicians attended this event, the largest of such continuing education sessions held in the state and jointly sponsored by the N.C. Veterinary Medical Association and the NCSU-College of Veterinary Medicine. Presentations were given by Drs. Peter Moisan, Jennifer Haugland, as well as a laboratory system overview given by myself. Additionally, we inaugurated and staffed a display booth to answer questions about the system and the services offered, and rolled out our updated User's Guide and specimen submittal forms. Interest and feedback was excellent, and will be used to enhance our services to our clients.

On November 18, we held our annual NCVDLs work conference in Raleigh. All Rollins and branch laboratory staff veterinarians convened to review case summaries and disease trends, procedures and protocols, and diagnostic testing updates. Coordination of activities between all five facilities within the system continues to be a major point of emphasis, and this gathering for professional knowledge sharing continues to pay dividends toward that end.

Thank you for your continued support of the laboratory system. We will continue to strive to provide the best in diagnostic services to veterinarians and animal owners, and to protect the livestock and poultry industries from catastrophic disease events. Best wishes for a wonderful holiday season, and do not hesitate to contact us for assistance.

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
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

Client Corner

Submitting Samples from Poultry Flocks for Examination by Dr. Tahseen Aziz

Birds from poultry flocks are regularly submitted to laboratories of the North Carolina Veterinary Diagnostic Laboratory System for necropsy and postmortem examination. The most common problems include increased mortality, clinical disease, and/or poor production performance in flocks.

Birds are submitted for diagnostic purposes. Diagnosis is defined as identifying the nature and/or the cause of a problem. Postmortem examination and laboratory testing are valuable tools in the investigation of flock problems. Growers and farm-service personnel should bear in mind that selecting the right birds for examination is a key element in the diagnostic process. Whatever the nature of the problem, submitting the right or wrong birds for examination may make the difference between a correct diagnosis, an incorrect diagnosis, or no diagnosis. If birds are exhibiting clinical signs, it is imperative to submit birds to the laboratory that have the representative signs. One should not walk in the poultry house and randomly pick up birds to submit for examination. For example, if birds in the flock cannot stand, exhibit lameness, and have swollen joints, then all the submitted birds should clearly demonstrate these clinical signs. If there is increased mortality, then freshly dead birds should be submitted. Decomposed birds are generally useless for diagnosis. Birds in a moderate or advanced state of decomposition usually have green discoloration of the skin over the abdomen; these should not be submitted. One should keep in mind that birds decompose quickly, especially in the hot days of summer. Decomposition also occurs more rapidly in heavy birds, such as broiler breeders. In general, birds that die within 12 hours before arriving to the laboratory are the best for necropsy and postmortem examination. Additionally, there is no reason to submit birds that have an obvious cause of death, such as severe pecking, severe prolapse of the oviduct, or severe traumatic injury.

**Submitting Samples from Poultry Flocks for Examination** (continued)

How many birds should be submitted for examination? The general answer is not too many and not too few. More are not necessarily better, and in fact, submitting too many birds, with some that are not representative of the flock problem, may confuse the diagnostician and make an accurate diagnosis difficult. As a guideline, submitting 8 to 10 birds (freshly dead and/or alive with typical clinical signs) is adequate for most situations. The diagnostician will ask for more birds, if necessary. If the grower or field-service person is not sure what kind of birds or how many birds he/she should bring to the laboratory, it is advisable to consult a laboratory veterinarian prior to submission. In closing, one should remember that good communication between the laboratory diagnostician and the person most familiar with the flock problem is essential to achieving the correct diagnosis.

Update of BVDV Testing in Camelids by Dr. Jennifer Haugland

Rollins Animal Diagnostic Laboratory is now accepting whole blood samples (EDTA tubes) for RT-PCR testing for Bovine Viral Diarrhea Virus (BVDV). Whole blood is the recommended sample for detecting BVDV in camelids. Camelids are considered to have low levels of the virus, making detection of the virus in serum less sensitive. Please call Dr. Gene Erickson or Dr. Jennifer Haugland at 919-733-3986 with any questions regarding testing for BVDV.

Cytology Submissions by Drs. Steve Rushton and Marti Hanes

Cytology can provide a quick and non-invasive way for diagnosing a number of common diseases involving the skin and subcutaneous tissues in large, small, and exotic animal practice. Cytology is able to provide reliable diagnoses of a number of benign and malignant tumors, such as: histiocytomas, mast cell tumors, and lipomas, as well as inflammatory reactions. Even in situations where the definitive diagnosis is difficult to make, cytology can often narrow the potential diagnoses down to epithelial, mesenchymal, or round cell tumors and may even help determine if the masses are inflammatory. Cytological diagnoses are only as good as the quality of the cellular infiltrates that are submitted, either on a slide (aspirate) or in a tube (body cavity fluids, urine, and cerebrospinal fluid). The number one reason for a non-diagnostic sample or inconclusive diagnosis is the absence or scant number of nucleated cells on the aspirated slides submitted.

Just as in surgical biopsies, a properly filled out Submittal Form is critical in helping the veterinary pathologist interpret the cells and the lesions.

NCVDLS Surgical Biopsy and Cytology SUBMITTAL FORM

Must include:

1. Identification of type of sample (aspirated mass, body cavity fluid, or urine).
2. Brief and thorough history (includes duration, size, and location of mass).
3. Species, Breed, and Age of animal.
4. Number of slides or tubes submitted.
5. Please indicate if a request for culture of fluid was submitted at the same time.



Cytology Submissions (continued)

Dermal and Subcutaneous Aspirates

- Use fine bore needles (23 gauge or less) to collect the cells. Larger needles tend to cause more bleeding and may not increase cell yield.
- Make squash preparations of samples with thick aspirated material. This helps to spread cells into a thin layer and reduce sections that are too thick to examine with the least disturbance of the cell membranes.
- Make 1 to 2 unstained slides with some material from different areas of the lesion, and mark them clearly.
- Adipocytes from lipomas rarely exfoliate on aspiration.

Lymph Node Aspirates

A properly performed lymph node aspirate may be a very useful tool in determining neoplastic versus reactive or inflammatory processes. The major limitation is that only a small section of the lymph node is being examined; therefore, a lymphosarcoma that has not diffusely affected the node may be missed and interpreted as a reactive node.

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

Body Cavity Fluids (Thoracic and Abdominal)

Examination of *pleural*, *peritoneal*, and *pericardial fluids* can help to define the nature of the disease process causing the effusion (true transudate, modified transudate, or exudate). In some cases, analysis of the cellular make up of these fluids will allow a specific diagnosis to be made (pyothorax, malignant neoplasia, bile duct rupture, etc).

- Minimize blood contamination and trauma of the sample by using fine gauge needles or butterfly catheters to collect samples.
- For pleural fluid collection, enter the rib space just in front of the rib to avoid laceration of the vessels running down its caudal aspect.
- Collect and submit the sample in EDTA (purple top) blood tubes. EDTA helps to prevent clotting and assists cell preservation.
- The fluid should be refrigerated and shipped with frozen gel packs to avoid cellular degeneration. Do **not** stain or fix the slides.

Urine

- Spin down the urine immediately and remove the supernatant.
- Place a drop of the sediment on several slides and let air dry.
- Do **not** stain or fix the slides.
- Samples obtained via cystocentesis are preferred.

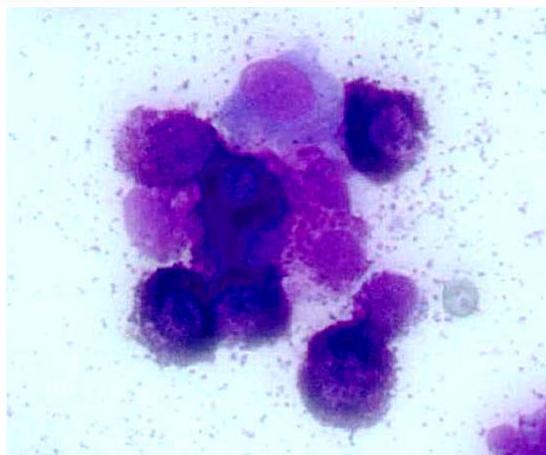


Figure 1. The above photomicrograph is of a dermal mass presented to the NCVDL for cytological examination. Numerous round cells with abundant deeply magenta, intracytoplasmic granules consistent with a Mast cell can be seen. These types of aspirates (Mast cell tumors) are commonly seen here at the NCVDL.



Cytology Submissions (continued)

Lavage (Nasal, Transtracheal, or Prostatic)

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

In conclusion, cytology can provide an effective, non-invasive diagnostic tool for many dermal and subcutaneous masses, lymph node aspirates, body cavity fluids, lavages, and urine specimens. The main limiting factor in reaching a cytological diagnosis is the number of nucleated cells that are present on the slides submitted. The veterinary pathologists at the Rollins Laboratory are available if you have any questions about aspiration techniques or any other questions on submitting high-yield (diagnostic) cytological specimens.

Disease Trends

Avian Botulism by Dr. Karen Post



Photo credit: G. Wobeser

Figure 1. Two Mallards exhibiting "limberneck".

Our laboratory system has recently been challenged with diagnosing two high profile cases of Type C avian botulism, both in ducks, one involving the City of Raleigh and the other involving the city of Swansboro.

Avian botulism is a paralytic disease caused by the ingestion of neurotoxins produced by the Gram-positive, spore-forming, obligately anaerobic, rod shaped bacterium, *Clostridium botulinum*. After ingestion, the toxin is absorbed into the bloodstream by passage across the intestinal wall. The toxin is carried to the peripheral nervous system, where it binds to gangliosides at the neuromuscular junction. Toxins then enter the nerve and bind to membranes, which become functionally changed so that vesicles containing acetylcholine are not able to function. Since

only cholinergic nerves of the peripheral nervous system are affected, the result is flaccid paralysis. In birds, this is most strikingly exhibited as "limberneck", as seen in Figure 1. If the diaphragm muscles are involved, death results from asphyxiation. Since treatment of affected birds is usually ineffectual, prompt removal and proper disposal of carcasses by burial or burning (in accordance with applicable ordinances) is recommended to remove toxin and maggot sources from the environment and prevent multiple death loss.

Clostridium botulinum is widespread in wetland soil and requires warm temperatures, a protein source and anaerobic (no oxygen) conditions in order to become active and produce toxin. Decomposing aquatic vegetation and/or invertebrates, combined with increased ambient temperatures provide an ideal environment for toxin activation and production. Birds may ingest the toxin directly or consume invertebrates which concentrate the toxin. Outbreaks in waterfowl typically occur in late summer or early autumn during periods of hot,



Avian Botulism (continued)

dry weather, generally on bodies of water with little or no outflow. Although there are several types of toxin produced by *C. botulinum*, waterfowl are most commonly affected by type C.

Diagnosis of botulism is dependent upon detection of pre-formed toxins in serum, vomitus/stomach contents, feces/intestinal contents or food. The mouse bioassay is considered to be the “gold standard” diagnostic test. This assay is performed by using aliquots of serum or supernatants from cultures of the patient's food, stool, or gastric contents, which are then injected intraperitoneally into mice. The mice are observed for clinical signs of botulism or death. Control mice are treated with 1 unit of botulinum antitoxin. Assays are performed at a reference laboratory and may take up to 4 weeks for completion. Because routine laboratory test results are usually unremarkable and confirmative toxin assays have a long turn-around time, clinical diagnosis is the foundation for early recognition, treatment and management decisions.

Although several other types of botulinum toxin are highly toxic to humans, cats, and dogs, there have been no recorded occurrences of intoxication in these species with Type C *C. botulinum* toxin. It is thus unlikely that Type C botulism in game birds poses a health hazard to humans or their pets.

General Reference:

1. Timoney JF, JH Gillespie, FW Scott, JE Barlough. 1988. The Genus *Clostridium* In: Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, pp. 214-240. Comstock Publishing, Ithaca, NY.

Porcine circovirus-2 (PCV-2) as a Major Etiological Agent in North Carolina Swine Disease

by Dr. Peter Moisan

Circoviruses of swine and birds have been described for the past 30 years. Over the last 15 years, Porcine Circovirus Type II (PCV-2) has been increasingly incriminated with the disease syndrome known as the Porcine Multisystemic Wasting Syndrome. As the evidence has become more convincing that PCV-2 is a central agent in this syndrome, the syndrome has been renamed Porcine Circovirus Associated Disease (PCVAD).

The diseases that fall under the broad category of PCVAD are quite varied. The most common manifestation is a wasting syndrome that is most commonly identified at 8 to 14 weeks of age in the late grower or early finisher stage of the production cycle. Infected pigs are predisposed to the resident illnesses on the hog farm. In addition, the wasting and subsequent weight loss can be profound. Survivors are not expected to regain profitability. Interstitial pneumonia, nephritis and dermatitis (the porcine dermatitis-nephropathy syndrome), diarrhea, stillbirths with mummification of fetuses, and icterus are described in decreasing order of frequency after the wasting disease.

Necropsy results from affected pigs vary, depending on the syndrome seen in the pigs. In most cases that are affected with the wasting syndrome, the 11-week-old pigs are the best for sampling. This preference is because they usually show the most prominent gross lesions and contain features that are most histologically pathognomonic. The necropsy specimens typically show interstitial pneumonia, with rubbery texture of the



Figure 1. Gross photograph of kidney from swine dermatitis-nephropathy syndrome. Glomerulonephritis is a prominent feature and evident from the capsular surface.



PCV-2... (continued)

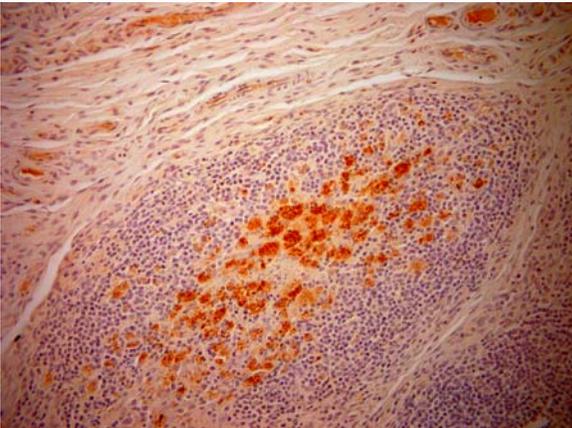


Figure 2. Photomicrograph of lymphoid follicle in ileum. There are amphophilic, botryoid inclusion bodies within the cytoplasm of macrophages in the germinal centers.

lungs and failure to collapse. Secondary bacterial bronchopneumonia and the effects of additional viral diseases (PRRS and/or swine influenza viruses) can also be seen in many cases. Lymphadenopathy of the inguinal and mesenteric lymph nodes is usually prominent at the gross examination. Evidence of diarrhea is seen in some animals. In a few animals, dermatitis and nephritis are seen (Figure 1).

Histopathological changes are most apparent in the lymphoid tissues and the lung. Lymphoid atrophy is usually profound in affected pigs. There is accelerated apoptosis of the lymphocytes of the gut-associated lymphoid tissue (Peyer's patches), lymph nodes, tonsil, and spleen. Macrophages and occasional multinucleated syncytial cells proliferate and are located within the vacated germinal centers of the lymphoid tissue. Intracytoplasmic "botryoid" inclusion bodies are seen within the histiocytic cells. Lymphohistiocytic inflammation

is seen in many other tissues as well, usually without characteristic inclusion bodies. These lesions include interstitial pneumonia, lymphohistiocytic meningoencephalitis, granulomatous or lymphohistiocytic myocarditis, granulomatous splenitis, dermal and subcutaneous vasculitis with epidermal necrosis, and necrotizing glomerulitis and interstitial nephritis. The destruction of lymphocytes by the virus is considered a major factor in the immune suppression induced by circovirus infection.

Concurrent diseases that are often seen with PCV-2 infection include PRRS, mycoplasmosis, and bacterial secondary infections (pneumonia and septicemia). In particular, mycoplasmosis appears to be exacerbated by concomitant infection with *Mycoplasma hyopneumoniae* and PCV-2. Circovirus should be considered a major part of the Porcine Respiratory Disease Complex. Porcine circovirus disease is also suspected to be exacerbated by concurrent porcine parvovirus infection.

Though the disease is the focus of intense research interest, there are many aspects of PCVAD that require further study. Initial studies indicate that Landrace cross pigs may be more susceptible than other breeds. An additional finding has shown that there are few strain differences between and among the genomic DNA in affected herds; however, even subtle differences in the genome of the circovirus may be responsible for marked increases in virulence. High death losses, occasionally 20 to 50 percent in North Carolina herds, have been recorded recently. It is probable that the concurrent diseases that we see in confined swine herds contribute to the heterogeneity in clinical signs.

Circoviruses are DNA viruses and are the smallest of animal viruses. They contain a circular strand of DNA that is single-stranded and contained within a naked protein capsid. The simplicity of the virus and the lack of genetic variability probably contributed to the development of a vaccine. A vaccination against PCV-2 has been employed in Europe for over a year and a vaccine is now available for use in this country.

In the North Carolina Veterinary Diagnostic Laboratory System, PCVAD has been recognized since 1999, and has been present as an epizootic in most corporate hog farms

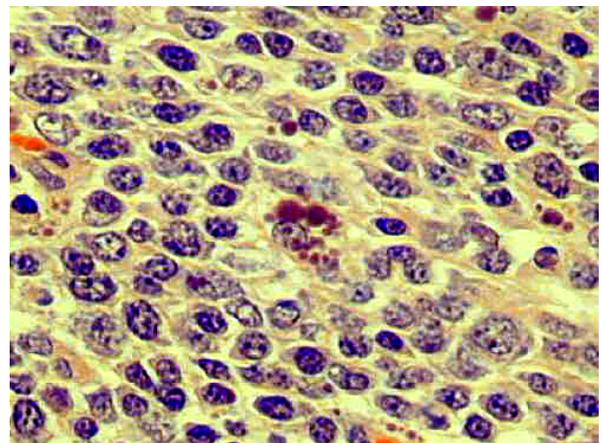


Figure 3. Photomicrograph of lymphoid follicle in ileum. Abundant Immunohistochemical staining of circovirus antigen in the germinal centers.

**PCV-2...** (continued)

since 2000. The ideal tissues for sampling for circovirus in pigs would be from 11-week-old pigs. We recommend submission of formalin-fixed lung, mesenteric lymph node, and ileum in order to identify the characteristic lesions by histopathology (Figure 2). The lesions are usually easily identified by histology in pigs 8 to 14 weeks old. In other cases, or when the lesions are not as clear, immunohistochemistry, another reliable test may be necessary in select cases (Figure 3). Of course, when respiratory or other diseases are suspected in the pigs, additional tissues are required. The laboratory can provide a list of tissues that should be submitted with each type of broad category of pig diseases.

General References:

1. Pallares FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Hanke BH, Yaeger MJ, Larson DJ, Schwartz KJ, Yoon KJ, Hoffman LJ. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). *J Vet Diagn Invest.* 2002, 14:515-519.
2. Segales J, Allan GM, Domingo M. Porcine circovirus disease. In: *Diseases of Swine* 9th ed. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, editors. 2006, pp299-307.

Interesting Cases

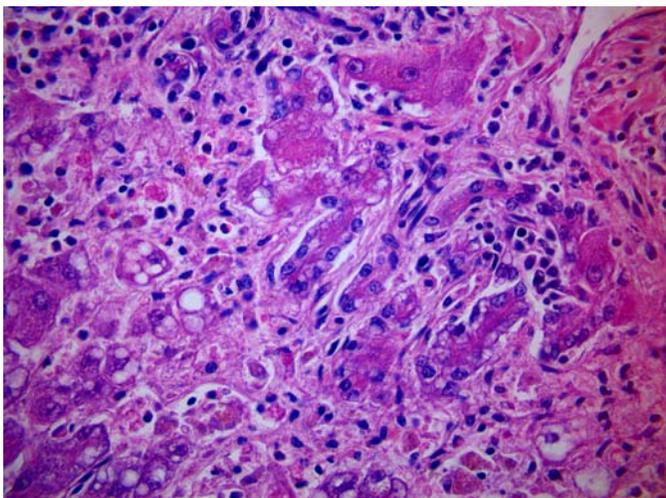
Equine Serum Sickness by Dr. David Drum

Figure 1. Equine liver, periportal lymphocytic hepatitis with submassive hepatocyte loss.

During the month of September 2006, an increase in the number of cases of idiopathic acute equine hepatic disease (IAHD) was presented to the North Carolina Animal Veterinary Diagnostic Laboratory System. Contacts with the owners indicated that tetanus antitoxin had been administered to the horses as a prophylactic measure after castration.

Idiopathic acute hepatic disease is the most common cause of acute hepatitis in horses. IAHD is also known as Theiler's disease, serum hepatitis, post-vaccinal hepatitis and acute hepatic atrophy. Theiler first recognized the disease when horses in South Africa developed acute hepatitis after being immunized against African Horse Sickness with a combination of live virus and hyperimmune equine serum. Since then, the disorder has been documented as a possible complication of the use of any equine serum product

in horses. It is most commonly associated with the administration of tetanus antitoxin (TAT).

Clinical signs of hepatic failure usually occur 4 to 10 weeks after receiving the equine origin products. On occasion, affected horses may have no prior history of exposure to such a product. Subclinical IAHD can also develop after administration of TAT. Most commonly, only one horse on the premises is affected, although outbreaks may occur or other horses on the farm may have evidence of liver disease (increased enzyme levels) without clinical signs. Occurrence of the disease in groups of adult horses during the late summer or early fall (August to November) suggests an infectious (viral) or vector-spread etiology. This seasonal occurrence could reflect the fact that many foaling mares receive TAT in the spring of the year along with their newborn foals. Lactating mares that receive TAT at foaling seem to be more susceptible. A Type III (immune-complex medi-



Equine Serum Sickness (continued)

ated) hypersensitivity reaction also has been proposed.

The onset of clinical signs is acute. Acute mortality may be 50 to 60 percent, with overall mortality as high as 88 percent in affected horses. Death usually occurs within 2 to 5 days of the onset of the first clinical signs. Horses with IAHD typically present with anorexia, hepatic encephalopathy, and icterus. The CNS signs are variable, ranging from lethargy to aggression or maniacal behavior, central blindness, and ataxia. Photosensitivity and discolored urine due to high bilirubin concentrations may be seen. Facial swelling is often described. Fever is present in about half of cases. Weight loss (uncommon), ventral edema with jugular pulses, and acute respiratory distress have also been seen in some horses with IAHD. Recognition of IAHD in one horse indicates that the other horses on the same premises should be carefully observed for clinical or serum biochemical signs of hepatic disease.

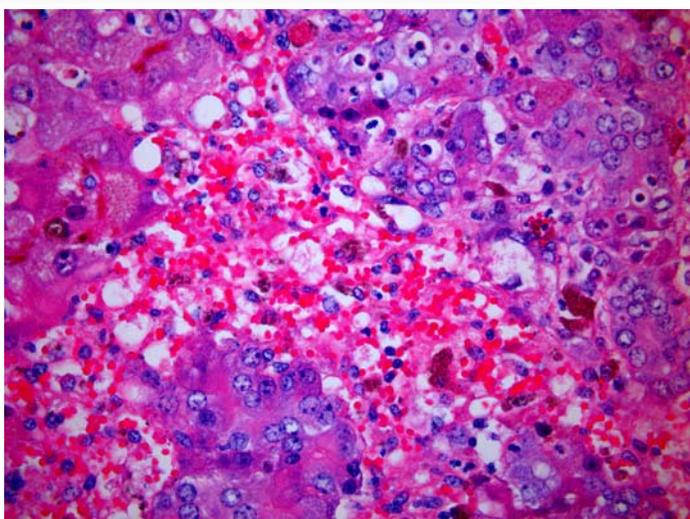


Figure 2. Equine liver, parenchymal collapse, with massive hepatocyte loss and bile duct hyperplasia.

The diagnosis of IAHD is based upon a history of prior administration of a horse serum biological product, clinical signs, and abnormalities of serum chemistries, biopsy, and necropsy. Serum levels of GGT, AST, and SDH are increased. GGT is frequently further increased during the first few days of illness. Total serum bilirubin concentration is generally higher in horses with IAHD than in horses with anorexia. Hyperbilirubinemia is common, with the unconjugated form being greater than 70 percent of the total. Serum total bile acid concentration will also be increased.

In some cases, the liver is shrunken and difficult to visualize with ultrasonographic examination. An ante mortem definitive diagnosis can be made only by liver biopsy. At necropsy, icterus and varying degrees of ascites are present. The liver is usually small and flabby to normal in size, but may be enlarged in peracute cases with a mottled and bile-stained surface. Histologically, there is marked periacinar-to-midzonal and occasional submassive-to-massive hepatocellular necrosis, mild to moderate mononuclear infiltrate and a few neutrophils, and moderate bile duct proliferation (Figures 1 and 2).

Other differential diagnoses for acute hepatic disease include acute pyrrolizidine toxicosis, acute infectious hepatitis, acute mycotoxicosis, cerebral disease, and hemolytic disease.

Because the use of TAT is not without risk, routine administration of TAT should be discouraged and routine vaccination with active tetanus toxoid should be emphasized in its place. Administration of TAT to parturient mares is strongly discouraged. Use of TAT should be restricted to situations necessitating tetanus prophylaxis and in which a history of active tetanus toxoid immunization is absent or unknown. The risks of post-vaccinal IAHD should be considered before the administration of TAT.

General References:

1. Guglick MA, MacAllister CG, Ely RW, Edwards WC. Hepatic disease associated with administration of tetanus antitoxin in eight horses. *J Am Vet Med Assoc.* 1995 206:1737-1740
2. Kelly WR, The liver and biliary system. In "Pathology of Domestic Animals, 4th edition, vol 2" Jubb KVF, Kennedy PC, Palmer N (ed.), pp. 319-406, 1993.



Phaeoophomycosis in a Cat by **Drs. Kim Hagans and Marti Hanes**

An 11-year-old neutered male Domestic shorthaired cat presented with a one-week history of sneezing that progressed to inappetence, lethargy and disorientation. The cat's pupils were asymmetrical and the cat had suffered one seizure. A Complete Blood Count and Serum Chemistry were unremarkable. Clindamycin was administered and the cat was referred to a neurologist for further evaluation. The cat died on the way to the neurologist and was presented for necropsy. The referring veterinarian's differentials included feline ischemic encephalopathy, infectious or inflammatory disease and rabies.

At necropsy, the 4.5 kg cat was in good body condition. External exam revealed severe calculus accumulation and pale mucus membranes. Pulmonary hyperemia and fluid congestion were noted. The left middle lung lobe was firm on palpation and congested. The trachea was clear. The heart weighed 19.5 g and the myocardium was pale. Abdominal organs were unremarkable. No ocular lesions were identified on gross exam. The right occipital lobe of the cerebrum was pale gray in color with pinpoint foci visible on the surface of the cerebrum. Cut surface revealed a 0.75 cm diameter circular dark, gray-green focus that encompassed approximately 33 percent of the right cerebral hemisphere. The cerebral hemisphere was normal in size when compared to the left hemisphere. No other abnormalities were identified on gross exam.

Histopathologic abnormalities were identified in the cerebrum, cerebellum, brain stem and frontal cortex. The abnormalities included:



Figure 1. Cut surface of the right cerebral hemisphere occipital lobe. Note the dark gray green invasive characteristic appearance of lesion.

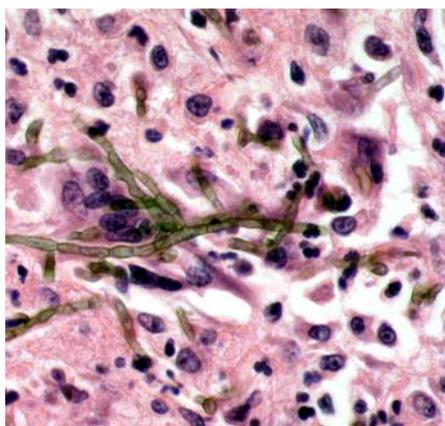


Figure 2. Cerebrum (Feline) (x400 H&E). Diffusely expanding and invading the neuropil of the cerebrum is focally extensive area of pyogranulomatous inflammation. Admixed with neurons, there are macrophages, neutrophils, a multinucleated giant cell, and lymphocytes with eosinophilic cellular and karyorrhectic debris (necrosis). Diffusely, there are many golden-brown pigmented fungal yeasts, pseudohyphae and hyphae. The branching septate hyphae are 7 to 20 μ m in diameter with central vaguely basophilic protoplasm. The walls are thin (2 to 3 μ m-thick), brown pigmented, and nonparallel.

1. Cerebrum: Diffusely expanding and invading adjacent neuropil was a large number of macrophages, pockets of viable and degenerate neutrophils, scattered multinucleated giant cells, rare lymphocytes and plasma cells surrounding eosinophilic cellular and karyorrhectic debris (necrosis). Diffusely, there were many golden-brown pigmented fungal yeast, pseudohyphae and hyphae. Yeasts were oval to round 7 to 20 μ m in diameter, with 2 to 3 μ m-thick brown walls and central basophilic protoplasm. Yeasts were often forming chains (pseudohyphae). There were rare, 5 to 10 μ m wide, septate, rarely branching hyphae with thin, pigmented, nonparallel walls. Multifocally, macrophages and giant cells contained fungal organisms. Adjacent neuropil demonstrated neovascularization, oligiodendrocytic and astrocytic glial proliferation rare fungal profiles and perivascular lymphocytic cuffing. Occasional meningeal vessels demonstrated fibrinoid vasculitis.

2. Cerebellum, brain stem and frontal cortex: Compression malacia, multifocal, mild.

Diagnosis: Cerebrum: Meningoencephalitis, Pyogranulomatous, chronic, focally extensive, severe, with many dematiaceous fungal yeasts, pseudohyphae and hyphae (Phaeoophomycosis).

Additional Diagnostic results: Rabies Fluorescent antibody test NEGATIVE.

Phaeoophomycosis is a collective term for cutaneous and systemic mycotic infections caused by several genera of black moulds that de-



Phaeohyphomycosis in a Cat (continued)

velop in tissue in the form of dark-walled (dematiaceous), septate hyphae and sometimes yeast or a combination of both. The hyphae may be short to elongate, distorted or swollen, or regularly shaped. Yeast, when present, will be variable in size and often exhibit budding.

Phaeohyphomycosis has been described in a number of animal species, as well as man. Multiple fungal species may be isolated including *Alternaria*, *Bipolaris*, *Cladophialophora*, *Curvularia*, *Exophiala*, *Fonsecaea*, *Moniliella*, *Phialophora*, *Ramichloridium*, and *Scolecobasidium*. These fungi are saprophytic and are commonly found in soil and decaying organic matter.

Infection occurs through wound contamination or inhalation of spores¹. Ulcerated cutaneous nodules, upper respiratory involvement and nasal-paranasal masses are the most commonly observed clinical signs. Lesions are primarily located on the face or distal extremities in feline cases. Fistulous draining tracts from enlarged nodules may be present. Cerebral or central nervous system (CNS) involvement, as in this case, is uncommon. *Cladosporium* sp. is the most common isolate from CNS disease. Clinical disease most often occurs in immunocompromised patients. Diagnosis is made by microscopic examination of biopsy specimens or exudate. Fungal isolation and PCR aid in determination of the causative fungus. Treatment for skin lesions includes wide surgical excision. Antifungals including itraconazole and amphotericin B may be effective for treating infections in inoperable locations². Prognosis is poor for animals with CNS involvement or widely disseminated disease.

References:

1. The Merck Veterinary Manual: Phaeohyphomycosis. 2006 (online).
2. Herraes P, C.Rees and R.Dunstan: Invasive Phaeohyphomycosis Caused by *Curvularia* Species in a Dog. Vet Pathol 38:456-459(2001).

Employee of the Quarter

Congratulations Lia!

The NCVDLs takes pride in naming Lia Collman as this summer's Employee of the Quarter. Lia is a Medical Laboratory Technician at the Western Animal Disease Diagnostic Laboratory in Arden. Her responsibilities include all laboratory areas, with the exception of necropsy. She takes the initiative in seeing that tasks are prioritized and that the components of these tasks are communicated to the appropriate individual or section, resulting in increased output and efficiency via optimized coordination. Lia tracks the needs of each laboratory section to ensure that tasks will not be subject to avoidable delay, as she monitors each task through to completion. With her consistent acknowledgement of her coworkers' efforts, Lia has augmented an elevated level of workplace morale and job satisfaction. Her energetic approach to the workday facilitates a smooth transition between various tasks and client needs that a small team (Seven total staff at WADDL) addresses each day. Congratulations Lia!!



Lia Collman receiving her well-earned Employee of the Quarter plaque from Dr. Richard Oliver.



Departmental News

As a general note, the following NCVDLs staff have attended Incident Command System Training, which is a course designed to better prepare personnel for emergency planning and response: **Drs. David Marshall, Kim Hagans, Carlton Rouse, Richard Oliver, Karen Post, Gene Erickson; Cheryl Hearn, Cindy Orlosky, Mechelle Johnson, Beverly Wood, Doug Carroll, Kim Bennett, Sandy Murphy, Lou Ann Risser, Herman Honeycutt, Jennifer Pruitt, Kathy Schmidt, Kim Howell, and Gina Lombardi.**

The annual American Association of Veterinary Laboratory Diagnostician's meeting for continuing education was attended by: **Drs. David Marshall, Gene Erickson, Richard Oliver, Karen Post, Peter Moisan, Tahseen Aziz, and Steven Rushton; Ms. Beverly Wood and Kathy Schmidt.**

ELKIN LABORATORY:

Dr. Mark Blakley has resigned his position as Veterinary Diagnostician to accept employment with Carroll's Turkeys.

ROLLINS LABORATORY:

Histology...Rollins Laboratory would like to welcome **Cynthia Nipper** in filling the Medical Laboratory Technician II position as of November 1, 2006. **Cynthia** comes to the laboratory after spending 18 years with the State Laboratory of Public Health.

Necropsy... We welcome back **Erica Savage**, a student from NCSU, who works part time as a necropsy assistant. We also would like to welcome **Cora Beth Lanier**, another NCSU student, as a new addition to the necropsy assistant team.

Quality Assurance...The NCVDLs would like to welcome **Ghazala Jawad, BS, MS**, who was recently hired to assist in the development and implementation of the laboratory's Quality Assurance Program. Ghazala is the wife of **Dr. Tahseen Aziz**, Rollins' Avian Pathologist, and has extensive veterinary diagnostic laboratory experience.

Reception...**Susan Gay** joined the Safety Committee September 20, 2006.

Serology...**Brandon Brown** attended the Leptospira MAT training course at the National Veterinary Services Laboratory in Ames, Iowa September 19 and 20. On October 5, **Jennifer Pruitt** amended her computer skills at a Microsoft Word XP workshop at the State Personnel Department Center. Also updating computer skills at the State Personnel Department Center, **Dawn Ventura** completed the Dream Weaver 8 workshop on September 26 and 28.

Veterinarians... **Dr. Steve Rushton** passed his pathology board exam as a new diplomate in Veterinary Anatomic Pathology of the American College of Veterinary Pathologists (ACVP). **Dr. Peter Moisan**, pathologist from the Rollins Laboratory, attended the Midwest Association of Veterinary Pathologists 25th Annual Meeting on August 17 and 18. He presented a case report entitled, "Braxy in Dairy Calves". The meeting was attended by approximately 150 pathologists and additional laboratory personnel, and was held at the new Animal Health Diagnostic Laboratory at Michigan State University in East Lansing, Michigan. There were a total of 52 presentations at the 2 day meeting. **Drs. David Marshall, Jennifer Haugland and Peter Moisan** all attended and made presentations at the North Carolina Veterinary Conference on November 2 and 3. A laboratory overview presentation was delivered by **Dr. David Marshall; Dr. Jennifer Haugland** presented the topic, "Small Ruminant Parasitism from a Diagnostician's Perspective"; and **Dr. Peter Moisan** discussed "Beef Practice Issues from a Diagnostician's Perspective".



Veterinary Staff

Rollins Laboratory (919) 733-3986

Director

Dr. David Marshall

Assistant Director

Dr. Karen Post

Veterinary Diagnosticians

Dr. Jennifer Haugland

Dr. Stacy Robinson

Vacant Position

Veterinary Pathologists

Dr. Tahseen Abdul-Aziz

Dr. Peter Moisan

Dr. Steven Rushton

Dr. Martha Hanes

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 Position

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

Vacant Position

