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Laboratory Diagnosticians

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

With another quarter comes another busy period in the evolution of the NCVDL system. We continue to make strides through the dedicated efforts of our talented staff, as well as equal parts of old fashioned hard work and support from our clientele. All of our exploits are being accomplished in an environment of increased responsibility secondary only to the avian flu issue. I continue to be amazed at the key role that our services provide to the viability of livestock and poultry industries, along with the general public, through surveillance for diseases of public health significance. The NCVDLs will continue to ardently accept that charge as long as there is need for our services.



Avian influenza (AI) continues to be a major issue with direct laboratory impact. The increasing spread of the H5N1 virus across Asia and Europe, coupled with the intense media attention, has increased submissions of dead and diseased poultry. In addition, we have conducted over 206,000 AI agar gel immunodiffusion tests during the past 12 months on individual commercial poultry serum samples as part of a surveillance program that is as robust as any in the country. Many of those samples were tested at Rose Hill, Elkin, and Monroe facilities, each of which is located in heavy poultry production areas of the state. Additionally, we have the PCR and viral isolation capability at Rollins, as well as our poultry pathologist, Dr. Tahseen Aziz, to complete a strong array of surveillance and response qualifications. In the coming months we will continue to build capability through the implementation of AI ELISA technology.

As of March, our laboratory in Rose Hill became the final facility to become fully functional with our new Laboratory Information Management System (LIMS). This milestone culminates a three year long project of replacing our antiquated DOS based system with more modern technology. Clients now have the ability to track and receive test results online through password protected web access. For more information, please visit the "How to Request Access to Online Test Results" link at the top of our NCVDLs home page (www.ncvdl.com). The inclusion of this feature is a valuable service to our clients, and I encourage routine users of our laboratory system to take advantage of it.

One of the major issues of attention over the past six months has been on upgrading overall laboratory safety. Our recently created Safety Officer position has paid great dividends with the employment of Kathy Schmidt. Kathy came to us with extensive experience in the Quality Assurance and Safety arenas bolstered by former

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- Biopsy Techniques

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

Holiday Closings...

Good Friday-April 14
Memorial Day-May 29

Our laboratories will be closed on the
above listed days.

**From the Director** (continued)

employment with the NCSU-CVM laboratory, as well as corporate research laboratories in the Research Triangle Park. At the Rollins facility, significant strides have been made in the development of a Safety Manual and a Chemical Hygiene Plan, disposal of excess chemicals, modernizing of the fire alarm system, employee safety training, and standardizing rabies vaccination protocols for high risk employees. Furthermore, Kathy has made an on-site review of the Monroe branch facility and will be visiting the other three branch laboratories in the coming months. Congratulations to Kathy on her hard work and accomplishments.

On May 3, we hosted several members of the Board of Agriculture (BOA) on a tour of the Rollins facility to better familiarize them with the nature of the laboratory's operations. The BOA members will be integral in support of several state legislative requests this short session of the General Assembly. Of particular interest is a \$250,000 request for funding to renovate and convert an underutilized section of our Chemistry laboratory into a BSL2+ molecular suite. This conversion will allow expanded domestic PCR capability for all species, as well as provide surge capacity for PCR diagnostics during a disease event. In addition, we are also pursuing \$1.25 million in funding to plan and design a much needed Rollins Laboratory expansion and renovation. Both of these needs are critical to the continued enhancement of our system capabilities, and I would encourage mentioning and supporting them in any future conversations with legislators.

Congratulations to our Winter 2006 Employee of the Quarter, Dr. Bing Tang, a Medical Laboratory Technologist in the Molecular Diagnostics section. Bing is a veterinarian trained in China who works in our BSL3 laboratory, performing PCR surveillance testing for a variety of domestic and foreign diseases. Bing was cited for her reliability, attention to detail, cooperative attitude, and willingness to do whatever it takes to get the job done. She is a valuable asset to our staff and a pleasure to work with.

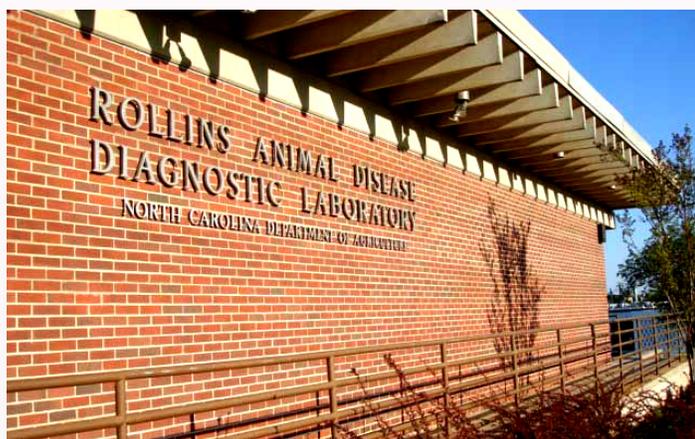
In January, we hosted the review team from the Centers for Disease Control as part of the formal review process to handle and work with select agents. This comprehensive review encompassed a BSL3 facility inspection, record keeping, training, security clearance documentation, and a host of other issues. While several minor discrepancies were noted, all were corrected immediately and approval was granted for another three year cycle. We handle very few select agents in our facility, but maintain the certification to enable us to work with them if the situation arises.

Thank you for your patronage as clients of the NCVDL system. We look forward to your continued support in the coming year. As always, I can be reached at (919)733-3986 for any feedback or comments.

Regards,

David Marshall, D.V.M.

Director

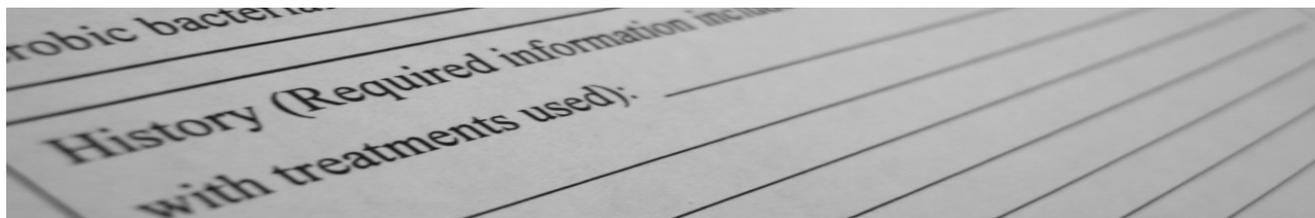


**Diagnostic Laboratory Advisory Committee**

Dr. Jim Floyd	NCSU- College of Veterinary Medicine
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Dr. Gene Nemecek	GIS-Hog Slats
Dr. David Marshall	NCDA&CS Veterinary Division

Client Corner**Patient History: Maximizing Results for Specimen Submissions** by **Dr. Peter Moisan**

We all learned in college about the value of a good history. The history helps the clinician put a case into perspective and often helps sort the differential diagnoses into “probable vs. improbable” diagnoses. In this respect, the management of a case is no different for the pathologist. We pursue differential diagnoses by intuition, and the history is an integral part of the reasoning process. In order for the clinicians to glean as much as possible from the pathologist’s report, we have included below a list of our recommendations:



1. Include the patient signalment. This includes the age, sex (neutered or not), and breed. Many breed predispositions are known and more are discovered all the time, making the inclusion of the breed an important part of the history.
2. Include clinical history and any relevant clinical observations. Including the complete patient record is neither necessary nor desirable. The pathologist needs a few lines of relevant history and observations about the patient. Irrelevant information is often misleading.

**Patient History: Maximizing Results for Specimen Submissions** (continued)

3. Previous biopsy reports from the patient may be quoted or included. Especially relevant would be the diagnosis for a lesion removed from the same or adjacent site, such as a mammary tumor. Also, necropsy results from other animals in the home or herd would be applicable.
4. Radiographs and clinical chemistry reports are occasionally included, but are frequently overlooked as useful sources of diagnostic information. In the case of any ancillary reports, the diagnosis from those reports should also be included. In other words, do not include the CBC or blood chemistry results without an interpretation.
5. Digital photographs are occasionally submitted with our accessions. A picture is truly “worth a thousand words”. Even drawings of a lesion are helpful.
6. A description of the necropsy lesions is critical to a diagnosis. The clinician acts as the gross interpreter for the pathologist in a case. We often receive necropsy specimens labeled “spleen, heart, lung, liver, etc”, with no description of any post mortem abnormalities. If no gross abnormalities are identified, please state that as well.
7. With small biopsies, a history becomes more critical. We receive many needle biopsies from masses or liver specimens. With these small specimens, there is very little tissue from which the pathologist can make an interpretation; therefore, the clinical data needs to be more reliable and complete in order for the diagnosis to be meaningful.
8. Often the best histories are spoiled by illegibility and abbreviations. We doctors have a reputation for poor penmanship. In order to communicate with the pathologist, legibility is critical. If legible handwriting is difficult, we recommend typing or dictating the material on the accession form. Additionally, abbreviations save time, however, abbreviations are often local phenomena, and a shortened form of a word at one college of veterinary medicine is not recognized by a graduate from another college.
9. Some clients do not give a history in order to prevent the pathologist from being biased. We are not biased in this regard and would discourage this practice. It delays the reporting of results and is definitely not in the best interest of the patient.
10. Finally, use anatomically correct terms wherever applicable.

Following these simple guidelines is not difficult. By adhering to the rules in submission of samples to your laboratory, more information will be obtained from your submissions and you will more often be able to achieve a positive diagnostic outcome.

Submission Form Revision by **Dr. Jennifer Haugland**

We are currently developing new submission forms for the NCVDLs. After receiving many comments on the last set of submission forms (which are currently available on the [website](#)), we are trying to create forms that are short and easy to use, yet thorough and complete. We have selected several veterinarians who regularly use the laboratories to test the new forms. This test phase is currently underway and will continue for several more weeks. These [newest forms](#) are also available for you to comment upon. Please email comments to Dr. Jennifer Haugland at Jennifer.Haugland@ncmail.net or call her at (919) 733-3986.



Equine Infectious Anemia Testing by **Denise House and Dr. Karen Post**

While spring ushers in a wide variety of horse-related activities, it also brings a large influx of blood samples to our laboratories for equine infectious anemia (EIA) testing. The Rollins Laboratory in Raleigh is the only facility within the NCVDLs that performs this testing. Our routine diagnostic assay is the agar gel immunodiffusion (AGID), better known as the Coggins test. Coggins tests are set up daily, Monday through Friday. Due to the nature of the test, samples received by 4 p.m. each day are batched and placed onto agar test plates. Since this is a 24 hour assay, the earliest that results are available is 4:30 p.m. the next working day after submission. This duration takes into account the time necessary for a technologist to interpret and record results. Rollins also has the ability to perform an Enzyme-Linked Immunosorbent Assay (ELISA) test. With this assay, results are usually available in less than 2 hours. Since this is an expensive test and because the laboratory is state-subsidized and cannot recoup test costs directly, these tests are reserved for emergency situations, with the laboratory retaining the right to decide what constitutes an emergency.

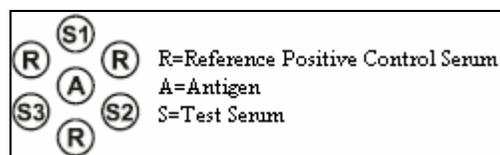


Figure 1. The seven well pattern used for EIA AGID testing.
Diagram credit: VMRD, Inc. : EIAV Antibody Test Kit.

Ideally, samples should be submitted 2 weeks prior to an event to allow ample time for testing and mailing of results. If there is a request for the owner/agent or veterinarian/clinic staff to pick up completed test forms at the laboratory, please effectively communicate this information to Ms. Kim Bennett, virology section supervisor, or Ms. Denise House, Medical Laboratory Technician, at the main Rollins number: (919) 733-3986. This request may also be written in the **upper left hand corner** of the test form. Results will only be faxed on a request basis. Again, that information must be effectively communicated to virology laboratory staff or written in the **upper left hand corner** of the test form. With the request, please do not forget to provide the number to which the report should be faxed.

All samples should be collected in tubes that hold approximately 3.0 ml of sample, can withstand the stresses of shipment, and are labeled as to source and tube number. Precautions should be taken to ensure that leakage and breakage do not occur during transit; tightly closing lids and using boxes and packing that will safely transport tubes are two invaluable precautionary examples. Small tubes such as snap cap bullets and small screw caps make processing samples much more difficult and should be avoided if at all possible. A minimum of 1.0 ml of serum is needed and hemolyzed specimens are unsuitable for performing a Coggins test. Unless serum or whole blood in a serum separator tube is submitted, the laboratory will assess a one dollar processing fee per test (up to \$5.00 per accession) for centrifugation of whole blood samples and serum harvesting.

To expedite results, all EIA test forms need to be signed and dated by the veterinarian who collected the sample(s) and filled out completely to include:

1. Veterinarian name and address
2. Owner's name and address
3. Where horse is stabled
4. Tube number
5. Horse's name, breed, color, sex and age
6. Identifying characteristics of the horse, such as markings, brands, whorls, etc.
7. Date the blood was drawn.

**Equine Infectious Anemia Testing** (continued)

Please note, laboratory staff will not make corrections to test forms after they have been submitted; therefore, it is important to check for accuracy prior to submittal. If results are not received within 2 weeks, please contact the Rollins virology laboratory in a timely manner for duplicate results. If a client has lost a copy of their test results and the submitting veterinarian cannot provide him/her with a copy, veterinary clinic staff or the submitting veterinarian must contact the virology laboratory to authorize the release of results.

With the proper submission procedures, clients will find a smooth process from sample submission to receiving results. The NCVDLs looks forward to continuing to serve our clients in the best and most efficient way possible.

Proper Biopsy Techniques by **Dr. Peter Moisan**

The Rollins Laboratory pathologists perform numerous biopsy diagnoses daily and appreciate the increased sophistication of surgical techniques. As a result, we would like to emphasize some of the best methods used in collection of biopsy material and some common pitfalls. Following these simple guidelines can dramatically heighten the success of your clinical efforts, boost client satisfaction, improve the chances of a correct diagnosis, and most of all, enhance patient quality of life. Our recommendations are as follows:

1. Send in a biopsy of sufficient size. Smaller biopsies frequently miss the site of interest. Others often distort the lesion. For punches, any size less than 5 mm is probably too small. This is because there is a 1 mm margin of distortion at either side of the site of incision by the punch. Two-millimeter and three-millimeter punches often do not contain diagnostic tissue.
2. Cautery artifact, from electrocautery, laser, or radiosurgery, is common. Cautery techniques should only be considered if there is at least a 1 cm border between the grossly visible edge of the margin and the cautery site; otherwise, sharp dissection is recommended.
3. Haired skin or the mucosal surface should be included in the biopsy specimen. Even if the skin or mucosa are not involved in the lesion, including the surface epithelium will help the pathologist orient the specimen and helps determine the depth of any tissue of interest.
4. Mast cell tumors are special circumstances. These neoplastic cells extend, in small numbers, to sites far from the center of the mass. As a result, wide excision is necessary in order to remove all of the "drifting" cells. Present recommendations are for the excision of lateral margins at least 2 cm from the visible edge of the mast cell tumor and at least a full fascial plane deeper than the visible deep margin of the tumor. Though these are wide and deep excisions, remember that surgical incisions always heal from side-to-side and not from end-to-end.
5. Lymph node biopsies are additional unusual circumstances. Needle, wedge, and punch biopsies of lymph nodes are often of limited value. This is because these partial biopsy techniques distort the architecture of the node and the lesion. The recommended procedure is excision and submission of an entire lymph node in formalin.
6. Special conditions exist. Needle biopsies of deep organs, digital amputations, bone biopsies, ear canal biopsies, and ocular biopsies are examples of special sites that require slightly different techniques or considerations. Suspected cases of autoimmune disease are additional special circumstances. Whenever there are questions about the correct types of sampling, calling the pathologists at Rollins Laboratory will often save time, help with a diagnosis, and may prevent the need for a second biopsy procedure.

**Proper Biopsy Techniques** (continued)

7. Finally, all fixed tissues should be submitted in 10 percent buffered neutral formalin. There should be at least a volume of 10 parts of solution to 1 volume of tissue. In addition, tissue sections should be no more than 1 cm in thickness in order for proper penetration of the formalin solution.

Disease Trends

What Should You Know About Bird Flu? by **Dr. Gene Erickson****Q: Should your clients be concerned for their personal health?**

- Not at the present time. The virus, Asian strain highly pathogenic (hi path) H5N1 avian influenza virus, has not adapted to humans and does not pass easily from person to person.

Q: How pathogenic is it for people infected with the virus?

- Currently, as of April 3, 2006, there have been 190 confirmed human infections, with 107 deaths for a case fatality rate (CFR) of 56 percent.
- If the virus adapts to humans, the CFR typically drops, but more people are infected.
- Human mortality has been restricted to Southeast Asia and the Middle East. Thirty-nine percent of the deaths have occurred in Vietnam with 49 percent of the human infections. China, the original source of the virus from 1996, has only reported 11 confirmed deaths (10 percent), and one third have been confirmed in Indonesia (22) and Thailand (14). Thus, 82 percent of human deaths due to hi path H5N1 have been in Southeast Asia.

Q: How is it being spread across the world?

- Some spread has been due to migratory waterfowl.
 - The first major waterfowl mortality was reported at Qinghai Lake in Northwestern China.
 - Subsequently, the virus has spread along waterfowl migratory flyways into Europe.
 - To date, the virus has been found in waterfowl and domestic poultry of 48 countries (OIE, 4-4-06):
Afghanistan, Albania, Austria, Azerbaijan, Bulgaria, Bosnia & Herzegovina, Cambodia, Cameroon, China, Croatia, Denmark, Egypt, France, Georgia, Germany, Greece, Hong Kong, Hungary, India, Indonesia, Iran, Iraq, Israel, Italy, Japan, Kazakhstan, Korea (Rep. of), Laos, Malaysia (peninsular), Mongolia, Myanmar, Niger, Nigeria, Pakistan, Palestine, Philippines, Poland, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Sweden, Switzerland, Thailand, Turkey, Ukraine, and Vietnam.
 - One of the major sources of movement of the virus into domestic flocks has been the export of day old chicks from China to other countries.

**What Should You Know About Bird Flu?** (continued)**Q: What is the most likely method for the virus to arrive in North America?**

- Just as the virus has spread from Northwest China to Europe and other countries via migratory waterfowl, that is also the most likely way it will enter the Western Hemisphere.
- With the arrival of spring to the Northern Hemisphere, migratory waterfowl will now return to their nesting grounds.
- The Siberian nesting grounds will be the epicenter for movement of the high path H5N1 among nesting birds there.
 - Survivors will resume their migration from those nesting grounds in late summer, beginning in July and August.
 - Of critical importance is the waterfowl that cross over the major migratory flyways,
 - Of those, the pintail duck and swans are the most likely to be infected.
 - They migrate along the Pacific flyway from Alaska down to Baja California. The Wildlife Service of USDA, through their NAHLN laboratories (including North Carolina), will be testing those migratory species as they begin their migration from Alaska, and when they reach the continental 48 states.
 - Testing will be concentrated on the Pacific flyway, where tens of thousands of birds will be swabbed for avian influenza PCR testing.
 - All four continental US flyways will be sampled, including the Atlantic flyway.
 - North Carolina is the winter migration destination for both pintail ducks and tundra swans.
 - Tundra swans nest near the Arctic Circle, north and slightly east of the Alaskan nesting grounds; but, there is overlap for the birds that winter here.

Q: Is it likely our domestic flocks will be infected if naturally infected waterfowl are in North Carolina?

- The experience of Europe is a good model for the US:
 - Housing conditions are similar.
 - Some free range bird raising is practiced, just as in the United States.
 - Only one commercial flock of turkeys has been infected in southeastern France.
 - Investigation of that outbreak has proven it was not due to exposure to migratory waterfowl.
 - Current suspicion is that the infection was introduced by bird or human fomite movement, but which occurred has not yet been reported or confirmed.
- Accordingly, the risk of domestic poultry flock infection in this country from migratory waterfowl is very low and unlikely, particularly if free range birds are moved indoors once the virus is confirmed in a state.

**What Should You Know About Bird Flu?** (continued)**Q: If domestic flocks are infected with bird flu should I be concerned?**

- Southeast Asian infections have largely occurred due to direct contact with infected birds and through cultural customs, such as the celebration of the New Year by eating raw blood pudding from sick birds.
- Domestic producers have an extensive preslaughter surveillance program in place for both chickens and turkeys. No house of birds serologically positive for H5N1 avian influenza will be processed for human consumption.
- An added safety feature is our modern meat inspection program.
 - No birds dying of avian influenza would be processed for human consumption due to the US Meat and Poultry Inspection System.
 - Typically, we do not kill and process dying chickens raised in our own back yards, which has been done in Asia.
 - Most people in this country do not consume raw poultry due to the risk of severe bacterial infections due to the surface bacterial contamination of the meat. This safety precaution has been taught to all children as part of their elementary school education, as well as by their parents.
- To summarize, you do not have to be concerned if domestic poultry are infected with bird flu. Our country and the poultry industry have the necessary safeguards in place to protect consumers.

Q: Are pigs a concern?

- Not at this time.
- Two independent experimental trials using high path Asian H5N1 avian influenza virus have documented the inability of the virus to be passed from pig to pig, just as is the case at present in people.
- The greatest concern will be if the virus successfully adapts to people, when they will then be able to transmit to the pigs, as has historically occurred since the great, worldwide pandemic of 1918.

Q: What should be our greatest concern?

- Human travelers, if the virus successfully adapts to people and is then easily able to transmit between people.



A dead mute swan, *Cygnus olor*, the species with the greatest die-off in Europe.



Recent Cases of Infectious Canine Hepatitis by **Dr. Peter Moisan**

Over the past 6 months there have been approximately 8 cases of infectious canine hepatitis reported in dogs from various regions of North Carolina. These cases have been fatal and necropsies were usually performed in laboratories of the NCDA. In our cases, as in others in the literature, the animals are usually puppies and have a history of inadequate vaccination against the virus causing canine hepatitis. Some have been adequately vaccinated against distemper and parvovirus, but not against canine hepatitis virus. Many reported outbreaks are in dog shelters where naïve, young animals are often present in crowded conditions.¹

Clinical signs of canine hepatitis are fairly specific, though, as the disease is only sporadic, it may not be readily recognized. These signs include vomiting and diarrhea, often with abundant blood in the stool. In terminal stages, there is icterus that is often quite profound. Necropsy lesions include small intestinal and colonic blood, swelling, pale liver, and yellow color of the subcutis and body fat. Petechiae and ecchymoses are located over the serosal surface of the small intestine. Features of disseminated intravascular coagulation may also develop, as the virus has tropism for endothelial cells, causing endothelial necrosis with induction of the intrinsic coagulation cascade. Because of this endothelial tropism, approximately 25 percent of affected dogs also develop corneal cloudiness (so-called “blue eye” syndrome), as the virus infects the endothelial cells of the cornea.

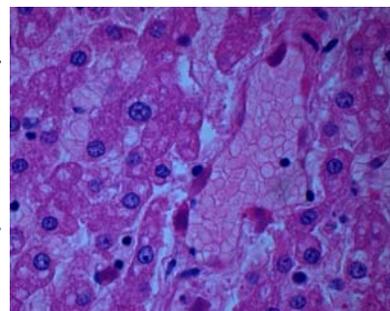


Figure 2. High magnification of canine liver with intranuclear adenovirus inclusion bodies (canine infectious hepatitis virus).

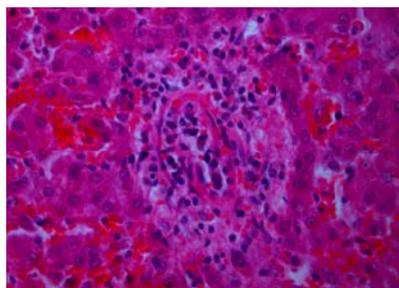


Figure 1. Periportal necrotizing hepatitis due to canine infectious hepatitis virus.

Canine hepatitis is caused by an adenovirus, canine adenovirus Type 1 (CAV-1). This is a DNA virus that forms intranuclear inclusion bodies and causes necrotizing hepatitis in the periportal regions of the liver. Along with tropism for endothelial cells, there is a tropism for hepatocytes. In the liver, the inclusions cause an expansion of the nuclei of the affected hepatocytes and are relatively abundant in the tissue sections (see photomicrographs). Splenitis and splenic necrosis are abundant in most cases as well. The diagnosis of canine hepatitis is by histopathology of sections from the liver and spleen. The inclusions are occasionally seen in other tissues, such as the endothelial cells of the kidney and small intestine. Vasculitis is occasionally also recognized in the small intestinal sections.

Canine adenovirus Type 1 is passed by the fecal-oral route primarily, with abundant virus passed in the bloody feces. There is also transmission by blood-sucking insects or by droplet secretions. Prevention of the disease is by proper vaccination. Infectious canine hepatitis is not seen in animals that are properly vaccinated against the disease. Differential diagnoses include parvoviral enteritis, coccidiosis, and aflatoxicosis, as well as other causes of diarrhea or liver failure.

Canine adenovirus Type 2 is a closely related virus that is part of the kennel cough complex. It is most often diagnosed in sections of lung from animals with chronic lung disease. The virus, CAV-2, localizes in the epithelial cells of the small bronchioles, as well as inflammatory cells and the Type II alveolar epithelial cells.² This virus is most often seen in combination with other agents such as *Bordetella bronchiseptica*, canine distemper virus, and parainfluenza virus. Vaccination against CAV-2 does not protect the patient against infection with CAV-1.

1. Pratelli, A, Martella V, Elia G, Tempesta M, Guarda F, Vapucchio MT, Carmichael LE, Buonavoglia C. 2001. Severe enteric disease in an animal shelter associated with dual infections by canine adenovirus type 1 and canine coronavirus. *J Vet Med B Infect Dis Vet Public Health* 48:385-392.
2. Grad R, Sobonya RE, Witten ML, Quan SF, Ray CG, Devine LC, Lentz LA, Lemen RJ. 1990. Localization of inflammation and virions in canine adenovirus type 2 bronchiolitis. *Am Rev Respir Dis* 142:691-699.



Interesting Cases

Arsenic Toxicosis in a Beef Herd by Dr. Steve Rushton

A seven year old Beefmaster cow was submitted to the Rollins Diagnostic Laboratory earlier this year, and at that time, was one of 14 cows to be found dead within a 20 hour period. Within the next three to four days, another 15 cows died and several were weak and very lethargic. The owner reported that many of the cows had watery diarrhea and severe dehydration prior to death. There were 41 cows in the affected pasture. Adjacent pastures of cows are unaffected. The cows were being fed cotteseed gin and coastal hay with no grain. The owner was concerned about the cottonseed since it contained abundant black moldy-like material. Upon further questioning, the owner stated that there had been some burning of treated wood piles within the last week.

The cow was examined within three hours of death and the examination only revealed severe (10-12%) dehydration. Ocular fluid along with rumen contents and fresh liver were sampled for suspected acute toxicosis, which is the top rule-out in acute multiple deaths in cattle. Rumen contents contained no pesticides and the pH was 7.0, interpreted as within normal limits. Ocular fluid for nitrates was negative. Lead was not identified in the liver, however, arsenic levels were 10.3 ppm, which is considered 20 times normal levels. Rumen contents also contained arsenic (12.9 ppm). Burned wood ashes were not submitted for arsenic levels.

Arsenic poisoning is rarely seen in cattle given that there has been a significant decline in the use of arsenic-containing compounds. As in this case, most occurrences of livestock intoxication are attributed to accidental access to arsenical compounds, such as exposure to burned wood that has been treated with an arsenical.

Arsenic, a heavy metal, is an essential trace element. Animals are able to tolerate low levels of arsenic; the normal level in cattle tissues is less than 0.5 ppm. Arsenic levels greater than 10 to 15 ppm in the liver, accompanied by clinical signs, are considered diagnostic of acute arsenic toxicosis. Arsenic poisoning may be peracute, with clinical signs including depression, prostration, and sudden death. The clinical signs displayed by the cows (watery diarrhea and severe depression) in this case were typical of acute arsenic intoxication, which develops 20 to 50 hours after toxicant ingestion. Animals that survive the acute phase may live for 2 to 7 days and exhibit clinical signs of ataxia and convulsions. Chronic arsenic poisoning is rarely seen in domestic animals because arsenic is rapidly excreted in the urine. Arsenic may be absorbed percutaneously, causing blistering, edema, and necrosis of the skin due to capillary dilatation and degeneration.

The trivalent form of inorganic arsenic is up to 10 times more toxic than the pentavalent form and causes most of the toxic effects. Pentavalent and organic forms of arsenic are reduced and metabolized in the rumen and converted to the trivalent form, producing toxicosis. Trivalent inorganic arsenic binds to and inactivates intracellular sulfhydryl-containing compounds, especially lipoic acid and α -keto oxidases, thereby disrupting cellular metabolism and inhibiting enzyme systems essential for oxidative phosphorylation. The tissues of most organs affected by arsenic have high oxidative energy requirements, principally the alimentary tract, kidney, liver, lung, brain, and epidermis. This disruption in energy metabolism most likely caused the diarrhea and lethargy displayed by the cows in this investigation.

A diagnosis of arsenic intoxication must be supported by clinical signs, identification of a source of the suspected toxicant, elevated levels of the toxicant in the tissues, and pathological lesions. The treatment regime for livestock includes supportive and decontamination procedures, administration of sodium thiosulfate, IV and PO, and antidotal therapy. Chelating sulfate antidotes contain sulfhydryl groups that compete with sulfhydryl-containing enzymes for available arsenic. The classic chelating antidote for arsenic toxicosis is dimercaprol (BAL). Thioctic acid, mesodimercaptosuccinic acid, and dimercaptosuccinic acid are alternative arsenic chelating agents. The prognosis following acute arsenic toxicosis is grave if the diagnosis and, therefore, treatment are delayed.

**Identification of Unusual *E. coli* in Calves with Diarrhea** by **Dr. Karen Post**

Non-pathogenic strains of *E. coli* are commonly found in the intestinal tracts of animals as normal flora; yet, *Escherichia coli* is also a major pathogen, primarily of neonates, causing diarrhea and septicemia. A diagnostic challenge is to differentiate normal flora strains from those that have acquired genes imparting virulence.

According to the history provided, a large dairy was experiencing scours in its Holstein calves from 2 to 10 days of age. There was high morbidity and low mortality in the herd. The attending veterinarian submitted five fecal specimens for diagnostic analysis.

All specimens tested negative for *Cryptosporidium* and *Giardia* spp. by the fluorescent antibody assay. No ova or parasites were observed upon fecal flotation examinations. Four of five specimens were positive for type A rotavirus by the latex agglutination test. Negative contrast microscopy confirmed the 4 positive samples. No salmonellae were recovered from fecal enrichment cultures, although heavy growth of a non-hemolytic *E. coli* was isolated from aerobic cultures of all five fecal samples.

The *E. coli* isolate was forwarded to our molecular diagnostics section for genotyping by multiplex PCR. It tested negative for all virulence factors in the assay, which included the pili K88, K99, F41, 987P, and the toxins STa, STb, LT and SLT-II. These are the most common virulence factors associated with neonatal colibacillosis. Additional in-house assays demonstrated that the isolate was negative for cytotoxic necrotizing factors type 1 and 2, SLT-I, and the attaching and effacing gene, which are other virulence factors that have been linked to bovine diarrhea.

To further rule in or rule out the *E. coli* isolate as a pathogen, it was forwarded to the [Gastroenteric Disease Center](#) at [Pennsylvania State University](#) for more extensive characterization. There it tested positive for the CS31A (colonization surface) antigen which is a capsule-like surface protein, immunologically related to K-88 and F41 pili, that mediates adhesion to enterocytes. Although there is a close association between these strains and cases of diarrhea and septicemia in calves, they have not been frequently reported in the United States.

Based on the laboratory findings, it was concluded that a co-infection with rotavirus and a CS31A-producing strain of *E. coli* were the etiologic agents of diarrhea in these calves. This case highlights the importance of molecular analysis to the diagnosis of colibacillosis. While serologic tests have traditionally been used to identify virulence factors associated with specific clinical syndromes, the application of molecular methods, mainly the polymerase chain reaction (PCR) assay, has increased both the rapidity of characterization and the amount of information which can be gleaned from an individual isolate.

***Coxiella burnetii* (Q Fever) Infection in a Boer Goat Herd** by **Dr. Jennifer Haugland**

Two Boer kids and two placentas from doe A, and one Boer kid and two placentas from doe B were presented for necropsy. According to the history provided, the two does kidded normally, but 3 out of 4 kids died within 3 to 5 hours of birth and the placentas looked abnormal. This was the third parity for both does, and they were in a group of 55 does ready to kid. There were a total of 700 does, which were split into 6 pastures on the farm. Two older does may have aborted about 1 month earlier. None of the other pastures have had reproductive problems. However, after further questioning of the owner, it was discovered that there have been several stillbirths and neonatal losses each year. These kids were only presented to the laboratory due to the abnormal placentas.

On necropsy examination all 4 placentas were confirmed abnormal. The placentas were thickened with multifocal to confluent copious tan exudate, which was more pronounced in the intercotyledonary regions. Hemorrhage within some cotyledons was also noted. No gross lesions were identified in the kids. The histopathologic examination revealed placentitis that was characterized as necrosuppurative, severe, diffuse, subacute,



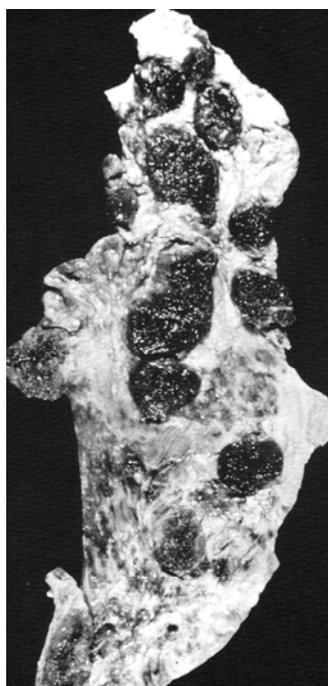
***Coxiella burnetii* (Q Fever) Infection in a Boer Goat Herd** (continued)

with mineralization and colonies of coccobacilli within the trophoblast cells. No histopathology lesions were seen in the fetuses.

Laboratory diagnostics included culture of lung, liver, and abomasal contents for aerobic bacteria, *Campylobacter jejuni*, *Campylobacter fetus*, *Brucella* spp., *Listeria* spp., and fungi. Placenta and fetus tissues were negative for *Chlamydia* spp. by ELISA. Placenta was negative for *Toxoplasma* spp. by IHC analysis, and the kidneys were negative for *Leptospira* spp. by PCR analysis. Placenta embedded in paraffin was submitted to the California Animal Health and Food Safety Laboratory at UC Davis for IHC analysis for *Coxiella burnetii* (Q fever). The placenta was positive for *Coxiella burnetii* (Q fever).

Q fever should be considered when caprine, ovine, or bovine placentas are thickened with white to tan exudate. Other differential diagnoses would include chlamydiosis and campylobacteriosis.

Abortion, stillbirths, or weak born neonates will likely occur with the thickened placentas. Many infections are asymptomatic. *Coxiella burnetii* can infect humans and cause disease that can vary from inapparent infections to flu-like disease, pneumonia, or heart disease.



C. burnetii abortion in a caprine.
Suppurative intercotyledonitis.

Photo Credit: Jubb, K.V.F., et al. Pathology of Domestic Animals, 4th Ed. Volume 3, 1993

The infected placenta, uterine fluids, and fetuses contain large numbers of the bacteria and should be handled only when wearing latex type gloves and a tight fitting mask over the face. *Coxiella burnetii* can also be shed in the milk and colostrum. It is resistant to drying with the result that animals and humans can be infected by inhaling dust. Grazing contaminated pastures and tick bites are other modes of transmissions. *Coxiella burnetii* can be shed even at a normal birth with a normal placenta. Stresses such as overcrowding or poor nutrition may play a role in determining if an infected animal will abort.

Abnormal placentas and the associated kids should be handled carefully by owners and veterinarians when submitted to the diagnostic laboratory. The tissues should be double bagged or boxed up and the differential diagnosis of Q fever should be written on the submission form to notify laboratory personnel of possible zoonosis. Laboratory personnel will examine and sample the tissues under a biological hood.

Serological diagnostic tests are not very sensitive or specific. It is difficult and likely impossible to verify that a herd or flock is negative for *C. burnetii*. Commercially available tests are CF ([NVSL, U. MN](#)), IFA, and ELISA ([Pan American Vet Labs](#)). A PCR test is also available at the [Wisconsin Veterinary Diagnostic Laboratory](#). IFA and ELISA are considered more sensitive than CF in cattle and humans. In humans, both phase I and phase II proteins are measured to differentiate between acute and chronic exposure. The Pan American Veterinary Laboratory's ELISA measures phase II antibodies.

Treatment with tetracycline is recommended by some to reduce or stop abortions but there is no treatment that will eliminate the shedding of the organism. No vaccine against *C. burnetii* is available in the United States.

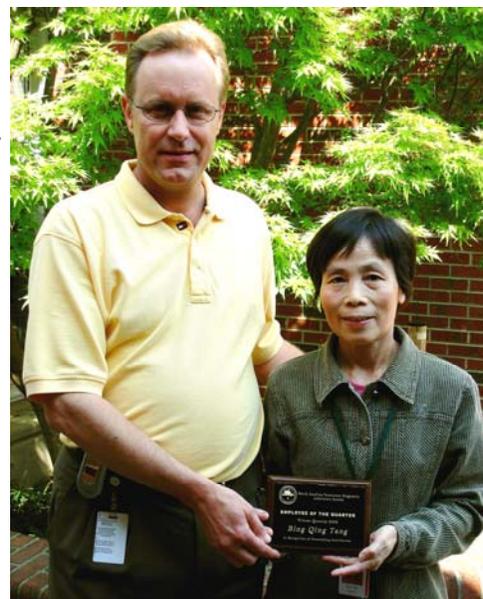
Coxiella burnetii infection is a reportable disease in North Carolina. Animals diagnosed definitively by IHC or PCR analysis done on infected tissues are to be reported to the State Veterinarian, (919) 733-7601. If the diagnosis is made through the North Carolina Veterinary Diagnostic Laboratory System, then the case coordinator will make the report.



Employee of the Quarter

Congratulations Bing!

Our honorary employee for the Winter Quarter is molecular diagnostician Dr. Bing Qing Tang. Bing is a self motivated individual who needs little direction and is always eager to work diligently. Flexibility, thoroughness, and a desire to learn are a few of Bing's attributes that exemplify an ideal employee. On a daily basis, she accurately completes an immense volume of molecular testing. Bing's dependability is demonstrated by her willingness to fill in whenever she is needed, even when she has worked a bench only a limited number of times. She is an optimal team player who adapts well to the needs of others in any work situation while still getting the job done. Bing has an easy-going personality and is always a pleasure to work with. Congratulations Bing!



Departmental News

ARDEN LABORATORY:

We are pleased to announce that the March 2006 Employee of the Month for the North Carolina Department of Agriculture is Paul Rector. Paul has worked in the Arden Diagnostic Lab as a Medical Laboratory Assistant since 1975. He was honored at the executive staff meeting in Raleigh on April 24, 2006. In the five year history of the Employee of the Month Program, Paul is only the third Veterinary Division employee to be so honored. Please congratulate Paul when you have an opportunity!

ROLLINS LABORATORY:

Bacteriology... Sadly, Carol Crabtree and Elizabeth Shelton, both Medical Laboratory Technologists, have resigned their positions. We appreciate their hard work and wish them success in all future endeavors!

Virology... On April 5, 2006, **Jeremi Brown** attended a PRV Latex training course at NVSL in Ames, Iowa.

Histology... **Faye Coombs** attended the NCSHT Spring Meeting in Greensboro, North Carolina on April 21 and April 22, 2006. Topics included Interpretation of Special Stains and Grossing for Histotechs.

Quality Assurance... On April 6, 2006, **Kathy Schmidt** attended the Laboratory Safety and Chemical Hygiene Workshop at NCSU. **Kathy** also attended the OSP Safety and Health Workshop in Apex on April 12.

Necropsy... Congratulations to **Allison Heatherly** for gaining admittance to the College of Veterinary Medicine at North Carolina State University for the upcoming year. Allison has worked as a necropsy assistant at the Rollins Laboratory since August 2004. **Dr. Jennifer Haugland** attended the program "Emerging and Re-Emerging Infectious Diseases of Man and Animals", which was held at Virginia-Maryland Regional College of Veterinary Medicine on April 22, 2006.



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[Dr. Stacy Robinson](#)

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[Dr. Tahseen Abdul-Aziz](#)

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Director

[Dr. Carlton Rouse](#)

Veterinary Diagnostician

[Dr. Hope Lucas](#)

