



Summer 2005

### From The Director

**W**ELCOME to the NCVDLs's inaugural quarterly newsletter. We are pleased to roll out this new feature as a tool for notifying our clientele about current disease issues, policy changes, and developments in our redesign and improvement of the laboratory system. We also would like for you to use this forum to provide feedback and suggestions as to enhancements we can consider to improve our service to you.

To say the least, things have been very active over the past four months since Agriculture Commissioner Steve Troxler assumed his position and designated me as Laboratory Director. I look forward to the challenge and am pleased with the progress that has been made to this point. Dr. Karen Post is serving as Assistant Director in addition to her duties as head of Bacteriological Services. Dr. Gene Erickson, who has served as interim director over the past year and a half in addition to his normal responsibilities, will continue as Head of Microbiological Services and the Molecular Diagnostics section, as well as direct activities involving the BSL 3 laboratory, our participation in the National Animal Health Laboratory Network (NAHLN) and the Laboratory Response Network (LRN), and facility structural issues. My thanks go out to these two professionals for the work they are doing in moving our system forward.

While still dealing with the traditional budget, personnel classification/compensation, and business model challenges of the past, we are pleased with some recent developments. Agriculture Commissioner Steve Troxler and his staff have made support of the veterinary laboratory system one of their top priorities for the upcoming years. This has already been reflected in the reclassification by the Office of State Personnel of our pathologist positions to allow the ability to recruit, retain, and compensate these specialists more effectively in a competitive environment. In addition, the department was successful in lobbying the General Assembly to include \$10 million in the proposed House budget as the first installment of a \$20 million Rollins expansion project. This funding was not included in the Senate portion of the budget, so the issue is currently being debated in conference committee. I urge all of you to personally contact your representatives and support this expansion request. Included in the discussion is a request for the laboratory to retain a portion or all of the fee revenue currently being generated to directly support the service, rather than going to the State's general fund. We will continue to build bridges with members of the General Assembly that will allow us to better position the lab system in the future as a key contributor to the State's animal and public health surveillance infrastructure.

Newly created is a Diagnostic Laboratory Advisory Committee, a group of fourteen representatives from all facets of the livestock, poultry, and companion animal veterinary community, as well as representatives from the NCSU College of Veterinary Medicine, State Public Health Laboratory, and the Farm Bureau. This group will advise our staff and help chart the course for future actions. The initial meeting of the group will be in early August.

In early November, 2004 we hosted the review team representing the American Association of Veterinary Laboratory Diagnosticians (AAVLD), the national accreditation body for veterinary diagnostic labs. The three-day review to renew our accreditation went well, and we have received the preliminary report indicating successful renewal. A formal announcement will be forthcoming shortly.

We are pleased that we have recently been able to successfully create and fill a Quality Control/Quality Assurance manager position. Lou Ann Risser comes to us with a strong



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comments and/or suggestions  
concerning The NCVDLs Report.

Arden Lab (828) 684-8188  
Elkin Lab (336) 526-2499  
Monroe Lab (704) 289-6448  
Rose Hill Lab (910) 289-2635



## From The Director (continued)

background in QA/QC, and will be focusing on the development of a Quality Manual and SOP's for all facets of operations consistent with meeting World Health Organization (formerly OIE) and International Standards Organization (ISO) requirements. This will be a very extensive task involving all system employees, but will pay great rewards in diagnostic and test result consistency and credibility, as well as facilitating international movement of animals and animal products through validation of our state's animal disease status.

The implementation of our new Laboratory Information System (LIMS) is nearing completion, replacing the archaic DOS-based product that could best be described as a "legacy system". As with any IT type project, bumps in the road and tweaking will be necessary and ongoing but the end result will be a web-based system for real time case reporting and tracking to aid you in managing your submissions.

Again, we ask for your patience and input as we make improvements and position the system for success in the next decade. One of our major goals is to improve communication between us and you, our stakeholders. Please visit our web site frequently at [www.ncvdl.com](http://www.ncvdl.com) for news and updates. You may also expect individual mailings, if the situation warrants. In addition, we can be contacted by email through the "Contact Us" link from the web site.

Regards,

*David Marshall, DVM*

## Client Corner

### Guidelines for Diagnostic Specimen Shipment

By Dr. Karen Post

U.S. Department of Transportation (DOT) rules in effect since February, 2003 have affected the definition of diagnostic specimens and how they are classified, packaged, and transported. These rules apply to the shipment of veterinary specimens to diagnostic laboratories. The rules are mandated by the federal government rather than the State or NCVDLs. A diagnostic specimen has been defined as "any human or animal material including excreta, secretions, blood and its components, tissue, and tissue fluids being transported for diagnostic or investigational purposes, but excluding live infected humans or animals."

Although previously exempt from regulation, Diagnostic Specimens are now considered Hazardous Materials and are listed in the Hazardous Material Tables of Title 49 Code of Federal Regulations. Veterinarians are subject to the new rules summarized below. Non-compliance can result in very stiff fines. These rules apply only to specimens that are considered as potentially infectious. More stringent requirements are in effect for known infectious agents. To ship specimens from suspected cases of foreign animal diseases (FADs) or other very highly infectious and virulent diseases, contact the State Veterinarian or the Federal Veterinarian-in-Charge. Formalin-fixed tissues are exempt, but should still be packaged in leak-proof containers with adequate absorbent material.

Diagnostic Specimens must conform to the standards which follow. These regulations APPLY to FedEx and other commercial shipping companies. The stringent parcel size limitation in the section "Shipments by Air of Diagnostic Specimens" is important for any clinic that routinely ships by air. Packing Instruction 650 includes additional requirements for air shipments and can be found at [www.iata.org/NR/ContentConnector/CS2000/SiteInterface/sites/whatwedo/dangerousgoods/file/PI650.pdf](http://www.iata.org/NR/ContentConnector/CS2000/SiteInterface/sites/whatwedo/dangerousgoods/file/PI650.pdf). Note: The US Postal Service also has additional regulations that may be found at [www.usps.gov](http://www.usps.gov) or [www.usps.com/cpim/ftp/pubs/pub52.pdf](http://www.usps.com/cpim/ftp/pubs/pub52.pdf)

Diagnostic Specimens must be packaged in triple packaging consisting of: 1. A primary receptacle: Primary receptacles (i.e. blood tube, specimen jar) must be packed in secondary packaging in such a way that under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. 2. Leak-proof secondary packaging: Secondary packaging(s) must be secured in outer packaging(s) with suitable cushioning material such that any leakage of the contents will not impair the protective properties of the cushioning material or the outer packaging. If several fragile primary

## Guidelines for Diagnostic Specimen Shipment (continued)

receptacles are placed in a single secondary packaging, they must be individually wrapped or separated to prevent contact between them. 3. Outer packaging: Outer packaging must be clearly and durably marked with the words "Diagnostic Specimens". The completed package must be capable of successfully passing a drop test at a drop height of at least 1.2 meters (3.9 feet). Shipping papers are not required under the 49 CFR rules, but may be required by some individual shippers. 4. Liquid Diagnostic Specimens must be packaged where the primary receptacle is leak-proof with a volumetric capacity of not more than 500 ml. Absorbent material of sufficient quantity to absorb the entire contents of the primary receptacle(s) is placed between the primary receptacle and secondary packaging. Multiple fragile primary receptacles placed in a single secondary package must be individually wrapped or separated as to prevent contact between them. Secondary packaging must be leak-proof. 5. Shipments by Air of Diagnostic Specimens additionally require that the primary receptacle or the secondary container is capable of withstanding without leakage an internal pressure differential of 95 kPa (14 psi). The outer package must display a diamond-shaped mark with UN3373 inside and the proper shipping name "Diagnostic Specimen" adjacent to the diamond-shaped mark. Please refer to Packing Instruction 650 for specifics on the size and lettering of this mark. The outer packaging does not exceed 4 L capacity for liquids or 4 kg for solids. Note: This volume limitation does not apply to parcels containing animal body parts, whole organs, or whole bodies. Training is required for those who offer packages for transport and they must know about and be able to apply the requirements of Sec. 173.199 (Title 49 CFR) to specific shipments. **Fines and penalties: According to 49 CFR Sec. 171.1 (c) "Any person who knowingly violates a requirement of the Federal hazardous material transportation law ..... is liable for a civil penalty of not more than ....\$27,500....and not less than \$250 for each violation. .... and shall be fined under Title 18, United States code, or imprisoned for not more than 5 years, or both."**

- Summaries and the complete regulation can be seen at:  
[www.epa.gov/fedrgstr/EPA-IMPACT/2001/January/Day-22/i92.htm](http://www.epa.gov/fedrgstr/EPA-IMPACT/2001/January/Day-22/i92.htm)
- [www.access.gpo.gov/nara/cfr/waisidx\\_03/49cfr173\\_03.html](http://www.access.gpo.gov/nara/cfr/waisidx_03/49cfr173_03.html)
- US Postal Service regulations may be found at [www.usps.gov](http://www.usps.gov) or [www.usps.com/cpim/ftp/pubs/pub52.pdf](http://www.usps.com/cpim/ftp/pubs/pub52.pdf)

When shipping specimens to the Rollins Laboratory use the address that corresponds to your method of shipment:

### United States Postal Service <sup>1</sup>

Rollins Laboratory  
1031 Mail Service Center  
Raleigh, NC 27699-1031

### United Parcel Service/Federal Express <sup>2</sup>

Rollins Laboratory  
2101 Blue Ridge Road  
Raleigh, NC 27607

<sup>1</sup> The United States Postal Service does not deliver to this address on Saturdays. Any specimens received by the state Mail Service Center after 7am on Friday will be delivered to Rollins Laboratory the following Monday.

<sup>2</sup> The United Parcel Service does not deliver to this address on Saturdays. Please contact your local United Parcel Service representative to determine shipping and delivery times.

## Get Your Results Online

NCVDLS clients of the Rollins and Griffin Laboratories now have the convenience of 24/7 electronic results reporting. Customers can securely access real-time results and download key data. While visiting our website, [www.ncvdl.com](http://www.ncvdl.com), click on "How to Request Access to Online Test Results" and simply follow the directions to become an online client. Please remember that all laboratory results are viewed in "real time" before final approval by laboratory staff. Thus, all results displayed on the website are considered preliminary and may be subject to change. Written copies of laboratory reports are considered final and should be reviewed before veterinary medical decisions are made.

## Necropsy Information

By Dr. Jennifer Haugland



*“A complete history that is provided prior to necropsy is invaluable.”*



### Current Fees

<b>Small animal/companion animal</b>	<b>\$30</b>
<b>Horses/large non-food animals (llama, etc)</b>	
0-100 lbs	\$30
101-500 lbs	\$40
Over 500 lbs	\$55
<b>Food Animals</b>	
0-100 lbs	\$ 5
101-500 lbs	\$15
Over 500 lbs	\$30

### Carcass Submission

Referring veterinarians or clients are encouraged to notify the lab and consult with an on-call veterinarian before sending a carcass. Coolers are located outside the Rollins, Elkin and Arden Labs for overnight drop-off of animals. The cooler at Rollins can accommodate animals up to 100 lbs., while those at Elkin and Arden can hold animals up to 200 lbs. In addition, the Elkin facility has outside access to a large, walk-in cooler which allows the acceptance of larger animals.

Submission of the entire intact carcass is generally preferred. It is extremely important that the animal be necropsied as soon after death as possible. Post-mortem autolysis and putrefaction lessen the chance of establishing a diagnosis with each passing hour. If the animal is small, it should be refrigerated. Do not enclose the animal in a plastic bag until the body is well-chilled. Bags hasten autolysis due to entrapped body heat. If long delays are anticipated, please contact a laboratory veterinarian. Freezing causes tissue artifacts that may preclude an accurate diagnosis via histopathology. Freezing may also be deleterious to the recovery of certain pathogens.

### Carcass Refusal

Refusal of a carcass for necropsy is at the discretion of the laboratory veterinarian assigned to the case and will be based upon the following: an animal is deemed too autolytic for optimal diagnostic testing; an animal has clinical signs that are consistent with a recent laboratory diagnosis in a herd or flock, therefore an additional necropsy is unwarranted; a diagnosis for an animal has already been obtained and confirmed, therefore a necropsy is unwarranted e.g. fractured leg, uterine prolapse, chronic laminitis.

### History

A complete history that is provided prior to the necropsy is invaluable. Please completely fill out the [necropsy submission form](#), which you can download at our website. Please provide as much information as possible. No detail is too insignificant. Be sure to indicate duration and description of clinical signs, as well as treatments, ancillary diagnostic tests, and vaccinations. Also, list impressions or provide a differential diagnosis list.

### Animals for Rabies Testing

Rabies testing in this state is performed exclusively by the North Carolina State Labora-

## Necropsy Information *(continued)*

tory of Public Health (NCSLPH) located in Raleigh. Heads from cats, small dogs and other small animals should be submitted directly to that lab via your county animal control agency. Sending these heads directly to the NCSLPH will enable you to get more rapid test results. Heads from large animals may be taken to any NCVDLs lab for brain removal; brains will then be forwarded on for testing.

All questions regarding human exposure, rabies vaccination, etc. should be directed to the state public health veterinarians at 919-707-5900 during normal business hours (M-F, 8am-5pm) or 919-733-3419 after hours. Emergency testing over a weekend or holiday may be requested by calling the pager at 919-310-5620. Circumstances which constitute an emergency situation for human exposure to rabies must satisfy one of the following criteria:

1. Unprovoked bite from a wild animal, such as raccoon, fox, skunk, bobcat, etc.
2. Unprovoked bite from an unvaccinated dog or cat.
3. Bite (provoked or unprovoked) resulting in skin breakage on either the head or neck.
4. Bites from bats.
5. Bat(s) found in domicile where people were asleep.

**Rabies test results** and answers to other questions can be obtained from the Rabies lab at 919-733-7544.

Please visit the NCSLPH website for additional information about specimen acceptance, state courier system and result reporting: <http://slph.state.nc.us/virologyserology/rabies/default.asp>

### Disposal of Animal Remains at the Rollins Laboratory

As a general rule, most carcasses are incinerated/rendered shortly after necropsy and no body parts can be returned. The only exception is that small companion animals may be collected by a private cremation service. **Arrangements with the cremation service must be made by the veterinarian or owner prior to the necropsy submission.** This must be indicated on the laboratory accession form; otherwise the carcass may not be retrievable.

Raleigh Area Crematory Services:

Faithful Friends Pet Cremation Services	919-874-0014
Buckleigh Hills Pet Cremation & Memorials	919-836-1815

### Change in After-hours Necropsy Policy

To improve our laboratory system, we are adopting changes in our current necropsy policy effective August 1, 2005. Necropsies will **not** be performed after 5pm on weekdays or state holidays, unless they qualify as an emergency. Weekend necropsies will be limited to 8am-12pm on Saturdays at Rollins Lab only; branch labs will not be providing the Saturday morning service. Only emergency necropsies will be performed after 12 pm Saturday on weekends. Emergencies are being limited to cases of multiple deaths within a herd/flock over a short period of time, instances where a foreign animal disease is suspected, and situations with zoonotic implications. All laboratory clients will receive a letter regarding these changes.

### Necropsy Training Session

Rollins Lab would like to host a necropsy review session for those veterinarians that might be interested in sharpening their skills. Tentative date is scheduled for Saturday, August 6 starting at 9am. We plan to have an equine and ruminant session. There will also be information available on appropriate selection, collection, and transport of specimens. For small animal practitioners, we may be able to arrange a training session during our normal work week. We welcome the opportunity to demonstrate how necropsies are performed in our lab and offer suggestions on how to optimize your field necropsies. Continuing education credits will be offered: 3 hours for one session and 5 hours for both sessions. Please contact Dr. Jennifer Haugland at 919-733-3986 if you are interested in attending this event.

## Client Billing

The NCVCLS is nearing completion of its migration to an Internet-based Laboratory Information Management System (LIMS). With the new system, in addition to obtaining test results online, you can now view individual case charges!

The migration to our new computer system, which began in January, also required the laboratory system to transition to a new accounting system. During the transition phase, we have gradually closed out client billing

accounts in our legacy system. Everyone has received new client billing account numbers, reflected on your invoices generated by the new system.

With our new accounting system, you have undoubtedly noticed changes in the appearance of your invoices. You can expect improvements in the appearance of your invoices in the months ahead.

Please note that the NCVCLS can currently only accept cash, check or money orders as payment for our

services. Members of our staff are working to incorporate the convenience of credit card payments into the system. You will be notified when we have fully implemented this form of payment.

We appreciate your patience during this time. If you have questions or suggestions for improvement, please do not hesitate to call Ms. Mechelle Johnson, our clerical supervisor, at (919) 733-3986

## Departmental News

**Chemistry...Robin Smith** retired as Chemistry Supervisor in December 2004 after 32 years of service. **Geoff Crissman** has been promoted to his position.

**Histopathology...** Congratulations to **Faye Coombs** for passing the Histology Board of Registry Exam in December, 2004. **Mary Horne** attended an instructional class in Torrance, Ca in July for the operation of our new tissue processor.

**Molecular Diagnostics...** **Chad Menard** attended a training program entitled "Training the Trainer," which dealt with foreign animal disease detection. The program was held in April in Athens, Ga. **Chad** and **Anita Quinn** participated in a teleconference on vector-borne diseases at the NC Laboratory of Public Health Lab held in Raleigh this past June.

**Serology...Vickie Pope**, former Rollins Lab serology section supervisor, retired in December 2004 after thirty years of service. She now works on a part-time basis at Rose Hill Laboratory. **Jennifer Pruitt** has been promoted to her position.

**Virology...New Test:** ELISA test for *Neospora* antibodies.

*Neospora caninum* is a protozoal (Apicomplexa) parasite that can cause abortion and neonatal morbidity and mortality in cattle, sheep, goats and horses. It has been reported in California that infection with *Neospora* is the most important diagnosed cause of abortion in dairy cattle. Neosporosis has been associated with bovine abortion storms frequently in North Carolina. The virology department of the Rollins Laboratory offers a serological test for rapid disease diagnosis, a competitive ELISA that detects serum antibodies to a *N. caninum* tachyzoite antigen. The test takes approximately two hours to perform and is run every Thursday. The assay has a sensitivity of 98.6% and a specificity of 98.9%.



*"Vickie Pope, former Rollins Lab serology section supervisor, retired in December 2004 after thirty years of service."*

## Service Awards

### 5 Years

Sherry Casey  
Dr. Peter Moisan  
Amy Stein

### 10 Years

Dr. Linda Kooistra  
Bing Qing Tang  
Dr. Reg Ridenhour

### 15 Years

Barbara Bright  
Lia Coleman  
Dr. Gene Erickson  
Beverly Wood

### 20 Years

Earlene Allen  
Sharon Graham  
Denise House  
Dr. Richard Oliver

### 20 Years (continued)

Dr. Carlton Rouse

### 25 Years

William Carpenter

### 30 Years

Geoff Crissman  
Diane Pearson

## Disease Trends

### Canine Brucellosis

By Dr. Steve Rushton

At Rollins Laboratory, we have recently necropsied animals from an abortion outbreak of Brucellosis in a kennel of breeding Beagles. Canine brucellosis is a contagious infection produced by a gram-negative coccobacillus called *Brucella canis*. *Brucella canis* is a bacterium that was first isolated from dead puppy fetuses in the middle 1960s. It is the most common cause of canine abortion that infects bitches and their fetuses.

#### Transmission

*Brucella canis* is sexually transmitted by the mating of infected males and females. *Brucella canis* in the female dog will live in the vaginal and uterine tissue and secretions for years and

except in rare cases, for life. The infected female usually appears healthy with no signs of disease or indication that she is a carrier of the organisms. She can spread the bacteria to other animals through her urine, aborted fetuses, or most commonly through the act of breeding. Once pregnant, bacteria will also infect the developing fetuses causing illness. In male dogs, *Brucella canis* resides in the testicles and seminal fluids. An infected male is just as dangerous as the female, as he can spread the bacterium via his urine or semen. Often times there are no signs except in advanced cases when the testicles may be uneven in size.

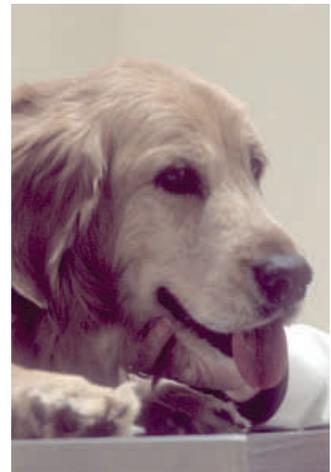
#### Clinical Findings

In female dogs, infection

leads to abortion or early death of infected puppies. Infected females may have no other clinical signs. In some cases, there may be decreased fertility rather than abortion. This may be due to resorption of fetuses early in their development.

In male dogs, infection of the testicles can lead to infertility due to anti-sperm antibodies developed as the body attempts to fight off the bacterial infection. The testes may atrophy after the initial period of swelling. Scrotal enlargement or infection of the skin over the scrotum may be seen.

In both female and male dogs, there may be infection of spinal discs (diskospondylitis) which can cause back pain and rear leg weakness or even paralysis.



## Canine Brucellosis (continued)

Eye inflammation may be seen in either sex.

### Diagnosis

The diagnosis of canine brucellosis warrants laboratory confirmation. Blood cultures are strongly recommended before declaring that an animal is truly infected. There are long and continual periods of bacteremia associated with this disease. Greater than 50% of infected dogs have a bacteremia lasting one year or longer. Most commonly available serologic assays are imprecise. Bacterial culturing of *Brucella canis* is the definitive test for diagnosis of aborted fetuses and breeding animals. *Brucella canis* is commonly cultured from aborted fetuses and their placentas in abortion cases. The organism may be recovered from ante-mortem blood cultures from the bitch and post-mortem cultures of lymph nodes, spleen, and reproductive organs from infected animals.

Serological testing is the most common and rapid method of detecting *Brucella canis* in dogs intended for breeding. The most widely available tests are:

1. Rapid Slide Agglutination Test (RSAT) – This test is an office screening test (D-Tec CB; Synbiotics Corp., Frontera, CA). The antigen is [rose bengal-stained B. ovis](#), which cross-reacts with *B. canis*. A negative slide test is strong evidence that the dog is not infected; only about 40% of dogs whose sera agglutinate in the slide test antigen are actually positive for canine brucellosis. With a positive slide agglutination test result, a dog should **not** be considered infected until additional laboratory tests are performed.
2. Tube agglutination (TAT) and Agar Gel Immunodiffusion (AGIDcwa) Tests - These tests utilize cell wall antigens. These are also flawed by false-positive reactions and difficulties in interpretation, especially with acute sera or sera from chronically infected

dogs. Results obtained by the TAT and AGIDcwa tests should also be confirmed by more specific tests and isolation attempts.

3. Indirect Fluorescent Antibody Test (IFAT) - An IFAT is used by several diagnostic laboratories in the U.S., but data on its accuracy have not been

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*“Serological testing is the most common and rapid method of detecting *Brucella canis* in dogs intended for breeding.”*

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published. Results from Cornell University's Diagnostic Laboratory indicate a high rate of false positive reactions.

Improved serologic tests include: (a) A RSAT that employs a mutant strain (less mucoid, "M-") of *B. canis* that has high specificity, (b) AGID test (AGIDcpa) that employs protein antigens extracted from the bacterial cytoplasm. These protein antigens are highly specific for the Genus *Brucella* and are useful in distinguishing between infected and uninfected dogs that possess antibodies reactive in agglutination or AGIDcwa tests, (c) enzyme-linked immunosorbent assay (ELISA) that employs as antigen cell wall lipopolysaccharide extracts of *B. canis* M- or cytoplasmic proteins extracted from *B. abortus*. Published results indicate significant advantages of the improved tests over those that are most widely available. These improved assays are currently available only at the Animal Health Diagnostic Laboratory, Cornell University [www.diaglab.vet.cornell.edu](http://www.diaglab.vet.cornell.edu) or (607) 253-3900.

### Treatment

There is no current treatment indicated for canine Brucellosis. Antibiotics have not been found to completely eradicate *Brucella canis* from infected

dogs. Dogs that are infected remain chronic carriers and potential focal points for further outbreaks of infertility problems within a kennel. These animals also pose a zoonotic hazard.

### Prevention

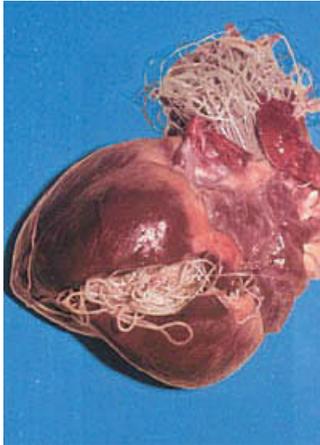
**All positive males and females should not be bred.** Surgical spaying or neutering of these individuals is recommended. All individuals used for breeding should be routinely tested prior to breeding.

### Human Health Hazards

**People can become infected with *Brucella canis*.** People should avoid contact with dead fetuses or the discharge from aborting dogs. Transmission has also occurred from contact with secretions from male dogs. Owners should always be informed to the potential health hazard in keeping any

## Canine Heartworm Disease

By Dr. Stacy Robinson



*“Despite the multiple monthly heartworm preventatives on the market, heartworm disease still remains a problem in North Carolina.”*

*Dirofilaria immitis* is the canine heartworm. Clinical signs of heartworm disease in dogs include dyspnea, coughing, lethargy, anorexia, weight loss, and exercise intolerance. At Rollins Laboratory, we have also had cases in which the dogs died suddenly with no prior clinical signs noted by the owner. Various pathological changes occur in the dog in response to the heartworms, which result in the symptoms forementioned. Villous nodular endoarteritis and pulmonary thromboembolism can occur, increasing pulmonary hypertension and ultimately resulting in right heart failure. Portal fibrosis in the liver may occur secondary to the impaired right heart. Glomerulonephritis often occurs in response to the microfilarial antigens.

Caval Syndrome is one complication of heartworm disease that we have seen recently. In caval syndrome, the heartworms fill the right atrium and caudal vena cava. They can interfere with the function of the tricuspid valve. Clinical signs include sudden onset of anorexia, weakness, hemoglobinuria, anemia, and bilirubinuria.

Heartworm disease can be prevented. Monthly preventatives such as ivermectin, milbemycin oxime, moxidectin, and selamectin are the most commonly used preventatives. It is recommended that puppies be started on preventative

medications no later than 8 weeks of age. In regions where the transmission season is more than half the year, year-round administration of a preventative is recommended to increase compliance especially during the critical transmission time period. As many of these products contain other anthelmintics against intestinal parasites, year-round heartworm preventative administration is also useful in prevention of those infections as well. Adult dogs should be tested before starting them on a heartworm preventative if they could have been infected at least 7 months earlier. In the event that a dog is found positive after the initiation of a heartworm preventative, testing prior to initiation helps to alleviate confusion later about the effectiveness of the preventative.

Despite the multiple monthly heartworm preventatives on the market, heartworm disease still remains a problem in North Carolina. Since January of this year, Rollins Laboratory has had at least 14 canine cases and at least two feline cases in which heartworm disease was determined to be the cause of death. At least four of the heartworm cases in dogs were complicated by caval syndrome. Nine of the 14 canine cases have occurred since April, which may suggest that these heartworm positive dogs are less able to com-

pensate during the warmer weather. In addition to those cases, there are numerous other cases in which heartworms have been found incidentally with some other condition or disease being the actual cause of death. In most of these cases, the dogs and cats were not on heartworm prevention at the time of necropsy or administration of a preventative had been discontinued for a period of time.

While prevention of heartworm disease should be the primary goal of the veterinarian, early detection of heartworm disease should be the second goal in dealing with this disease. Antigen tests are generally more sensitive than microfilaria tests. Timing of your testing is as important as which test you use. Since the prepatent period is six to seven months, if an animal is not given the preventative for a period of time during the transmission season, the animal should be tested six to seven months after the end of the unprotected time period. Annual testing without regard to this prepatent period will not necessarily meet the goal of early detection of infection. Usually annual testing is recommended when there is no known gap in heartworm preventative administration to make sure that the prevention program is working as expected. Puppies do not need to be tested for heart-

## Canine Heartworm Disease (continued)

worms until they are 7 months old.

Melarsomine dihydrochloride is the treatment of choice for killing adult heartworms. The manufacturer's instructions describe a two-injection protocol and a three-injection protocol. The manufacturer recommends that the severity of the heartworm disease and risk of complications during treatment be the determining factors in deciding which protocol to use. The three-injection protocol is recommended by the American Heartworm Society for all dogs no matter what their stage of disease. The three-injection protocol has a worm kill efficacy of 99% and a lower risk of compli-

cations. Many universities, including NCSU College of Veterinary Medicine, use this protocol for most heartworm disease cases.

Another possible method of killing adult worms is by using ivermectin at prophylactic doses monthly. However, this method may require more than two years of continuous administration to completely eliminate the infection. This method may be worth a try in cases in which melarsomine dihydrochloride is declined. However, the American Heartworm Society Guidelines report that a recent study indicated that ivermectin should not be used as an adulticide in symptomatic or very active dogs. If this method of treatment is used, it is recommended that

the veterinarian recheck the dog every four to six months until the worms are dead. At Rollins Laboratory, we recently had a case in which heartworm disease had been previously diagnosed antemortem, and the dog was currently on the monthly chemoprophylaxis agent for treatment of heartworm disease. The owners were surprised at the death of the animal, as the dog was being treated for the disease. If ivermectin is used as an adulticide, the owners should be made aware that while the dogs are being "treated for the disease," the process is slow and the worms continue to cause disease and pathological changes in the animal.

The American Heartworm Society 2005 Guidelines for the Diagnosis, Prevention and Management of Heartworm infection in Dogs and Cats should be accessed and can be printed from their website at [www.heartwormsociety.org](http://www.heartwormsociety.org) for additional and more detailed information about prevention, treatment and testing. Much of the information in this article was taken from the 2005 guidelines, as well as, from a recent talk given by Dr. Teresa DeFrancesco at the North Carolina Small Animal Academy veterinary meeting.

## Equine Leukoencephalomalacia (ELEM, Moldy Corn Disease)

By Dr. Jennifer Haugland

An 11 month old Belgian- Paint cross filly was found wandering blindly into the barn and fences. She became recumbent 3 hours later and was euthanized. She had a large area (5X5 cm) of necrosis in the left cerebral cortex. The rest of the rostral cerebrum was very soft and yellow. Histopathological diagnosis was leukoencephalomalacia. Seven horses were being fed a grain mix that contained corn, oats, alfalfa, molasses, and minerals. The corn was home-grown shelled corn and the feed was ground at the farm. The grain mix had a level of 11.0 PPM of fumonisins. Only 1 other horse developed any neurologic signs and that horse was blind.

ELEM is a sporadic disease of horses that occurs when the horse



consumes corn that is infected with the fungus *Fusarium moniliforme*. This fungus produces Fumonisin mycotoxins and stressed corn appears to produce more mycotoxins. The mycotoxin impregnates the kernel during the growing season and

when eaten in sufficient quantities, the fumonisins damage the brain and liver. It seems that horses need to eat infected corn for 1-2 weeks before clinical signs appear.

Clinical signs of fumonisin toxicity will appear between 10-90 days after consuming the infected corn. The neurological form of the disease is manifested by reduced responsiveness to external stimuli, incoordination, aimless wandering, head pressing, circling, blindness, hyperexcitability, paresis, and eventually recumbency. The onset of clinical signs is usually sudden. Depending on the severity of brain damage, the horses usually die within 24-48 hours after clinical signs start. Horses that do survive will have life-long neurological defects that make them unproductive



## Equine Leukoencephalomalacia (ELEM, Moldy Corn Disease)

(continued)

and possibly dangerous. Another form of ELEM is liver disease. The clinical signs of liver failure are icterus, edema, hemorrhage, weight loss, and elevated liver enzymes and bilirubin levels. Clinical signs of liver failure may also be neurological from hepatic encephalopathy.

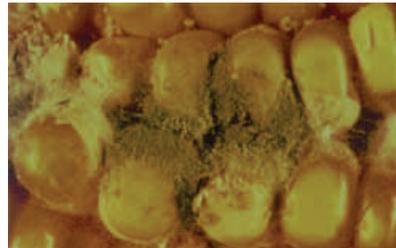
A combined level of Fumonisin B1 and B2, as low as 5 PPM, has been associated with ELEM in horses. Swine are not usually affected until the levels reach 15 PPM. Cattle have shown decreased weight gains and feed refusal with levels of 50 PPM. Horses, poultry, and swine are considered the most sensitive species. Fumonisin is found in all parts of the contaminated corn kernel. Cracking, rolling, steam flaking,

or pelleting processes will not change the toxicity. This toxin is present in the corn at harvest and does not increase or decrease with time or normal storage conditions. The kernels that are infected with *Fusarium* are usually more brittle and will break during harvesting and handling. So fumonisin is usually higher in the corn particles that fall through the screen (fines or screenings). There is some risk in all stressed corn, but the risk is much higher when feeding corn screenings, discolored, shriveled, dirty, and weed-seed infested corn.

If horse owners feed concentrates that contain any corn, they should be aware of the source of that corn. Commercial grain mixes (e.g. Purina) would be

considered the safest source as these companies are considered to have high quality standards. Feed that is milled at a local feed mill or corn that is grown by the owner should be considered risky. The local feed mill should have quality assurance programs that include sorting corn loads by quality, ingredient testing, random or spot-check fumonisin tests, and fanning or screening the corn to remove fines or damaged corn. If there are any questions regarding contaminated corn, contact:

Sheila Jordan, Feed Administrator  
Food and Drug Protection Division  
North Carolina Department of Agriculture and Consumer Services  
1070 Mail Service Center  
Raleigh NC 27699-1070  
Phone (919) 733-7366  
Fax (919) 733-6801



## Interesting Cases

### Canine Herpes Virus

By Dr. Steve Rushton



A 7-week old English Bulldog was presented to the Rollins Laboratory for sudden death with no clinical signs. Four other littermates died within a five day period with similar signs. Gross necropsy revealed numerous ecchymotic hemorrhages within both kidneys and well as within all liver lobes and within the small and large intestine. The lungs were congested and did not fully collapse. Histologic examination revealed random necrosis within the kidney, liver, intestine, and lung with small numbers of eosinophilic, intranuclear inclusions within the remaining epithelial cells.

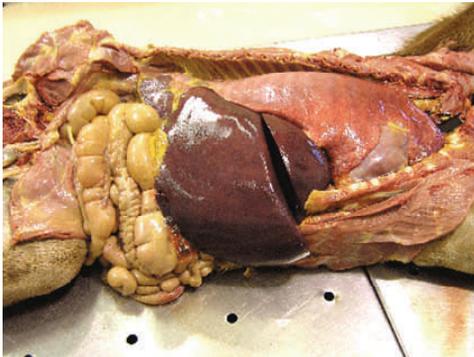
Canine Herpesvirus is generally asymptomatic in dogs infected when older than 1-2 weeks at the time of exposure. Disease caused by CHV is generally fatal in neonatal pups that lack immunity derived from their dams. Neonatal pups may be infected during passage through an infected dam's birth canal or, more commonly, by contact with oronasal secretions of the dam or other dogs in a kennel. Infected littermates, or neighboring dogs that are shedding virus, also serve as sources of infection. Deaths of 1- to 4-week old pups are most common. Pups rarely die if they are 2-3 weeks old at the time of exposure. We have been seeing an unusual correlation lately of English and French Bulldogs pups, greater than 4 weeks old, with Herpesviral deaths here at the Rollins Laboratory.



## Tyzzer's Disease (*Clostridium piliforme*)

By Dr. Steve Rushton

A 10-day old Quarter horse foal was submitted to the Rollins Laboratory for sudden death. Gross necropsy revealed a markedly enlarged liver with myriad, 1-3 mm beige nodules within all liver lobes. Histological examination revealed large multifocal zones of necrosis and neutrophils that effaced around 40% of the hepatic parenchyma. The margins of the necrotic foci had numerous long, thin bacilli that were arranged in "haystacks."



*Clostridium piliforme* (formerly *Bacillus piliformis*) is a gram-negative, intracellular, pleomorphic, spore-forming, motile bacillus that is an environmental pathogen. Pathogenesis involves the oral route of infection with a primary enteric infection that subsequently disseminates to the liver. Tyzzer's disease is not common in horses but has been reported in foals between 7-40 days old. Laboratory animals such as rats, mice, gerbils, hamsters, rabbits, guinea pigs, and rhesus monkeys are frequently affected. The liver is the most common organ affected with occasional involvement of the intestines.

## Comings and Goings

### Arden Laboratory

**Dr. David Drum** has replaced Dr. David Waldrep as veterinary diagnostician at Arden Laboratory. A specialist in small animal medicine, Dr. Drum worked for USDA-APHIS in Pennsylvania before moving to North Carolina. He decided to move partly due to an interest in diagnostics and partly for the milder climate in North Carolina, as opposed to Pennsylvania.



### Rollins Laboratory

**Bacteriology...** **Carol Crabtree** and **Angie Murphy** joined the Bacteriology department at Rollins Laboratory in April. **Carol** has several years of experience as a medical technologist and previously worked at the NC Laboratory of Public Health. **Angie** has a Bachelor's degree in microbiology and previously worked at the Constable Lab in the Food & Drug Protection Division.

**Clerical...** **Amy Dean** joined the Clerical department in January as a transcriptionist. She previously worked for Dean Interior Trim, Inc. **Tamara Seago** joined the Clerical department as a data entry clerk. She is also the backup for Accounts Payable and Accounts Receivable. **Tamara** previously worked for Safelite. **Judy Smith** has resigned her position in Accounts Receivable effective July 1.

## Rollins Laboratory (continued)

**Histopathology...** The histology lab welcomed **Elaine Erwin** as their new medical laboratory assistant on July 1. **Drs. Linda Kooistra** and **Jim Cooley** resigned their positions as pathologists on May 31. **Dr. Kooistra** continues to provide us with avian necropsy service on a part-time basis. **Dr. Cooley** accepted a position in academia with Mississippi State University. Both of these fine pathologists will be sorely missed.

**Necropsy...** **Jessi Hamilton** and **Cat Hefley** have joined Rollins Laboratory as necropsy assistants. **Cat** is a third year veterinary student and **Jessi** is in her second year at NCSU/CVM. They will work in necropsy until classes start in August.

## Veterinary Staff

### Rollins Laboratory (919) 733-3986

Director

- Dr. David Marshall - [David.Marshall@ncmail.net](mailto:David.Marshall@ncmail.net)

Assistant Director

- Dr. Karen Post - [Karen.Post@ncmail.net](mailto:Karen.Post@ncmail.net)

Veterinary Diagnosticians

- Dr. Jennifer Haugland - [Jennifer.Haugland@ncmail.net](mailto:Jennifer.Haugland@ncmail.net)
- Dr. Stacy Robinson - [Stacy.Robinson@ncmail.net](mailto:Stacy.Robinson@ncmail.net)
- Dr. Steve Rushton - [Steve.Rushton@ncmail.net](mailto:Steve.Rushton@ncmail.net)

Veterinary Pathologist

- Dr. Peter Moisan - [Peter.Moisan@ncmail.net](mailto:Peter.Moisan@ncmail.net)

Veterinary Microbiologist

- Dr. Gene Erickson - [Gene.Erickson@ncmail.net](mailto:Gene.Erickson@ncmail.net)

### Arden Laboratory (828) 684-8188

Director

- Dr. Richard Oliver - [Richard.Oliver@ncmail.net](mailto:Richard.Oliver@ncmail.net)

Veterinary Diagnostician

- Dr. David Drum - [David.Drum@ncmail.net](mailto:David.Drum@ncmail.net)

### Elkin Laboratory (336) 526-2499

Director

- Dr. Darrell Rector - [Darrell.Rector@ncmail.net](mailto:Darrell.Rector@ncmail.net)

Veterinary Diagnostician

- Dr. Mark Blakley - [Mark.Blakley@ncmail.net](mailto:Mark.Blakley@ncmail.net)

### Monroe Laboratory (704) 289-6448

Director

- Dr. Kim Hagans - [Kim.Hagans@ncmail.net](mailto:Kim.Hagans@ncmail.net)

Veterinary Diagnostician

- Dr. Reg Ridenhour - [Reg.Ridenhour@ncmail.net](mailto:Reg.Ridenhour@ncmail.net)

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

Director

- Dr. Carlton Rouse - [Carlton.Rouse@ncmail.net](mailto:Carlton.Rouse@ncmail.net)

Veterinary Diagnostician

- Dr. Hope Lucas - [Hope.Lucas@ncmail.net](mailto:Hope.Lucas@ncmail.net)

