Summer 2006

The NCVDLS REPORT

Accredited by the American Association of Veterinary Laboratory Diagnosticians

Veterinary News and Information From North Carolina's Diagnostic Laboratories

From The Director by David T. Marshall, DVM

The hot days of summer this year have again contributed to an active case load over the past two months, as the added stress of the heat and humidity seem to have exacerbated underlying disease conditions in livestock, poultry, and companion animals alike. At this writing, the temperature in Raleigh is approaching 100 degrees Fahrenheit, and the threat of arbovirally transmitted disease looms on the horizon. Laboratory system employees continue to work diligently in providing reliable, cost effective diagnostic services to our veterinary and citizen stakeholders, and will continue to do so in the coming years.

North Carolina Department of Agriculture and Consumer Services

Steve Troxler, Commissioner

Major decisions affecting the laboratory system emerged from this session of the General Assembly, which ratified a budget and concluded their work during the first week of July. Of particular note is the creation of a newly funded Laboratory System Administrator position to oversee all day to day operations and governing of the system. The recruitment and filling of this position will be a challenge due to the advanced skill set necessary for the qualified candidate. In addition, several other veterinary diagnostic laboratories throughout the country are currently addressing vacancies in similar Director positions. Our intent is to advertise nationally with the hopes of securing a candidate that will provide outstanding direction for the system into the future.

The department was successful in securing $250,000 in Capital Improvement funding to renovate a section of the Rollins Chemistry Laboratory into additional molecular BSL2+ testing bench space. The department will attempt to supplement this funding with 2006-2007 Repair and Renovation Funds, bringing the budget for the entire project into the $550,000 range. The expansion will be maximized by USDA grant funding to secure testing equipment. These enhancements will expand the molecular surveillance capabilities for endemic diseases, as well as surge capacity during a foreign or emerging disease event. Expansion of this capability is consistent with our responsibilities as one of the original twelve members of USDA's National Animal Health Laboratory Network (NAHLN).

I have previously addressed in this column an ill-conceived study commissioned by the General Assembly last session to study the feasibility of consolidating the laboratories of the NCDA&CS, State Laboratory of Public Health, State Bureau of Investigation, as well as others. I am pleased to share that the study has been canceled and will no longer be conducted. Thanks to those who expressed to their legislators concern regarding the potential negative impact of this study on veterinary diagnostics.

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Points of Interest...

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Holiday Closings...

Independence Day-July 4

Labor Day-September 4

Our laboratories will be closed on the above listed days.

From the Director (continued)

Congratulations to Ms. Kim Howle, our Spring 2006 Employee of the Quarter. Kim is a Medical Laboratory Technologist III in our Virology section, and in addition to her electron microscopy duties, serves as coordinator for sample referral, biological sample shipping, receiving of results, and communication with clients and practitioners. She is truly a pleasure to work with, and completes every task efficiently and with a smile on her face. Her dependability and focus on customer service benefits the laboratory system well. Congratulations and thanks, Kim.

Several members of the staff will be attending the annual American Association of Veterinary Laboratory Diagnosticians (AAVLD) meeting in October, to be held this year in Minneapolis. One of the special daylong break-out sessions this year will focus on Emergency Preparedness in a laboratory environment. We are in the early stages of developing an emergency preparedness plan, including how to better incorporate the laboratory functions during a highly infectious disease outbreak into the overall response of state and federal health officials. In addition, we have begun the process of training staff in Incident Command System (ICS) protocol. This unified command structure is being used by most human and animal health authorities, including the Centers for Disease Control and the USDA, to administer a coordinated response to disease events. We anticipate this session to be beneficial in further developing our response capabilities.

Again, thanks for your continued support of the NCVDLS. Please feel free to contact me with any comments or concerns.

Regards,

David T. Marshall, DVM

David Marshall, D.V.M.

Director

Diagnostic Laboratory Advisory Committee

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

Client Corner

New Laboratory Submission Forms by **Dr. Jennifer Haugland**

New laboratory submission forms have been developed. There are 3 different 1 page forms; general submission, surgical biopsy/cytology submission, and avian submission. The new forms have been designed to be shorter, more thorough, and more user friendly for the clients and laboratory staff. The first draft of the submission forms were actually tested by 19 veterinary clients and were available online for review. Comments and suggestions were taken into consideration when the final version was completed. The anticipated date to mail the new submission forms to all clients is October 1, 2006. **Once you receive the new submission forms, please discard all other NCVDLS laboratory submission forms.** The new forms will also be available on our website, www.ncvdl.com.

Rollins Institutional Animal Care and Use Committee (IACUC) Established: Governing the Care of Live Animals by **Dr. Marti Hanes**

Rollins Animal Diagnostic Laboratory maintains several animal species in support of the ongoing need for fresh blood used as testing reagents. The blood of chickens, turkeys, sheep and guinea pigs are used for several serology and bacteriology protocols at both the Rollins and Rose Hill laboratories. Dr. David Marshall, acting as Institutional Official, has appointed several members to the newly formed Rollins Institutional Animal Care and Use Committee (Rollins IACUC). The Chairman of the newly formed IACUC, appointed by Dr. Marshall, will be Ms. Anita Quinn-Rudd. In this capacity, she will schedule and conduct meetings, as well as

IACUC Established: Governing the Care of Live Animals (continued)

maintain records pertaining to the use of live animals at Rollins. Additionally, she will assess activities for compliance with Federal regulations and help create local policies and standard operating procedures (SOPs).

The policies addressing day-to-day care of animals maintained for agricultural research is somewhat different than the guidelines used in biomedical research and industry. The IACUC at Rollins will use the guidelines formulated and approved by Federation of Animal Science Societies (FASS) for the care of sheep, turkeys and chickens supported in the Rollins Animal Facility. This guide is commonly called the "Ag Guide". Additionally, the IACUC will use the "Guide for the Care and Use of Laboratory Animals", commonly called the "Guide", as its basis for care of the guinea pigs retained at the same facility.

The composition of the Rollins IACUC committee must follow regulations provided in the USDA's Animal Welfare Act and its amendments. Specifically, the IACUC members will be composed of at least 3 members: the Chairperson; a veterinarian, with training in laboratory animal medicine; and a public member to represent the general public (non-affiliated). The Rollins IACUC will also have the voting representation of the departments of Virology and Serology.

The first IACUC meeting was composed of the following appointed members: Chairperson Ms. Quinn-Rudd; Attending Veterinarian: Dr. Marti Hanes; Kim Howle; Jennifer Pruitt; and the absence of a non-affiliated member (to be announced). Dr. Hanes is a Diplomate of the American College of Laboratory Animals. Both Ms. Howle and Ms. Pruitt will develop the animal use protocols for their respective departments for presentation to the newly formed committee.

While we have a dedicated staff that has always provided humane and excellent care for our on-site animals, the creation of this committee and formalization of an accredited oversight process is a step we are proud to take in the evolution of the laboratory system.

Quality Assurance and Safety Update by **Kathy Schmidt**

Upon becoming the new Director of Rollins a little over a year ago, Dr. Marshall made a huge commitment to improve both the quality and safety of all processes in the veterinary division. This pledge included operations here at Rollins, in the branch laboratories and the field service staff. Shortly thereafter, a QA/Safety Officer position was created to help make this vision a reality and I, Kathy Schmidt, was selected to fill this position last October. This endeavor has brought forward many challenges, yet I am extremely pleased with what we have been able to accomplish within such a brief amount of time.

The initial task consisted of purging all old, obsolete and expired chemicals that had accumulated over the years at Rollins. This cleanup not only made the laboratory a safer environment, but a little more spacious as well. The next task involved creating chemical inventories in which all chemicals within Rollins could be tracked. The supervisors developed chemical inventories for their respective departments and collected MSDS sheets for each chemical listed. These MSDS sheets are now stored alphabetically in each department to assure accessibility in the case of an emergency. An electronic chemical inventory list is also available, which allows for chemicals to be sorted by hazard and location. Copies of MSDS sheets for chemicals used in the field were distributed to the Animal Health Technicians (AHTs) for their reference as well. The Department of Agriculture (DOA) has also recently purchased an online MSDS program that allows electronic access to over a million MSDSs that are available to all of the laboratories. This electronic database can be used to update MSDSs, store electronic versions, as well as serving as a resource for both information on any new chemicals and for general safety information.

Safety Documentation including the Chemical Hygiene Plan, Emergency Response Plan and Hazard Com-

Quality Assurance and Safety Update (continued)

munication Plan has been reviewed, updated and made available to the branch laboratories for their own personalization. A schedule has been established and I have begun conducting the annual trainings necessary to fulfill OSHA requirements. New and replacement safety equipment has been purchased, and SOPs have been established for handling emergency situations. A practice fire drill and tornado drill have been held, and revised evacuation route postings and safety equipment charts have been created.

An evaluation visit has been made to all of the four branch laboratories. Our goal is to assist them in resolving questions and providing them with information to insure compliance with Safety By Objective (SBO) requirements.

An effort has been made to include AHT needs and requests, and I have given them an avenue to have concerns addressed. An information packet has been developed for each of the AHTs, including the forms necessary for work related accidents or injury. An emergency contact information sheet has been developed and completed by each employee, listing the name and contact information in case of accident or injury; this information will be updated annually. We are continuing to identify and provide Field Service personnel with Personal Protective Equipment (PPE) that they request to make their jobs safer as well.

I thoroughly enjoy working with everyone in this department, assisting in improving the working environment and serving as a resource for any concerns they may have. A great deal has been accomplished but much more remains to be done. Everyone truly wants to be safe wherever they are, may that be at home, work or on the road; and, I am happy to continue aiding them with the best information and equipment possible.

Disease Trends

Infectious Laryngotracheitis by **Dr. Tahseen Aziz**

Infectious laryngotracheitis (LT) is an acute, viral respiratory disease of chickens characterized by tracheal moist rales, gasping, coughing, blood-stained expectoration, elevated mortality, and decreased egg production. Laryngotracheitis was first described almost 80 years ago, and it is still a significant threat to the poultry industry today, especially in areas of high poultry production.

As in years past, North Carolina is currently experiencing a significant LT outbreak within the commercial broiler industry. As of July 20, 2006, thirty-eight farms in 8 counties had been confirmed infected through diagnostics conducted at NCVDLS. Other major poultry producing states in the southeast are experiencing similar, if not more economically devastating, outbreaks. Eradication efforts are focusing on enhanced biosecurity, controlled marketing, and vaccination. Laryngotracheitis is a notifiable disease, and must be reported to the office of the State Veterinarian within 24 hours of suspicion.

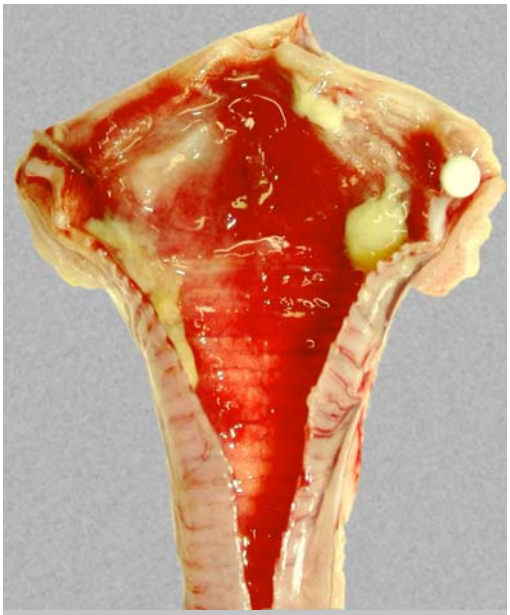

Figure 1: Larynx and trachea of a broiler chicken affected with LT. The mucosal surface has a hemorrhagic appearance. There is a small amount of yellowish exudate.

Laryngotracheitis is caused by a herpesvirus. Only one serotype of LT virus has been recognized, but naturally occurring strains vary in virulence from highly virulent strains that produce high morbidity and mortality to strains of low virulence that produce mild-to-

Infectious Laryngotracheitis (continued)

subclinical infection. Laryngotracheitis occurs primarily in chickens; although, a form of LT has been reported in pheasants. Turkeys are susceptible to experimental challenge, but naturally occurring disease has not yet been described.

Laryngotracheitis is a highly contagious disease. Under field conditions, the virus is transmitted from bird to bird by shedding of virus-containing airborne droplets. These droplets enter the upper respiratory tract through inhalation and/or intra-ocular routes. It has been shown that infectious virus remains in the trachea and tracheal secretion for up to 10 days post infection. Some recovered birds become carriers and intermittently shed the virus for long periods of time, thus becoming a source of infection to other birds. Transmission by people is an important form of spread between farms. Contaminated supplies, equipment and vehicles moved between farms are also potential sources of infection.

"It is important to emphasize that achieving successful diagnosis of LT depends on collecting the right samples from the right birds in an infected flock."

All age groups are susceptible to infection, but young birds appear to be more resistant to natural infection leading to clinical disease. Compared to other viral respiratory diseases, LT spreads slowly in an infected flock. In natural infection, the incubation period (time between exposure and appearance of clinical signs) is about 6 to 12 days. During this period between infection and onset of clinical signs of the disease, infected birds shed the virus, and infective particles may be transferred by people into susceptible flocks. Outbreaks vary widely in severity. Determinant factors in the severity of an outbreak include the virulence of the virus strain involved, and the susceptibility of the flock. It is important to note that the majority of outbreaks occur during the cooler months of late winter and early spring. Outbreaks are rare in summer. It is speculated that the heat may inactivate the LT virus.

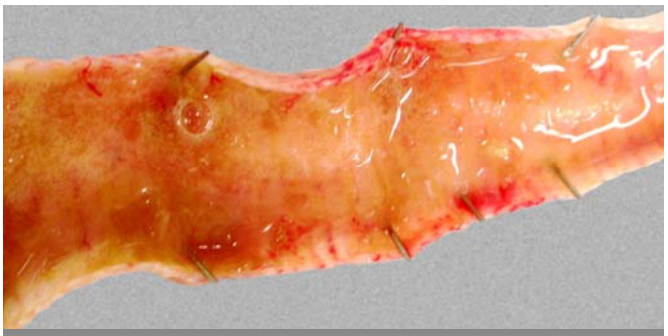

Figure 2: Trachea of a broiler chicken affected with LT. The mucosal surface is covered with excessive amount of mucus. Note the reddening of the mucosa.

The most common clinical signs observed in birds in an infected flock are dyspnea (gasping for air), tracheal moist rales (gargling sounds), coughing, head shaking, and expectoration of blood-stained mucus. Reddening of the conjunctivae, foamy lacrimation, and variable facial swelling around the eyes are seen in some birds. Mortality can vary from 5-70% but usually is in the range of 10-20%. Gross lesions are found primarily in the larynx and trachea. Generally, lesions are more severe in the larynx than in the trachea. There are considerable variations in the severity and type of lesions among birds. The mucosa may have a rough appearance, show variable degrees of yellowish discoloration or reddening, and in some cases may appear hemorrhagic (Figure 1). The mucosa may be covered with excessive mucus that may be blood-tinged (Figure 2). A yellowish or reddish caseous exudate is sometimes seen in the lumen of the larynx and/or trachea. Dead birds may have an occluding pseudomembrane or plug in the trachea. Death may be by suffocation. When the lungs are cut, a white to yellowish exudate may come out of airways (bronchi).

Timely and accurate diagnosis of LT is important. In most cases, clinical signs and gross lesions are distinctive enough to incite suspicion of LT. The laboratory method most commonly used to confirm diagnosis is histopathologic examination of larynx, trachea, eyelid and lung. The decisively characteristic and diagnostic

Infectious Laryngotracheitis (continued)

histopathologic lesion of LT is the formation of syncytial epithelial cells (multinucleated and fused cells) with intranuclear inclusion bodies.

Samples from live birds showing clinical signs are preferred over samples from dead birds. It is imperative to collect samples from several birds, because syncytial cells and intranuclear inclusions are generally present only in the early stages of the disease (1-5 days). Samples from 8 to 10 birds with clinical signs are sufficient in most cases. The larynx and upper third of the trachea should always be included in the samples. The samples should be placed immediately in a container with a sufficient amount of 10% formalin solution.

Virus isolation is another method to confirm the diagnosis of LT. Samples for virus isolation may include larynx, trachea, lung, conjunctiva, or exudate collected by swabbing these sites. Although diagnosis of LT based on the demonstration of inclusion bodies has been shown in published research to be less sensitive than virus isolation, our experience at Rollins Animal Disease Diagnostic Laboratory contradicts this finding. It is important to emphasize that achieving successful histopathologic diagnosis of LT depends on collecting the right samples from the right birds in an infected flock.

BVDV Testing in Camelids by **Dr. Jennifer Haugland**

Bovine Viral Diarrhea Virus (BVDV) was first reported in a stillborn alpaca fetus by the Minnesota Veterinary Diagnostic Laboratory in 2002.[1] Since then, BVDV has been found in other alpaca herds, causing unthriftiness and reproduction losses. Most notably, the syndrome of persistent infection (PI), which is seen in cattle, has also been diagnosed in alpacas.[2-4] As of February 1, 2006, the Alpaca Research Foundation (ARF) reported that 40 PI alpacas were identified in North America.[5] A PI calf is defined as an animal that has detectable virus in serum or buffy coat cells in 2 samples collected at least 21 days apart. This criteria, which has been established for cattle, is currently being used for camelids.[4] Experimental infections and serological testing of herds with viremic alpacas has shown that alpacas and llamas do develop an antibody response after being exposed to an animal (cattle or camelid) that is shedding the virus; although, clinical signs of non-pregnant camelids are generally minimal while the pregnant animals may experience pregnancy losses.[1,3,6] The majority of non-pregnant alpacas exposed to the virus at the Ontario farm where the first PI was identified were subclinically infected, and it was only apparent by antibody testing that they had been infected. A few alpacas that became sick had clinical signs that ranged from having the appearance that their mouths were uncomfortable eating their pelletized supplement for a few days; to being off feed and depressed; to one death from hepatic lipidosis. Many of them, even those subclinically infected, had stress breaks in their fleece. The majority of the clinical disease experienced on this farm included several early pregnancy losses, an aborted fetus that tested positive for BVDV, and the birth of a persistently infected cria.

The discoveries of seroconversion to BVDV in alpacas and llamas, viremia, and the birth of persistently infected crias obviously concerns owners and veterinarians. Subsequently, camelid shows, owners, and breeders are requesting that their animals be tested for the presence of BVDV. Testing recommendations can be found at the diagnostic laboratory websites of Washington State University, Cornell University, University of Guelph, and the Alpaca Research Foundation. All of the universities recommend that polymerase chain reaction (PCR) or viral isolation (VI) testing be done on whole blood samples. At this time, serum is not considered a reliable sample because serum concentrations of virus of camelids may be significantly lower than in cattle. Immunohistochemistry (IHC) on skin biopsies is not a test that is widely recommended for camelids.

**** Please note that at this time NCVDLS can not perform PCR or viral isolation on whole blood samples for BVDV. We are considering adding PCR analysis on whole blood for the future. ****

BVDV Testing in Camelids (continued)

Following are some of the testing recommendations found at the above websites.

1. All aborted and stillborn fetuses, crias that die, and any unexplained deaths should be necropsied and viral isolation of tissues be done for BVDV.
2. All premature, low birth weight, poor doing crias or camelids with unexplained chronic illnesses should be tested by PCR or VI using whole blood samples. Animals less than 12 weeks of age should be tested only by PCR because maternal antibodies may interfere with viral isolation.
3. If an animal is positive for BVDV, the result may represent only an acute infection. A second blood test must be collected 3 weeks later and tested to confirm that the animal is indeed persistently infected. **The second test is essential because the animal will be euthanized on the basis of this testing.**
4. If BVDV has been diagnosed in the herd, the remainder members of the herd should be tested for the presence of the virus. If the entire herd can not be tested due to economic reasons, it is important to test all animals less than 2 years of age and reproductively active females.
5. Any females that may have been exposed to BVDV during pregnancy should have their crias tested soon after birth.
6. If BVDV has not yet been detected in the herd, but you want to know if the herd, especially pregnant females, has been exposed to the virus, you can test serum for antibodies to both BVDV Type 1 and Type 2. **This serum test (SN) can be done at NCVDLS.**

For additional information please review the testing recommendations at each of the above websites and the following references:

1. Goyal S, Bouljihad M, Haugerud S, et al. Isolation of bovine viral diarrhea virus from an alpaca. *J Vet Diagn Invest* 2002;14:523-525.

2. Carr N, Carman S. BVD virus: a newly recognized serious health problem. *Alpacas Magazine* 2005;summer:2-7.

3. Carman S, Carr N, DeLay J, et al. Bovine vial diarrhea virus in alpaca: abortion and persistent infection. *J Vet Diagn Invest* 2005;17:589-593.

4. Mattson D, Baker R, Cantania J, et al. Persistent infection with bovine viral diarrhea virus in an alpaca. *J Am Vet Med Assoc* 2006;228:1762-1765.

5. ARF Research Update. Bovine viral diarrhea virus (BVDV) in camelids. *Alpacas Magazine* 2006; Herdsire:236-238.

6. Wentz P, Belknap E, Brock K, et al. Evaluation of bovine viral diarrhea virus in New World camelids. *J Am Vet Med Assoc* 2003;223:223-228.

Staphylococcus Arthritis in Broiler Breeder Chickens by **Dr. Tahseen Aziz**

Bacterial arthritis is characterized by inflammation of the membrane that lines the joint cavity, with effusion of exudate (fluid) into the cavity of the joint. In many cases, there are also periarthritis (inflammation of tissues around a joint) and tenosynovitis (inflammation of a tendon and the sheath around it). Sporadic cases of bacterial arthritis/tenosynovitis may be found in most broiler breeder chicken flocks. Although a variety of bacteria have been associated with bacterial arthritis/tenosynovitis in broiler breeder chickens, *Staphylococcus aureus* (*S. aureus*) is the bacterium most commonly isolated from affected joints. *Staphylococcus* arthritis can cause economic loss through reduced weight gain, mortality, and culling.

Staphylococci are very common and widespread. They are normal inhabitants of the skin and upper respiratory tract (nasal cavity, mouth, trachea) of healthy chickens, and are ubiquitous in the chicken-house environment. The route by which joints become infected is not always apparent. It is most likely that *S. aureus* reaches the joints via the blood following entry through the skin or the respiratory tract. *Staphylococcus aureus* appears to be a typical opportunistic bacterium; it waits for appropriate conditions or predisposing factors for invasion and initiation of infection. The skin is a natural barrier to microorganisms, but when there is a large or small break in it due to wounds or abrasions, *S. aureus* may invade the subcutaneous tissues (tissues under the skin).

***Staphylococcus* Arthritis in Broiler Breeder Chickens** (continued)

The bacterium may then reach the joints either via the blood stream or through direct spread from the injured site. Under field conditions, environmental factors such as sharp projections and wood splinters in litter may result in skin injuries or abrasions. Feed restriction programs which are practiced in broiler breeder flocks may play an important role in the increased incidence of staphylococcal arthritis in these flocks; minor scratches and abrasions of the skin may occur when birds rush to feeders at feeding times. Thickening of skin of the foot-pads brought on by excessive weight or poor litter conditions may predispose to cracks and ulcerations; these cracks and ulcers on the foot-pads can be routes for infection with *S. aureus.* A high incidence of staphylococcal arthritis may be seen in breeder flocks with aggressive males. One can associate the problem with peck wounds and fighting injuries; also, mating injuries may lead to staphylococcal arthritis in breeding hens. An increased number of cases of staphylococcal arthritis may occur in the flock following a period of severe stress (environmental stress, handling the birds, or other diseases), possibly lowering the resistance of birds to *S. aureus*. Since the bacterium is ubiquitous in the environment, bird-to-bird spread of infection is not of importance.

Arthritis may affect one or more joints at the same time. The most commonly affected joints are intertarsal (hock) and interphalangeal (toe) joints. Early signs include lameness, reluctance to move and/or limping on one leg. Birds with severe bilateral arthritis are usually unable to stand. Mildly affected birds may drop one wing for support during moving. Severely affected birds appear depressed and lose condition gradually due to inability to reach feed and water; they become dehydrated and emaciated and eventually die from starvation. In some recumbent birds, abscesses may be evident in the bodies of thoracic vertebrae with subsequent pressure on the spinal cord. Swelling of the foot, probably caused by infection of joints between toes, is a common manifestation of *S. aureus* infection in some broiler breeder chickens breeder flocks.

Lesions in the affected joints vary in severity. In the early stages and mild cases, lesions may not be apparent externally but examination of the joint cavity reveals that the synovial fluid is increased in amount and opacity. In severe cases, affected joints are swollen and hot with a painful reaction elicited when the affected area is squeezed or manipulated. Incision into affected joints reveals the presence of various amounts of creamy, yellowish thick-fluid in the joint cavity and surrounding tissues. In many cases, there is also involvement of the tendons above and/or below the hock joint.

Diagnosis of staphylococcus arthritis can only be confirmed by the isolation of *S. aureus* from infected joints. A sterile cotton swab should be used to culture the cavity of an affected joint. Because *S. aureus* is normal flora on the skin of birds, one should be careful when opening and swabbing the joint cavity to avoid any contact between the swab and the skin. In staphylococcus arthritis, usually a pure growth of *S. aureus* is obtained from aerobic culture of the swab, especially in the early stages of the disease. However, in advanced or chronic cases, *Staphylococcus* may disappear from the lesion, or may only be present in low numbers. One should keep in mind that arthritis/tenosynovitis could be caused by other disease agents such as *Escherichia coli*, *Pasteurella multocida*, *Mycoplasma synoviae*, and reovirus (viral arthritis). Laboratory testing is necessary to differentiate staphylococcal arthritis from other types of arthritis. Lesions in the tendons must be particularly differentiated from lesions of viral arthritis.

Treatment of staphylococcal arthritis involves the use of antimicrobial medications. Although *S. aureus* is susceptible to many antimicrobials, it is advisable to conduct antimicrobial-susceptibility testing since many isolates are resistant to one or more antimicrobials. In general, *S. aureus* is susceptible to one or more of the following antimicrobials: Penicillin, erythromycin, tetracyclines, lincomycin, sulfonamides, and sulfonamide-ormetoprim. However, it is extremely important to keep in mind that in most cases, treatment with antimicrobials is rarely effective in clearing the arthritis in affected birds, probably due to failure of the drug to reach effective concentrations in the joints. Severely affected birds are unlikely to recover and should be culled.

***Staphylococcus* Arthritis in Broiler Breeder Chickens** (continued)

Sporadic cases of staphylococcal arthritis are probably unavoidable in any broiler breeder flock. Because affected birds usually do not respond to treatment, the medication of an affected flock may not be cost-effective, and therefore, this problem is more effectively prevented than treated. Staphylococcal arthritis is best prevented by practicing good husbandry, management and high standards of hygiene. Attention to details of proper management, as they affect the integrity of the skin, and proper health of the musculoskeletal system can result in effective prevention of this condition. The most important management factor is avoidance of any condition that may cause injuries to the skin and joints, several examples follow:

- Provide sufficient floor space, i.e. avoid high stocking density.
- Provide sufficient feed and water space.
- Ensure that feeders are in good condition with sharp or rough edges, and that they are installed at correct height.
- Eliminate any sharp objects on the floor of houses.
- Use good quality litter that is soft and free of splinters.
- Use good litter management. Eliminate all factors (management and diseases) which increase litter moisture; remove caked litter regularly.
- Avoid crowding by good management practices, particularly good ventilation and dry litter.
- Avoid disturbance of pens; do not disturb the birds more than is absolutely necessary.
- Keep to a minimum the number of times the birds are handled, and be very careful when handling the birds. If at all possible, birds should be handled by the wings. If this is not possible, use both legs. Never handle birds by one leg. Also, birds should be placed on the ground carefully and not thrown down.
- Avoid excessive light intensity to prevent or reduce the problems of fighting and pecking.
- Feed the birds regularly, and make sure that feeding and watering systems are working properly to avoid stress and crowding.
- Beak-trimming should be performed well to reduce the damage of pecking. In males, clipping of the back toes should be performed well to prevent injuring the back of the female during mating.
- Optimize all aspects of management to reduce stress. Management is a broad term that includes many components such as temperature, ventilation, availability of feed and water, litter condition, crowding, etc. Stress can increase the susceptibility of birds to *S. aureus*.
- The use of antimicrobials in the feed may be useful but should not be relied upon to prevent staphylococcal arthritis. Antimicrobial therapy should only be used in cases where good preventative measures have failed.
- Early recognition of the problem is very important. Whenever there are alarming numbers of cases of staphylococcal arthritis in the flock, the poultry veterinarian or farm manager should check all possible factors that could contribute to the problem.

Interesting Cases

***Halicephalobus gingivalis* in a Horse** by **Dr. Kim Hagans, Monroe Branch Laboratory**

A 20-year-old Standardbred gelding had been lame in the right rear leg for 7 days, and then became lame in the right front leg for 2 days. The horse became recumbent, and was euthanized. As this horse was recently purchased, vaccination status was unknown. No other horses on the farm were affected. The referring veterinarian's differentials included Rabies, EEE, WNV, EPM and neoplasia.

At necropsy, the 430 kg gelding was in good body condition. External exam revealed palpable soft tissue edema of the right front fetlock extending to the carpus. No evidence of musculoskeletal trauma was identified. A 2.0x1.0x1.5 cm lobulated white mass was identified in the left ventricular free wall of the heart. A white firm mass measuring 4.0x4.0x2.5 cm was identified in the right renal cortex. Examination of the brain revealed a rough irregular nodular texture on the left side of the corpus callosum and accumulation of debris in the lateral ventricle. The associated cerebral cortex was soft. No abnormalities of the spinal cord were identified upon gross exam.

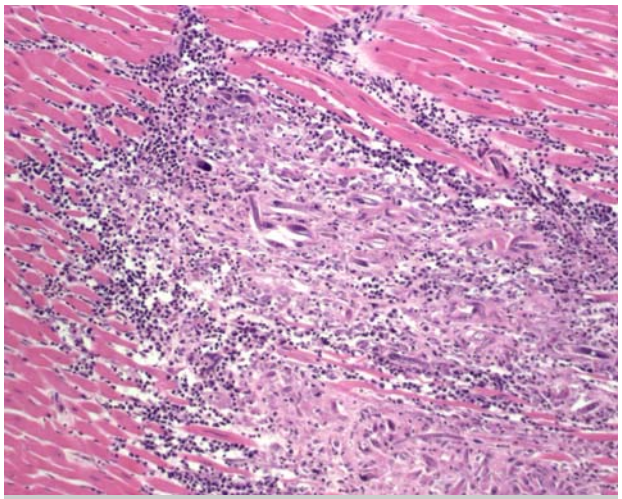

Photo courtesy of Dr. Marti Hanes

Figure1. (Magnification 200x) *H. gingivalis* in the myocardium. The parasite is surrounded by a mixture of fibrin, neutrophils, cellular debris, epithelioid macrophages and multinucleated giant cells that are contiguous with coalescing infiltrates of plasma cells, lymphocytes, and eosinophils in a loose fibrovascular stroma.

Histopathology abnormalities were identified in the cerebellum, heart and kidney.
The abnormalities included:

1. **Cerebellum**: Meningoencephalitis, granulomatous and eosinophilic, multifocal, moderate, with necrosis, and few adult nematodes, etiology consistent with *Halicephalobus gingivalis*.

2. **Heart and Kidney**: Granulomas, multifocal, coalescing, moderate to severe with intralesional nematodes.

3. Additional diagnostic results:
   - Rabies Fluorescent antibody test NEGATIVE
   - West Nile Virus-PCR NEGATIVE
   - Eastern Equine Encephalitis-PCR NEGATIVE

Halicephalobus gingivalis is a free living nematode (order Rhabditida) of soil and decaying organic matter. This facultative parasite causes disease in man and horses. Organisms identified in the tissues are limited to female adults, larvae, and eggs. In the horse, granulomatous lesions are most common in the brain, spinal cord, oral and nasal cavities, lymph nodes, lungs, kidneys, and adrenal glands. Granulomatous lesions are less frequently reported in cutaneous wounds, bone (mandible, maxilla, femur, and nasal bones), heart, stomach, liver, testes, eye, optic nerve, and other organs. The route of infection is thought to be through contaminated wounds, via ingestion, or inhalation with hematogenous dissemination. The nematodes gain access to the brain through hematogenous spread.

***Halicephalobus gingivalis* in a Horse** (continued)

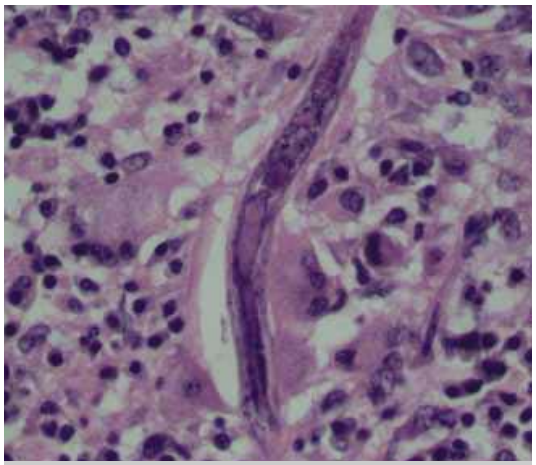

Photo courtesy of Dr. Marti Hanes

Figure 2. Higher Magnification (400x) of the nematode. Note the nematode with a smooth cuticle, pseudocoelom, a tubular digestive tract lined by low cuboidal epithelium, tapered tail, and rhabditiform esophagus with a corpus, isthmus, and bulb.

Clinical findings may include lethargy, ataxia, loss of condition, progressive neurologic signs and multiorgan dysfunction. Laboratory work abnormalities include elevations in indicators of inflammation, elevated creatine kinase and elevated creatinine. Other clinical pathology values may be abnormal depending on the organs involved. Diagnosis is determined by histopathology findings in sections submitted and may be difficult to determine antemortem depending on the locations of the lesions.

While this infection is uncommon, at least 31 cases of *H. gingivalis* infections have been described in published literature. *Halicephalobus gingivalis* appears to have a wide geographic distribution. Recommendations for treatment are unclear due to the lack of knowledge of the organism's susceptibility to anthelmintic drugs and route of entry of the organism. With definitive diagnosis of this parasite from biopsy sections, a poor prognosis is warranted due to the potential for rapid neurological deterioration.

1. Johnson JS, Hibler CP, Tillotson KM, Mason GL. Radiculomeningomyelitis Due to *Halicephalobus gingivalis* in a Horse. *Vet Pathol* 2001; 38:559-561.
2. Brojer JT, Parsons DA, Linder K, Peregrine A, Dobson H. *Halicephalobus gingivalis* encephalomyelitis in a horse. *J Can Vet* 2000; 41: 559-561.

Bovine Viral Diarrhea (BVD) in a Beef Calf

by **Dr. Darrell Rector, Northwestern Branch Laboratory, Elkin**

A two-month-old female Hereford calf was submitted for diagnostic necropsy. Two days previously, the animal was found in lateral recumbency with a loose, bloody stool. The calf had been treated by the owner with tilmicosin, sulfa drugs, fluids and electrolytes. Ten remaining calves of the same age in the herd showed no abnormal clinical signs.

The calf weighed approximately 130 kg and had good body stores of fat. The gastrointestinal tract was essentially empty, containing no ingesta and minimal blood. The intestinal mucosa did not contain grossly evident erosions or ulcers. The liver was dark orange and swollen with rounded edges. The lungs were hemorrhagic and edematous. The urinary bladder was grossly normal, but contained red-tinged urine. There were multiple petechial hemorrhages in the thy-

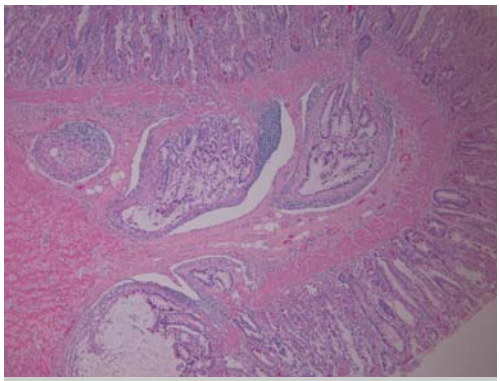

Figure 1: Sections of bovine ileum with severe atrophy and necrosis of the gut-associated lymphoid tissue.

Laboratory test results:

1. Chemistry–Kidney contained 3.4 ppm of copper; normal levels are 4–6 ppm.
2. Bacteriology–*E. coli* was isolated from the lung, lymph node, liver, kidney, and intestine.
3. Molecular diagnostics–PCR testing was negative for *Leptospira* spp.
4. Immunohistochemistry–Positive for BVD virus.

Laboratory Diagnosis: **BVD**

Bovine Viral Diarrhea (BVD) in a Beef Calf (continued)

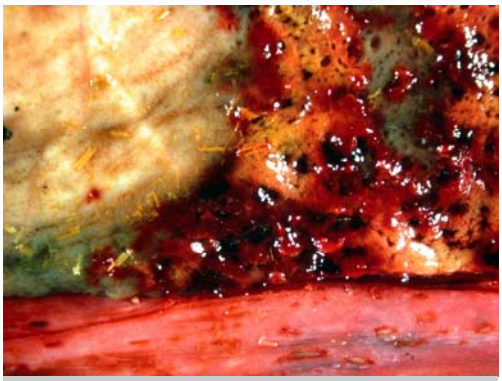

Figure 2: Gross photographs of colonic and esophageal ulcers from an animal with acute mucosal disease (A persistently infected animal with acute BVD infection).

mus, epicardium, and brain. No gross lesions were found in the remaining organ systems. Differential diagnoses at the time of necropsy included: hemolytic anemia (unknown cause), coccidiosis, copper toxicity, leptospirosis, sepsis, and BVD (Type II serotype).

Bovine Viral Diarrhea is an acute, contagious disease of cattle. It is widespread and may be observed in a variety of clinical and pathological manifestations. Most field infections are acute and inapparent or masked by concurrent infections. Clinical signs of uncomplicated post-natal disease are: fever, nasal and ocular discharge, anorexia, diarrhea, lameness, and oral erosions or ulcerations. Some strains (most notably Type II strains) of BVD virus have been shown to cause a hemorrhagic syndrome in immunocompetent animals. The mechanism for this bleeding tendency is the tropism of the virus for the megakaryocytes in the bone marrow. Destruction of these cells leads to thrombocytopenia and subsequent hemorrhagic disease. Affected animals bleed into their eyes and profusely from injection sites. The key to prevention and control is a vaccination program. It is unclear if vaccination can completely protect the animal from the myriad of different strains of this complex virus, but the available vaccines will produce some degree of immunity if properly used. The most appropriate time to vaccinate the breeding female is prior to the breeding season. This timing is in order to prevent infection of naïve breeding cattle by strains of BVD virus that can cause persistent infection (PI) of the fetal calf. These PI calves serve as the main survival means for the BVD virus and the vector for introduction of the virus into post-natal cattle. Vaccination is necessary for prevention of the animal-to-animal transmission of BVD virus.

Employee of the Quarter

Congratulations Kim!

Dr. David Marshall and Employee of the Quarter honoree Kim Howle.

Many congratulations are in order for this spring's Employee of the Quarter, Kim Howle, a Medical Laboratory Technologist in the Rollins Virology Section. Kim is well known for her continuous smile and superior work ethic, as well as being highly organized, conscientious, and dependable. Any task or question given to Kim is completed in a professional and thorough manner. Laboratory clients find Kim to be extensively knowledgeable about where to outsource laboratory specimens when services are not offered in-house. Kim is responsible for packaging and shipping infectious materials for our laboratory system and has completed extensive training in that area. The Federal Aviation Administration (FAA) has commented on her proficiency and she consistently receives excellent performance grades on evaluations. Kim is a tremendous asset to the NCVDLS, and we look forward to her continued success here at Rollins Laboratory! Congratulations Kim Howle!!!!!

Departmental News

MONROE LABORATORY:

Dr. Kim Hagans attended the Fourth Biennial Foreign Animal Disease Training Course in Madison, Wisconsin from July 30 to August 4, 2006.

NORTHWESTERN LABORATORY:

Euretta Herko-Pierce, Medical Laboratory Technician in Serology, will be retiring effective August 31, 2006. She will be missed after her 21 years of service. Her vacancy is being advertised.

ROLLINS LABORATORY:

Bacteriology... Two of the vacant positions within the department have been filled by internal promotions. We would like to congratulate **Karen Surratt** on her acceptance of the Medical Laboratory Technologist II position and **Rob L'Heaureux** for his receiving the Medical Laboratory Technologist I position. **Katie Wilkins** and **Lee Goldbach** have accepted positions as Medical Laboratory Technician and Medical Laboratory Technologist, respectively, effective August 8, 2006.

Histology... The departmental supervisor position has been filled by **Mary Horne**, who also accepted the position as a promotion. Mary's vacancy is in the process of being filled.

Quality Assurance...Anita Quinn, Medical Laboratory Technologist, will officially resign from her position effective August 31, 2006. Anita has accepted a position as a research scientist at Almac Diagnostics and we wish her the best of luck there.

Veterinarians... Drs. Steven Rushton and **Peter Moisan** attended the Southeast Veterinary Pathology Conference on May 20-21, 2006 in Tifton, Georgia. **Dr. Rushton** presented a case of ependymoma in the brain of an alpaca, while **Dr. Moisan** presented a case of *Salmonella dublin* septicemia in a group of dairy calves. The *Salmonella* case was initially coordinated by **Dr. Blakley**, Veterinary Diagnostician, Northwestern Laboratory. **Dr. Marti Hanes** attended the Fourth Biennial Foreign Animal Disease Training Course in Madison, Wisconsin from July 30 to August 4, 2006.

Virology... Effective August 31, 2006, we will sadly be saying goodbye to Medical Laboratory Technologist **Eve Erastus**. Eve has honored the laboratory with 26 years of service. We wish her the best in all her future endeavors. A welcome is extended to **Jean Kennedy**, Medical Laboratory Technologist, who started at Rollins on August 7, 2006.

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Dr. Jennifer Haugland
Dr. Stacy Robinson
Veterinary Pathologists
Dr. Tahseen Abdul-Aziz
Dr. Peter Moisan
Dr. Steven Rushton
Dr. Martha Hanes
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Dr. Reg Ridenhour

Rose Hill Laboratory (910) 289-2635

Director
Dr. Carlton Rouse
Veterinary Diagnostician
Vacant Position