The University of Georgia

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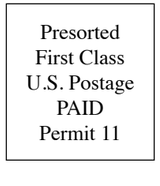
Meet The Faculty :

Dr. Murray Hines

Dr. Hines has just been appointed as the Acting Director of the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) effective April 1. After receiving his veterinary degree in 1980 from the University of Tennessee, he completed his PhD and pathology residency at Louisiana State University in 1991. Prior to his employment at the University of Georgia in January 1994, he was a faculty member in the Department of Comparative Pathology at the University of Miami, School of Medicine. He is a full professor in the Department of Pathology, an ACVP board certified veterinary pathologist, and has served as a diagnostic pathologist at the TVDIL for more than 15 years. He also serves as director of pathology resident training, immunohistochemistry section head, and pathology research resources head. His research interests are Johne's disease and other mycobacterial infections. Dr. Hines has earned a strong national and international reputation for his expertise in Johne's disease research. He is a co-investigator and core leader in the multi-institutional Johne's Disease Integrated Project (JDIP), chairs the JDIP Animal Model Standardization Committee, served on a National Academy of Science committee on Johne's disease, is proceedings editor and section moderator for the International Association for Paratuberculosis, and is the conference coordinator for the Southeastern Veterinary Pathology Conference. He has also served on the editorial board of the *Journal of Veterinary Diagnostic Investigation*, and has been a reviewer for several other veterinary journals.

Dr. Susan Sanchez



Dr. Sanchez received her BS degree in 1986 from the Universidad Complutense in Madrid, Spain and completed her MS at the Royal Veterinary College in London, UK in 1989 and her PhD from the Universidad Complutense and Autonoma in Madrid, Spain in 1990. Prior to her employment at UGA in 1999, she was Director of Surrey Diagnostics, Surrey University, UK. She is an associate professor in the Department of Infectious Diseases, a charter microbiologist by the British Institute of Biology and has served as clinical microbiologist at the Athens Veterinary Diagnostic a laboratory (AVDL) for nearly 10 years. She also serves as the Section Head for Microbiology and Molecular Diagnostics at the AVDL. In this capacity, Dr. Sanchez has greatly expanded AVDL's ability to diagnose multiple bacterial, fungal, and viral diseases by using molecular techniques (PCR tests), thereby improving the turn-around time for test results. Her research interests are antimicrobial resistance mechanisms and their spread as well as the ecology of pathogenic bacteria such as *Salmonella* and *Staphylococcus aureus*. She has earned a strong national and international reputation in both of these fields. In addition to her successful diagnostic and research endeavors, Dr. Sanchez also serves as the Director of the Georgia Veterinary Scholars Program, a highly competitive 12-week paid research program for veterinary students funded by the National Institutes of Health, Merck-Merial and the Veterinary Medical Experimental Station.

The University of Georgia College of Veterinary Medicine
Diagnostic Veterinary Matters
A newsletter from the Georgia Veterinary Diagnostic Laboratories
Volume 2, Issue 1 • Spring 2009

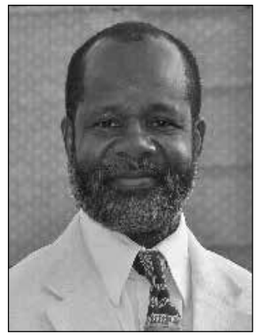
Georgia Veterinary Diagnostic Laboratories
Athens • Tifton

Note from the Directors

We are very pleased to present to you the second volume of our re-established newsletter. Henceforth we plan to publish the newsletter semi-annually – in March and October. As you all know, economic times are tough and the diagnostic laboratories are witnessing the same budget cuts and business income shortfalls that now plague multiple sectors nationwide. Although we do not know how much more this crisis will affect our services, we are pleased to inform you that we do not plan any cuts in diagnostic services during the current fiscal year.

The quality of the results we deliver to you is critically important. Following a recent audit, we are proud to report that both the Athens and Tifton laboratories received full accreditation from the AAVLD until December 2012. We are thankful to our faculty, colleagues, and staff for working hard to earn AAVLD's stamp of approval on our quality systems. In this newsletter and subsequent issues, we will present to you the remarkable individuals that make up the faculty at both laboratories.

As you will read in the following pages, we continue to work hard to improve our services to you by ameliorating the quantity and quality of test systems and the delivery of test results to you. In the face of a serious budget shortfall, these improvements are only possible because of the dedication and hard work of our faculty and staff. Indeed, these trying times are being met by our continuing resolve to cut costs without cutting services, by putting technology to work and by training personnel to operate more efficiently. However, in view of rising operational costs (labor and supplies), we realize that cost-cutting measures alone may not be sufficient to absorb decreased state subsidies and increased operational costs. Therefore, in an effort to maintain the current level of service (which is needed to protect animal health across Georgia), we believe it will soon be necessary to implement a small and selective test fee adjustment. Our test fees were last adjusted in October 2006 and currently recoup only around 30% of the actual cost of our services. A fee adjustment proposal is being submitted to the Commissioner of Agriculture and we ask for your support and understanding. Working together with you, we are confident that we will not only survive the current economic turmoil, but emerge from it stronger and better prepared to continue serving you.



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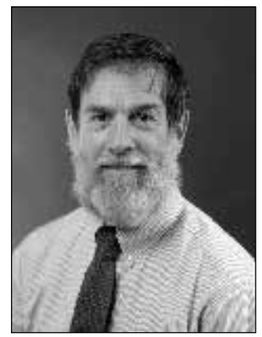

Charles A. Baldwin

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Personnel Highlights

TVDIL Director Dr. Sandy Baldwin retires from UGA

On March 31, 2009, Dr. Charles (Sandy) Baldwin will retire from the University of Georgia. Sandy came to UGA in 1992 after an already impressive career of six years at Cornell University and seven years at Oklahoma State University. He wears many hats: Professor of Virology, Board Certified Virologist, and director of the Tifton Veterinary Diagnostic and Investigational Laboratory.

Since 1992, Sandy has served as the Virologist of the TVDIL. In 2000, he added the role of Director to his duties. Sandy has led the TVDIL in the National Animal Health Laboratory Network for surveillance testing of diseases that threaten the livestock industry, domestic animals, and human health--including classical swine fever, brucellosis, spongiform encephalopathies, eastern equine encephalitis (EEE), West Nile virus, and avian influenza. Sandy was instrumental in investigating EEE in pigs; and in exploring the importance of MIF-A3 in mycobacterial disease. He served on the committee that developed the Georgia poultry plan for screening for highly pathogenic avian influenza. Additionally, Sandy has worked to develop better methods for BVD diagnosis in Georgia cattle herds. During the past four years, he has explored the role of amphibians as indicators of environmental health.

We are likely familiar with Sandy's contributions to expanding the diagnostic capabilities of the TVDIL. He has been instrumental in ensuring that the TVDIL always has the latest diagnostic tests for bovine, equine, porcine, and small animal diseases. Sandy has been instrumental in adding molecular testing to our diagnostic capabilities for diseases such as canine herpes, canine distemper, eastern equine encephalitis, and feline respiratory viruses, among others. He developed a *Brucella canis* cytoplasmic antigen for use in the AGID test offered by the TVDIL (the TVDIL serves as the official *B. canis* testing laboratory in the state of Georgia).

Virology is not the only area in which Sandy has contributed to the TVDIL. He has been instrumental in initiating endocrine testing and protein electrophoresis in the clinical pathology laboratory section, establishing laboratory ranges for these tests, and overseeing the interpretation of test results. Over the past two years, Sandy has been dedicated to making certain that the TVDIL has succeeded in meeting all of the newly established requirements for AAVLD diagnostic laboratory accreditation. He has authored numerous book chapters and published countless manuscripts, ranging from viral diseases in cattle, horses, dogs, cats, pigs and wildlife species, including Florida panthers and amphibians. Additionally, he has served as Chair, Vice Chair and Board Member for the American College of Veterinary Microbiologists.

Sandy has been dedicated to the success of the TVDIL in its role of assisting practitioners in their care of Georgia's animal population. He has always been willing to help in whatever capacity is needed, be it taking in animals on holidays, driving a package to an overnight delivery service for shipment, or taking the time to speak to a group of school children. Sandy and his wife, Bette, will be moving to Florida where they plan to enjoy retirement by spending the winter months with their Florid, grandchildren and their summer months with their New England grandchildren. We thank Sandy for his years of dedicated service to UGA, to Georgia veterinarians, and to safeguarding animal and human health within the state. We wish him a happy and healthy retirement.

AVDL

1. Dr. Cathy Brown, pathologist at the AVDL, won one of the two best article awards of the American Association of Veterinary Laboratory Diagnosticians. The awards, announced at the 51st Annual Conference of the AAVLD in Greensboro, N.C., recognize the best papers published in the Journal of Veterinary Diagnostic Investigation. Dr. Brown's award was for the paper entitled "Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007," published in September 2007. It is noteworthy that this paper was the very first published description of renal disease resulting from melamine and cyanuric acid exposure in pet animals; similar pathology was recently observed in Chinese children exposed to melamine-contaminated baby formula. Congratulations to Dr. Brown and her co-authors for bringing this honor and national recognition to themselves and to the UGA College of Veterinary Medicine.

2. Dr. Angela Ellis, pathologist at the AVDL, completed her PhD from UGA in December 2008.

3. Mr. Charles Hong, Dr. Saliki's graduate student, completed his master's degree from UGA in December 2008. His research focused on canine parvovirus (CPV) and resulted in the first description of CPV type 2c strain in the United States (for more information, see article in J Vet Diagn Invest 2007, vol. 19: 535-539).

Science-at-a-Glance: On the Wilder Side.... Ranavirus in Amphibians

by Debra Miller, DVM, PhD

Ranaviruses have been implicated as one of the causes of worldwide amphibian declines. These viruses can be deadly to amphibians and have caused mass mortality events in North America. Clinical signs of ranavirus infection may be vague but include hemorrhaging of the skin, lethargy, and swelling (edema). Multiple internal organs may be affected; however, the kidney and liver often are targeted, resulting in hemorrhage and necrosis. In some species, mortality can occur as early as within a few days of exposure. Subclinical infections also occur in some (less susceptible) species. Although this pathogen can infect all life stages of amphibians, larvae (tadpoles) are most often affected. Transmission is most commonly through casual contact, cannibalism, or water exposure. Ranaviruses can infect other lower vertebrates as well, including reptiles and fishes, and have been known to cause extensive morbidity and mortality in these groups. Treatment is currently not available; however, Japanese researchers have developed a vaccine for fish that may eventually prove useful in captivity. Additionally, we found that Nolvasan® can be used to inactivate the virus, which is especially important for equipment (e.g., nets, boots) used in the field or in captive facilities.

The University of Georgia has been collaborating with other researchers, veterinarians and biologists to investigate this pathogen. One example is a large scale collaborative study between the UGA Veterinary Diagnostic and Investigational Laboratory and the University of Tennessee Center for Wildlife Health. This study is designed to investigate ranaviruses in free-ranging amphibians. The preliminary findings suggest that anthropogenic stressors (i.e., those that affect water quality) may cause some species (e.g., green frogs) to be more susceptible to the virus. When coupled with metamorphosis, these stressors can prove especially devastating because during metamorphosis, cortisol levels increase and the larval immune system is dismantled so that an adult immune system can be built. During this critical time period, these creatures logically have an increased susceptibility to pathogens. Thus, additional stressors may prove detrimental to wild populations as well as captive ones (e.g., zoological and ranaculture facilities).

Amphibians are used as fish bait, pets, exhibit specimens (e.g., in zoos), food (e.g., frog legs), and research specimens, all of which may result in shipment of live animals. In many cases, animals are either collected from the wild (generally as egg masses or larvae) or obtained from captive facilities. Recent studies suggest that ranavirus strains found in captive amphibian facilities (e.g., bait shops, ranaculture facilities) may be more virulent than wild strains. Because people frequently buy amphibians from captive facilities and accidentally or intentionally release them into the environment, novel and highly virulent strains can be released into natïve amphibian populations. Moreover, humans can inadvertently transport virus particles among watersheds on footwear, clothing, or recreation equipment. Thus, humans potentially contribute to the spread of this pathogen.

Of course ranavirus is not the only pathogen that negatively impacts amphibians. During the past few years, we have investigated die-offs of various amphibian species in captivity and in the wild. In some cases, other pathogens such as *Batrachochytrium dendrobatidis* or *Aeromonas hydrophila* were involved. *Batrachochytrium dendrobatidis* is a fungus that causes chytridiomycosis in amphibians, and has contributed to worldwide amphibian declines and even extinction of some species. *Aeromonas hydrophila* is a bacterium that is commonly associated with infections (some severe) in lower vertebrates, but generally is a secondary invader. It was previously thought to be the primary agent in 'red leg' disease; however, recent evidence suggests that ranaviruses are the primary pathogen of this disease and *A. hydrophila* is secondary (opportunistic).

The good news is that, in May 2008, the World Organization for Animal Health (http://www.oie.int/) listed both ranavirus and chytridiomycosis as reportable diseases. Currently, the OIE is in the process of establishing guidelines for pre-shipment testing and disease surveillance. These listings will serve as valuable tools to unite veterinarians and biologists in attempting to control the spread of these deadly pathogens, and hopefully halt the worldwide amphibian decline.

For more information on ranavirus in amphibians, contact Dr. Debra Miller (229-386-3340) at TVDIL.

Science-at-a-Glance: Issues Associated with *Salmonella* in Pet Foods

Angela Ellis, DVM, PhD & Susan Sanchez, BSc, MSc, PhD, MIBiol, CBiol

Earlier this year, an outbreak of salmonellosis was associated with tainted peanut butter products from the Peanut Corporation of America, and products are still being recalled as this article is being written. Although most of the products involved were for human consumption, some dog biscuits, rawhides, and other pet treats were among the recalled items. In addition, many pet owners admitted to having fed peanut butter crackers and other recalled human foods to their pets. Given recent deaths in animals due to melamine/cyanuric acid and aflatoxin contaminated dog and cat foods, it is understandable that owners are concerned about food recalls. However, *Salmonella*-contaminated pet treats and foods actually pose a risk to owners as well as to their pets.

Although most animals are susceptible to infection with *Salmonella*, infection does not necessarily result in clinical disease. In dogs and cats, a carrier state is far more common than clinical disease. *Salmonella* has been isolated from feces of 1-36% of healthy dogs and 1-18% of healthy cats, and the actual prevalence of infection is probably higher than these estimates. Dogs and cats with healthy immune systems or that are infected with low numbers of organisms typically do not develop clinical signs or will have only mild, transitory illness.

Clinical disease, when it occurs, may include gastroenteritis, bacteremia with or without endotoxemia, abscesses, pyothorax, meningitis, osteomyelitis, cellulitis, mucoid or bloody diarrhea, abortions, stillbirths, or birth of weak puppies/kittens. Fewer than 10% of infected dogs and cats die during the acute phase of *Salmonella* infection. Clinical signs are more often than not associated with stress. Stress may also increase shedding of *Salmonella* in healthy companion animals.

Salmonella may persist in intestinal epithelial cells and lymphoid tissue, and infected animals typically shed bacteria for 3-6 weeks and rarely up to 12 weeks. Phagocytic cells in the spleen, liver, and lymph nodes may harbor bacteria even longer, and animals may become persistently infected. These animals may then start to shed bacteria again following episodes of immunosuppression due to stress, certain drugs, or systemic diseases.

Humans are more susceptible to clinical disease than are dogs and cats, and salmonellosis can be a serious disease in infants, the elderly, and those with poor immune systems. In addition to possible exposure from fecal shedding of *Salmonella* from asymptomatically infected pets, owners may also be exposed to *Salmonella* directly through handling of contaminated food. This occurred previously in 2006-2007 when 70 people became ill with a strain of *Salmonella* (*S. schwarzengrund*) that was traced back to contaminated dry dog food. Earlier outbreaks have been associated with contaminated pig ears and dog treats containing dried beef or seafood.

Pet food is one of the highest risks for pet owners as many of these foods may be contaminated with pathogenic *Salmonella*, but we assume that food is safe and it is very unlikely we engage in hand washing after handling such products.

The Centers for Disease Control and Prevention recommends the following steps to prevent *Salmonella* infections. Persons should wash their hands for at least 20 seconds with warm water and soap immediately after handling dry pet foods, pet treats, and pet supplements, and before preparing human food and/or eating. Infants should be kept away from pet feeding areas. Children under 5 years should be kept away from pet food, treats, or supplements.

The Athens diagnostic laboratory has a *Salmonella* diagnostic test that is more sensitive than culture that can help identify healthy carriers. These newer molecular tests allow for diagnosis and carrier identification within 24 hours. Furthermore, once the organisms are cultured we can type isolates and determine if they are in any way associated with the current human outbreak strains.

Data thus far from the past year does not indicate an increase in isolation of *Salmonella* of any serotype from clinically sick dogs. None of past isolates have been of the serotype *Typhimurium* currently involved in the outbreak. If you have any questions on how to submit samples or determining the most appropriate test, please call Dr. Susan Sanchez, section head of Microbiology and Molecular Diagnostics at the AVDL 706-542-5568.

TVDIL

1. Dr. Murray Hines was appointed acting director of the Tifton Veterinary Diagnostic and Investigational Laboratory, effective April 1, 2009. See page 8 for Dr. Hines's biographical sketch.

2. Dr. Jolade Sansi won the best poster presentation award at the 51st annual meeting of the American Association of Veterinary Laboratory Diagnosticians in Greensboro, N.C., in October 2008. The title of the work presented was "Evaluation of Polymerase Chain Reaction using multiple primer sets for the detection of Leptospira serovars." Co-authors were M. Pence, N. Sheikh, S. Rajeev. Dr. Sansi is a principal lecturer and a Ph.D. candidate at the Federal College of Animal Health and Production Technology, Institute of Agricultural Research and Training, at the University of Ibadan, Nigeria. She is completing her Ph.D. research project on "Prevalence of Leptospira species in domestic and wild animal populations in South Georgia" under the guidance of Dr. Sree Rajeev, a veterinary bacteriologist at TVDIL.

What's New?

1. Coggins submission & resulting online

Our laboratories now offer online Coggins (EIA) certificates through GlobalVetLink Services, a Web-based service, that enables faster reporting. To learn more about GlobalVetLink applications, visit www.globalvetlink.com or call 515-296-0861. GlobalVetLink's Electronic EIA certificates and electronic health certificates are the next steps toward a paperless practice. There are many additional benefits in making Coggins "paperwork" paperless:

a. No mailing. With electronic certificates, state officials automatically receive information via GlobalVetLink's online services.

b. Even faster Coggins results. Results are delivered to you and your clients via the GlobalVetLink online system, real-time, as soon as tests are completed.

c. Eliminate the paper shuffle in your practice. Simply print out your certificate when it's done and provide it to the client - or enter the owner's e-mail address into GlobalVetLink for clients to receive certificates real-time, following test completion.

d. Digital photos. Stop sketching - provide clients an improved form of identification for their horses.

e. Database capabilities. Reuse owner and animal information year after year without re-entry. When the information is entered into GlobalVetLink, there's no need to repeat that action for each certificate.

2. Online Results: By May 2009, you will be able to view, print, and query all your results of submissions to the AVDL dating back to July 2001. All existing clients in our database will be assigned login information, which will be communicated to you. Beta testing of the system is ongoing. If you are interested in participating in the beta test, send an e-mail to athndlab@uga.edu, and a trial account will be set up for you. The Tifton laboratory will be providing access to results of submissions to Tifton at a later date. Online results are the first installment of our electronic services delivery upgrade. Future installments will include: view/pay your invoices and online submission of your paperwork. Online submission of paperwork will help improve our turn around time because the laboratory will receive submission information prior to arrival of samples.

3. New tests:

AVDL: We strive to adapt and evolve with the needs of the farming industry. Newly-introduced species such as alpacas and llamas are becoming increasingly popular. We have developed and validated a new fast PCR test for detection of *Mycoplasma haemolamae*, the causative agent of hemolytic anemia. Tick borne diseases are on the rise in our companion animals. We have validated new molecular tests to cover five additional species. Currently we can test for eight bacterial tick-borne pathogens (*Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi* [Lyme disease agent], *Bartonella* sp., *Bartonella henselae, Ehrlichia* sp., *Ehrlichia canis, Ehrlicia chaffeensis, Ehrlichia ewingii, Rickettsia* sp.).

TDVIL: Virology/Serology has added a new *Neospora caninum* ELISA test that will be batch processed, but run at least once weekly. Clinical Pathology is now performing cortisol testing for low-dose and high-dose dexamethasone suppression, baseline cortisol and ACTH stimulation tests. Please contact Anita Merrill at the TVDIL (229-386-3340) for additional information on cortisol-related testing.

Science-at-a-Glance: Blood and Cytology Submission Guidelines

By Paula M. Krimer, DVM, DVSc, DACVP

General Guidelines and Principles

• **Label slides, tubes, and slide holders:** Use patient name and site. Labeling is necessary to prevent and correct sample errors, and to preserve chain of custody. A key, explained in the submission form, can be used for multiple masses/ specimens.

• **Submit premade smears:** Submit premade unstained smears prepared immediately (<1 hour) after sample collection and quickly air dried. Cells degrade extremely quickly in fluids, and premade smears are necessary to preserve cellular morphology. Contaminating organisms may grow in transit, but premade smears can confirm in vivo sepsis.

• **Send unstained smears:** Stains are standardized in laboratories, so slight differences in staining of cellular components, infectious agents, and foreign materials are better identified with staining done at the laboratory. Some quick stains do not stain granules of mast cells, eosinophils and basophils.

• **Send smears/samples with submission form in safe packaging:** Complete the submission form as completely as possible. Keep slides dry and clean - do not refrigerate or expose to formalin fumes. Package well to prevent breakage.

What to submit

• **Blood**: Properly labeled EDTA blood and labeled unstained direct smears.

• **Body Cavity fluids:** Properly labeled EDTA fluid and labeled unstained direct smears. Sedimented smears are acceptable if identified as such. Collect and reserve a sterile red-top tube and/or culturette swab for potential aerobic and anaerobic culture or other additional tests (EDTA is bacteriostatic).

• **Joint fluids:** EDTA fluid for analysis and labeled unstained direct smears. Collect and refrigerate a culturette swab for potential aerobic and anaerobic culture (EDTA is bacteriostatic). Due to excessive sample thickness, sedimented slides are not useful.

• **Washes:** Unstained, properly labeled direct smears of fluid, sedimented smears, and smears of any floating particulate matter. A fluid analysis is not necessary. The typical blood smear technique with a very small droplet is best for direct and sedimented slides. Pull smears are best for floating particulate matter (transferred with a wooden stick). Note that cells degrade very quickly (15 minutes) because of the low protein content of wash fluid. Collect and reserve a sterile red top sample and/or culturette swab for potential aerobic and anaerobic culture, fungal culture, or PCR testing.

• **Urine:** Unstained, properly labeled, sedimented smears and a sterile red top tube of urine. Note that crystals degrade quickly in urine and cannot be seen on most cytology preparations.

Smear Techniques

Blood Smear Technique – Figure A (ideal with any fluid)
Step 1: Use a needle tip or microhematocrit tube to place a very small drop on a clean slide near the white or frosted end of a properly labeled slide.
Step 2: Place spreader slide above the drop away from the frosted edge, parallel to the slide below at a 30-45° angle.
Step 3: Draw the spreader slide back into the drop, allowing the fluid to spread across the tip and make a line of fluid.
Step 4: Quickly push the spreader slide away from the frosted edge to form a cone-shaped layer of blood/cells. The pointed end is the feathered edge.
Step 5: Air dry quickly (not shown).
Practice Tips: Reduce droplet size if a feathered edge is not created. Change the angle of the spreader slide to adjust the length (a higher/larger angle shortens the smear while a smaller/lower angle lengthens it).

Pull Smear Technique – Figure B (do not use with blood)
Step 1: Hold the slide with a small drop of fluid by the labeled white/frosted end in your non-dominant hand.
Step 2: Place a clean slide, held in your dominant hand, over the fluid perpendicular (at 90°) to the bottom slide.
Step 3: Lower the spreader slide onto the slide with fluid. When the two slides meet, the capillary action will hold the two slides together forming the shape of a cross.
Step 4: While keeping the top slide perpendicular to the bottom slide, gently pull away from the frosted/white labeled end until the top slide is pulled completely off the bottom slide. When you are done, the slides will create the shape of a T (the bottom slide being the standing half and the top slide is the top half of the T).
Step 5: Air dry quickly (not shown).

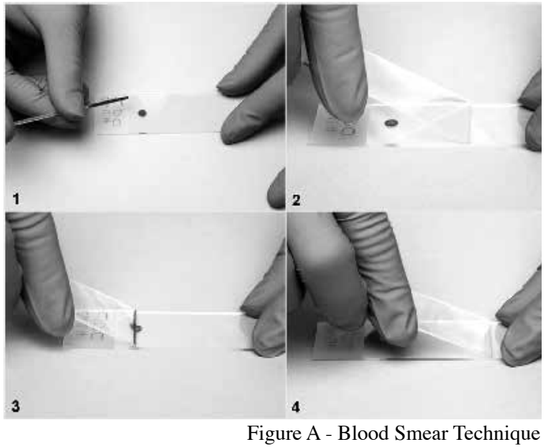

Figure A - Blood Smear Technique

Practice Tips: The capillary action causes significant cell trauma/disruption, and the pull smear technique is only best for thick samples such as particulate materials and transtracheal washes. Reduce droplet size if a feathered edge is not created.

Sediment Smears

Sedimented smears are made after centrifuging very dilute fluids such as body cavity effusions and transtracheal washes. Carefully decant all but about 0.5 ml of the supernatant fluid without disturbing the sediment. Gently mix the sedimented cells with the remaining fluid (do not shake) and make several smears of this sediment mixture using either the previously described Blood Smear or Pull Smear technique. Be sure to label sediment slides for accurate interpretation.

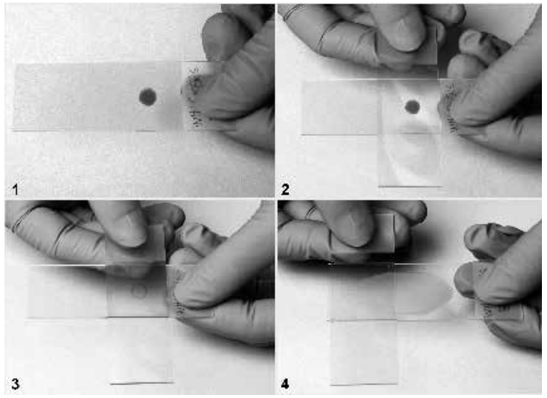

Figure B - Pull Smear Technique

Science-at-a-Glance: *Leptospira* Infection in Animals

by Sree Rajeev BVSc, PhD, DACVM

Leptospirosis is a potentially fatal and zoonotic bacterial disease caused by pathogenic bacterium *Leptospira*. There are over 200 serovars maintained in renal tubules of many domestic and wild animal species.

The organism is maintained in renal tubules without causing disease. The organisms are excreted through urine and can contaminate the environment or result in infection of susceptible animals and humans through contact with urine. Accidental/incidental hosts may be infected by accidental exposure, and infection may result in severe disease.

Leptospira are transmitted through contact with urine of infected animals or contaminated environment. The bacteria will enter the blood stream through the skin and mucosa and invade the tissues. If the host's immune responses are adequate, the infection will be cleared. The organisms may persist in the kidneys and eyes for a period of time.

In acute leptospirosis, the disease has a protean manifestation, which will resemble many other bacterial and viral infections. Acute infections may result in renal and hepatic failure, cardiopulmonary failure, hemorrhage, jaundice, abortion and stillbirth. Recurrent uveitis is common in horses and humans as a result of *Leptospira* infection.

Cattle may drop in milk production, and early embryonic death or abortions are attributed to *Leptospira* infections.

For animals in early stages of infection, heparinized blood and urine for fluorescent antibody (FA) staining and polymerase chain reaction (PCR) are recommended. A paired serum sample is also recommended for serological testing. Antibodies appear five to 10 days after infection. Therefore, samples collected at the initial stage of infection and after 14 days of infection will give an accurate estimate. The microscopic agglutination test (MAT) is available for six serovars of *Leptospira,* which may provide information on the specific serovar involved.

In cattle, when reduced fertility is suspected due to leptospirosis, submit 10 ml of a midstream-collected urine sample for FA and/or PCR. Midstream urine (second or third void urine) collected after the administration of a diuretic such as furosemide (Lasix®) is ideal. Using a combination of PCR and FA is ideal to minimize false negative or false positive results.

Postmortem specimens of internal organs such as liver, kidney and heart blood, collected aseptically soon after death, should be transported immediately to the laboratory. If there is delay in transporting the specimens, samples should stored at 4°C.

FA, PCR and Immunohistochemistry are available for testing tissue specimens. Culture is available, but is not recommended due to low sensitivity resulting from contamination and a prolonged incubation period. All samples for PCR and FA should be shipped overnight with ice packs.

This bacterium is susceptible to a wide variety of antibiotics. Commonly used antibiotics are: penicillin G, amoxicillin, ampicillin, doxycycline and erythromycin. Vaccines containing some serovars are available for prevention.

Humans mainly acquire infection by occupational and recreational exposure to infected animals' urine in a contaminated environment. Fatal disease may develop.

Through research at the TVDIL, we are attempting to isolate strains of *Leptospira* prevalent in animal populations from this geographic region. This is important for developing improved vaccines and control measures. We are also studying the association of *Leptospira* with reduced fertility in cattle. If you have a case or questions concerning leptospirosis, please contact Dr. Sree Rajeev at the TVDIL (229-386-3340).