**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 compromising 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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Sandy has been dedicated to making certain that 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 at the AVDL’s annual meeting 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 melanoma and cyanuric acid in dog 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 melanoma and cyanuric acid exposure in pet animals; similar pathology was recently observed in Chinese children exposed to melanoma-contaminated baby formula. Congratulations to Dr. Brown and her co-authors for an important contribution to 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).

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 stiffness (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 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 raniculture 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 ranaviruses are found in captive amphibian facilities (e.g., bait shops, raniculture 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 native 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-shipping test 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.
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 dietary trends 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 do not develop clinical signs or will only have mild, transitory illness. Clinical disease, when it occurs, may include gastroenteritis, bacteremia with or without endotoxemia, abscesses, pyothorax, menigitis, 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 salmonellosis 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 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 foods. Human food handlers should be kept away from pet feeding areas. Children under 5 years should be kept away from pet foods, 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.
General Guidelines and Principles
• Label slides, tubes, and slide holders: Use patient name and number. 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 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 smear.
• Body Cavity fluids: Properly labeled EDTA fluid and labeled unstained direct smear. 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 smear. 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 the 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).

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 one sedimented smear made from 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.

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 viremia.

Leptospirosis is transmitted through contact with urine of infected animals or contaminated environment. The bacteria will enter the blood stream through the skin and mucous 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 protein 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 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 serovor 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).