The University of Georgia

College of Veterinary Medicine
Veterinary Diagnostic Laboratories
DW Brooks Drive
Athens, GA 30602
Editor: Jerry Saliki

We're on the Web!
www.vet.uga.edu/dlab

Contact Us

Athens
Phone: 706-542-5568
E-mail: athndlab@uga.edu

Tifton
Phone: 229-386-3340

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Accessions
Johnne T. Graves, Administrative Associate
Mary Ann Ethridge, Administrative Assistant

Administration
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Krista A. Mattocks, Associate Accountant
Ken West, Systems Administrator Associate
Debra E. Webb, Administrative Assistant

Clinical Pathology
Anita L. Merrill, Medical Technologist, Laboratory Manager
Tammie T. Vann, Medical Technologist

Electron Microscopy
Deborah A. Blakey, EM Technician

Histopathology
Diane S. Rousey, Laboratory Manager
Kimberly A. Bridges, Histology Technician
Melissa P. Gandy, Histology Technician

Immunohistochemistry
Lisa W. Whittington, Research Technician

Maintenance
Randall O. Gay, Maintenance Foreman
T. Greg Owens, Animal Caretaker/Groundskeeper
Gerald Kendrick, Lead Building Services Worker
Shirley A. Watson, Building Services

Microbiology
Cynthia (Cindy) K. Watson, Laboratory Manager
W. Gail Clifton, Laboratory Technician
Teresa (Teri) T. Register, Medical Technician
Jill U. Johnson, Laboratory Technician
Debra G. King, Laboratory Technician
Candice J. Jackson, Laboratory Technician
Barbara J. Goins, Laboratory Assistant

Necropsy
Brian E. Bourgeois, Laboratory Technician

Toxicology
Teresa M. Cook, Laboratory Technician

Virology/Serology
Dallas R. Ingram, Laboratory Manager
Kristie L. Goins, Laboratory Technician
Michele D. Farrar, Laboratory Technician
Julie Y. Musgrove, Laboratory Technician

The University of Georgia College of Veterinary Medicine

Diagnostic Veterinary Matters

A newsletter from the Georgia Veterinary Diagnostic Laboratories
Volume 1, Issue 1 • Fall 2008

Georgia Veterinary Diagnostic Laboratories
Athens • Tifton

Message From The Directors

We are very pleased to publish a newsletter again after a 5-year hiatus. We thank you for your support of the Georgia Veterinary Diagnostic Laboratory System. During the last few years, we have made great strides, tailoring our services to better meet your needs in these changing times. As you will see in the following pages, numerous new and improved tests have been implemented in both laboratories, and the modernization of our information technology services will soon help you view your test results and securely pay your bills from anywhere using the Internet.

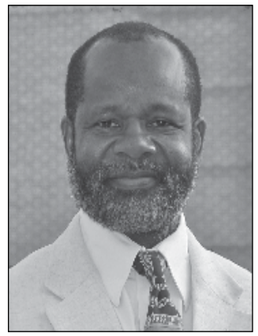
There have been many personnel changes at the laboratories since the last newsletter. Dr. Jerry Saliki has been named the new director of the Athens laboratory. Dr. Doris Miller, who served as director for 20 years, now serves the two laboratories and the University of Georgia's College of Veterinary Medicine as associate director for state government relations, while maintaining her duties as pathologist at the Athens laboratory. Both laboratories have many new faculty and technical/clerical staff and we will be introducing these remarkable individuals in upcoming issues of the newsletter.

Both laboratories continue to monitor our animal population for infectious diseases; some of these are particularly noteworthy as potential zoonotic agents. The Tifton laboratory continues to diagnose Eastern Equine Encephalomyelitis (EEE) virus; the lab has had several cases in horses and has also isolated the virus from two dogs. In the Athens laboratory, methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly detected in domestic animals. To ensure that we continue to produce reliable test results and diagnoses, the laboratories have enhanced our quality-assurance system by producing written documentation of procedures (quality manual and standard operating procedures).

A small dormitory was renovated in the Tifton laboratory to house faculty and students from the UGA Veterinary College who visit Tifton on clinical rotations. In Athens, the building for a tissue digester (an environmentally friendly carcass disposal system) has been completed, and we expect the digester to be installed in 2009.

Again, we want to thank you for your continual support of the Athens and Tifton Veterinary Diagnostic Laboratories. We value the trust that you place in us to assist you in the diagnostic evaluations of your patients.

We pledge to you that we will continue to strive to provide rapid and reliable results on all of your submissions to our laboratories, and will enhance our ability to make this data available to you in a timely manner. Please do not hesitate to call on us with questions or suggestions that can improve our service to you and your clients.


Jeremiah [Jerry] T. Saliki


Charles [Sandy] A. Baldwin

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Personnel Highlights

Athens

Dr. Jerry Saliki was appointed Director of the Athens Veterinary Diagnostic Laboratory, effective August 1, 2008. Dr. Saliki served as Acting Director from August 2007 to July 2008. **Dr. Angela Ellis**, pathologist at the Athens Veterinary Diagnostic Laboratory recently defended her PhD thesis and is expected to be conferred with the degree at the December 2008 graduation. **Dr. Pauline Rakich**, clinical pathologist at the Athens Diagnostic Laboratory, was promoted to the rank of full professor. She also won the College of Veterinary Medicine Outstanding Laboratory Service Award in 2008. **Paula Bartlett**, manager in the Bacteriology & Molecular Diagnostics section, won the 2008 Clinical Services Staff Award. **Sabrina Bailey,** manager of the Accessions section received a 15-year service certificate; she has spent all 15 years with the Athens lab.

Tifton

Dr. Moges Woldemeskel became a board certified pathologist with the American College of Veterinary Pathologists in 2008. **Cindy Watson** received an award for 25 years of service. **Kristie Goins**, **Krista Mattocks** and **Gail Clifton** received awards for 15 years of service; **Kim Bridges** amd **Mary Ann Ethridge** received awards for 5 years of service.

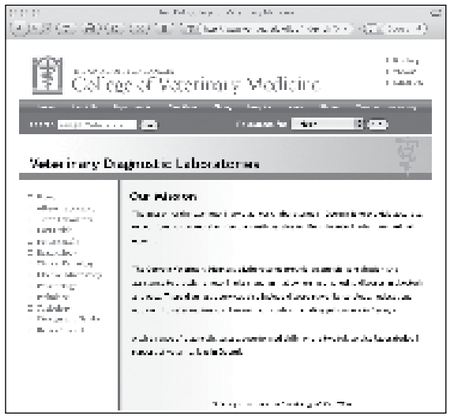
What's New?

Submission forms online in fillable PDF format

You can locate the new submission forms at www.vet.uga.edu/dlab. You can fill in the forms, print, and submit with your samples or fax the forms to the laboratories (Athens: 706-583-0654; Tifton: 229-386-7128).

For Athens, click on Athens Laboratory on the left hand column; the new fillable PDF form, "Athens Diagnostic Lab Submission Form" is at the bottom of the page.

For Tifton, click on Tifton Laboratory and then click on the "Routine Accession Form" link to open the fillable PDF form.



New diagnostic programs:

In order to serve the needs of the Georgia Aquarium and marine mammal stranding/rehabilitation programs, the Athens lab has recently implemented an aquatic animal diagnostic program. The increasing use of laboratory animals in biomedical research at UGA, and at other Georgia-based and regional institutions has also created a need for a laboratory animal diagnostic program, which was implemented at Athens in 2007. These two programs, which are fully paid for by user fees, supplement and enhance our strong diagnostic programs already in place for food and companion animals.

New tests:

Athens: Many new PCR tests have been added: canine respiratory coronavirus, canine distemper, canine parvovirus, *Mycoplasma* spp., *Anaplasma phagocytophilum, Borrelia burgdorferi* (Lyme disease), *Chlamydophyla felis,* Feline herpes virus, *Salmonella enterica, Streptococcus equi, Coronavirus,* papillomavirus, morbillivirus, *Mycobacterium* spp., Tyzzer's, *Mycoplasma pulmonis*, mouse parvovirus (NS-1, MPV, MVM), Rat parvovirus (RPV), mouse polyoma virus (MPV) and *Pasteurella multocida*. Serology tests have been added for canine distemper virus, type 2 BVD virus, marine mammal morbilliviruses, and several laboratory animal pathogens.

Tifton: Real-time PCR tests for *Ranavirus* and for *Batrachochytrium dendrobatidis* (amphibian chytridiomycosis); PCR tests for CDV, EEE, WNV, feline herpesvirus, feline calicivirus, and several bacterial pathogens (*Streptococcus equi*, *Mycoplasma* spp., *Salmonella* spp., *Mycobacterium paratuberculosis*, and *Leptospira* spp.); SN tests for canine herpesvirus and for equine herpesvirus-1. In Clinical Pathology, numerous assays have been added to our repertoire: progesterone, T4, Free, T4, T3, Free T3, cortisol (serum and urine), estradiol, testosterone, vitamin B12, folate, digoxin, phenobarbital, fructosamine, and serum protein electrophoresis.

Meet the Faculty and Staff

FACULTY

Athens Veterinary Diagnostic Laboratory

Jeremiah T. Saliki, DVM, PhD, DACVM
Director & Professor; Virology/Serology Section Head
Viral diseases of domestic & marine mammals, development of diagnostic assays
jsaliki@uga.edu

Uriel Blas-Machado, DVM, PhD, DACVP
Pathologist & Assistant Professor
Farm animals, laboratory animals
ublas@uga.edu

Cathy A. Brown, VMD, PhD, DACVP
Pathologist & Professor; Pathology Section Head
Renal disease in dogs and cats
cathybro@uga.edu

Angela Ellis, DVM, PhD Candidate
Pathologist & Assistant Professor
Pathology of wildlife and exotic species
aellis@uga.edu

Paula M. Krimer, DVM, DVSc, DACVP
Clinical Pathologist & Assistant Professor
Hematology, hepatic disease and coagulation, biochemistry interpretation and cytology
krimerp@uga.edu

Doris M. Miller, DVM, PhD, DACVP
Pathologist & Professor; Associate Director for State Gov't Relations
Reproductive pathology; human/animal bond; forensic pathology; Scrapie program
miller@uga.edu

Pauline M. Rakich, DVM, PhD, DACVP
Pathologist & Professor; Accessions Section Head
Dermatopathology, neoplasms, cytology, clinical pathology
rakich@uga.edu

Susan Sanchez, BSc, MSc, PhD, MIBiol, CBiol
Microbiologist & Associate Professor; Bacteriology & Molecular Diagnostics Section Head
Antibiotic resistance, development of molecular assays, laboratory and aquatic animal diagnostics, infection control.
ssanchez@uga.edu

Tifton Veterinary Diagnostic & Investigational Laboratory

Charles A. Baldwin, DVM, PhD, DACVM
Director & Professor; Virology/Serology Section Head
Viral diseases of animals, development of diagnostic assays
cbaldwin@uga.edu

Murray E. Hines, II, DVM, PhD, DACVP
Pathologist & Professor; Immunohistochemistry Section Head; Director of Resident Training
Mycobacterial diseases of animals, immunohistochemistry, Johne's disease, food animal pathology
mhinesii@uga.edu

Debra L. Miller, DVM, PhD
Pathologist & Associate Professor; Necropsy/Receiving Section Head
Pathology of wildlife and exotic species (especially amphibians, sea turtles and marine mammals)
millerdl@uga.edu

Melvin E. Pence, Jr., DVM, MS, DABVP
Food Animal Health Field Investigator
Beef animal production, Johnes Disease, BVD
mepence@uga.edu

Sree Rajeev, BVSc, PhD, DACVM
Assistant Professor; Microbiology Section Head
Leptospirosis, Johne's disease, bacterial zoonotic disease
srajeev@uga.edu

Eloise L. Styer, PhD
Public Service Associate, Electron Microscopy Head
Virology, ultrastructure, parasites, identification of toxic plants
estyer@uga.edu

Moges Woldemeskel, DVM, PhD, DACVP
Pathologist & Assistant Professor; Histopathology Section Head
Nephropathy in domestic animals, tumor biology (IHC), and respiratory disease in ruminants (infectious)
mwoldem@uga.edu

STAFF

Athens Veterinary Diagnostic Laboratory

Accessions
Sabrina Bailey, Administrative Associate, Accessions Manager
Wendy Counkle, Administrative Assistant
Jennifer Still, Administrative Assistant

Administration
Natasha Liburd, Business Manager
Beverly Arnold, PhD, Quality Manager
Sherry Hawkes, Accountant
Amy Lavender, Data Management Specialist, Receptionist
Shirley Smith, Administrative Assistant

Bacteriology/Molecular Diagnostics
Paula Bartlett, Laboratory Manager
Sarah Bates, Laboratory Technician
Franchesca Brothers, Laboratory Technician
Ingrid Fernandez, Laboratory Technician
Amy McKinney, Laboratory Technician
Ashley Phillips, Laboratory Technician

Pathology
Pauline Groh, Laboratory Manager
Kelley Gibson, Laboratory Technician
William (Mike) Watson, Laboratory Technician

Serology/Virology
Pamela Currin, DVM, Laboratory Manager
Terry Bennett, Laboratory Technician
Jennifer McClain, Laboratory Technician
Gulnaz Shaikh, Laboratory Technician
Rachel Steffens, Laboratory Technician

Notes and Alerts

1. Routine versus extended pathology reports
When submitting specimens to the Athens Lab for histopathology (biopsy, dermatopathology, or mail-in necropsy), the type of report you desire should be checked on the second page, i.e. Routine or Extended Report. The Routine Report includes the diagnosis and a comment that provides information about the diagnosis, such as: adequacy of excision, mitotic index for certain tumors, biologic behavior, differential diagnoses, suggestions for additional diagnostic testing or treatment, and any other useful information. In addition to the diagnosis and comment(s), the Extended Report includes a histologic description of the tissue(s). The higher fee for the Extended Report reflects the additional time required to compose and record the description. The Tifton Lab only completes an extended report.

2. "Necropsy-in-a-bottle" histopathology submissions
Submission of large, often unfixed tissue specimens, including entire plucks, entire necropsies, excised mammary chains, whole hearts, and surgically removed limbs, require additional dissection and, in the case of large bones, additional processing techniques to properly prepare the tissues for processing, cutting, and staining. The AVDL will charge additional fees in unit increments of $10 to offset the additional time required for evaluating, dissecting, and collecting appropriate samples for processing such submissions. Please call and speak to a pathologist when in doubt about the size and thickness of organs to submit.

3. Communicating results:
a. All results will be communicated directly to the veterinary hospital/clinic using the medium (e-mail, phone, fax, or regular mail) you select at the time of submission. We cannot discuss results with animal owners. Please do not instruct owners to contact the laboratories directly.

4. General submission guidelines:
a. Submission forms should be placed in the OUTSIDE BAG only to protect the integrity of the form from breaks, leaks, or spills.
b. Ship all blood samples with ice packs, but the blood should be protected from freezing.
c. Fixed tissue in neutral-buffered 10% formalin.
*Approved containers – Please use only approved containers labeled with a bio-hazard sticker indicating the container is approved for use with 10% formalin. Approved containers can be purchased from most major vet supply companies.
*DO NOT submit formalin-fixed tissue in pill bottles or Ziploc® bags. Improper containers are dangerous to our accessions receiving employees as well as postal and courier employees.
*Place the approved container in doubled plastic Ziploc® bags. Remember to include enough absorbent material in the primary container to absorb all of the liquid in a sample package should there be a leak or break in the primary container. Everything is supposed to be within a secondary container for shipping (see below).
Following this protocol will protect our accessions receiving employees from repeated contact with this hazardous material. It also will alleviate any concerns with FedEx, UPS and the USPS. We greatly appreciate your cooperation.

5. Regulations on shipping samples to veterinary diagnostic laboratories
On February 14, 2003, new federal regulations concerning shipping of animal diagnostic specimens became effective. **These regulations affect all animal samples sent in to veterinary diagnostic laboratories.**

If you ship unfixed tissues, blood or fecal samples through the USPS or by a courier (FedEx, UPS, DHL, etc.) you must be aware of your responsibilities **as the shipper**. The major points of the regulations are summarized below.

1. All animal materials transported for diagnostic purposes are defined as "diagnostic specimens."

2. All diagnostic specimens must be "triple packed" consisting of:
a. The Primary Receptacle (the container that holds the sample) Examples: The tube containing blood or urine, the bag containing the liver sample, the cup containing the fecal sample, the specimen swabs.
b. The Leak-Proof Secondary Packaging (this will contain the primary receptacle in case it breaks.) Examples: A sealed plastic bag around the primary receptacle, a larger bottle or tube that contains the primary receptacle.
You must also have enough absorbent material to cushion the secondary packages and absorb any fluid that leaks. Example: Absorbent paper toweling around the plastic bag containing the bottle or tube. Additional packing paper is used to cushion and fill the outer package.
You must also include enough ice packs to keep the fresh specimens cool during shipment. Specimens not shipped with ice packs or shipped with too few ice packs, often arrive in poor condition, rendering them non-diagnostic.
c. The Outer Package: The normal cardboard box or other shipping container can be used. This must be labeled "Diagnostic Specimen."

3. Your employees who package or ship materials MUST know how to properly package and ship diagnostic specimens. Complete regulations can be found on the EPA Web site: www.epa.gov/fedrgstr/EPA-IMPACT/2001/January/Day-22/i92.htm
A brochure from FedEx can be found at: www.fedex.com/us/services/pdf/PKG_Pointers_Specimens.pdf

4. Unfixed specimens should be shipped overnight or next day air to avoid autolysis. Also, avoid Friday shipments. Specimens collected Friday through Sunday, should be kept refrigerated and shipped overnight or next day air on Monday.

5. Any diagnostic specimens shipped by air will require specific additional packaging as well as documentation. Contact your courier company (FedEx, UPS, DHL, etc.) for more information.

6. Diagnostic specimens transported by private or contract motor carriers are not covered by these regulations. However, safe and appropriate packaging should be used.

Please remember that the onus to comply with these regulations is on the shipper, which is your veterinary clinic.

Working For You

In response to your requests we have implemented the following:

1. The two laboratories now accept credit card payments (Visa, Mastercard, American Express, and Discover).

2. Both laboratories have third-party billing from FedEx and UPS whereby you package your samples, place the appropriate shipping label, check the "Bill third party" box, and provide our billing account numbers. Then call FedEx or UPS for pick up. FedEx and UPS bill us at a discounted rate for the cost of shipping the samples, and we add the cost to your monthly bill. They deliver it to us by noon the following day. The Athens Lab uses both FedEx and UPS; the Tifton Lab uses only FedEx. Call the laboratory at 706-542-5568 for more information.

We are also working to make our web site more user-friendly, and make the information you need easy-to-find such as: submission forms, sample requirements, test turn-around times, and more. Additionally, we are currently working on the following projects, which will be implemented first at Athens and shortly thereafter, at Tifton:

1.**Online results:** Immediate reporting of results for all submitted cases. View them securely and confidentially at your convenience and from any location.

2. **Online invoice:** View current details of your account.

3. **Online credit card payments:** Make secure, hassle-free credit card payments to your diagnostic laboratory account.

We will keep you updated on these and other efforts to modernize our services to you and your clients.

Science-at-a-Glance: Laboratory diagnosis of Johne's disease

by Sree Rajeev, DVM, PhD, DACVM

Johne's disease (JD), caused by Mycobacterium avium subspecies paratuberculosis (MAP), is an economically important disease of ruminants. The disease is characterized by chronic, progressive granulomatous enteritis and affects a wide variety of hosts including cattle, sheep, goats, llamas, alpacas, bison and deer. The U.S. dairy cattle industry estimates an annual economic loss of $220 million due to JD. The presence of MAP in milk, its resistance to pasteurization temperatures, and its possible association with Crohn's disease in humans suggest a significant public health risk.

Rapid and accurate diagnosis of MAP infection is critical for JD control programs. Fecal culture has merit over serological methods because of its higher sensitivity and specificity, and it is the method of choice for the detection and removal of individual infected animals from a herd. The Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL) uses an automated liquid culture system (ESP® para-JEM System, TREK Diagnostic Systems Inc., Cleveland, OH) for culture of MAP. The cost of testing is $20 per sample. Our experience indicates that the sensitivity of this method is much higher than the previous culture method using solid media. The detection rate of this technique is 40–50% more in some heavily infected herds principally because of the higher chance of detecting low shedding animals.

In the MAP diagnostic procedure, fecal samples and tissues go through an extensive decontamination process for 48 hours, and then the samples are incubated in the media in the ESP system. As we obtain positive signal from the system, the samples are taken out and confirmed by acid-fast staining and PCR using a MAP-specific genomic target. Results from heavy shedding animals are obtained before the 25th day of incubation, and final results on all animals can be obtained by about 8 weeks after sample submission as opposed to 16 weeks previously required for incubation on solid media. Direct detection of MAP in fecal samples by PCR is available on request. However, direct detection by PCR is not done routinely due to low sensitivity in detecting low shedding animals. In order to assure our clients with quality services, TVDIL participates in annual proficiency testing for MAP culture offered by the National Veterinary Services Laboratory.

Tips for sample collection and shipping

Collect fresh fecal samples in a screw-capped plastic container and ship them overnight with ice packs. Freezing samples in a household refrigerator is NOT recommended since this may reduce the viability of the organism. If storage is required, it is advisable to store samples in a refrigerator. Herd-level detection of disease can be done either by culturing pooled fecal or environmental samples and by ELISA on serum samples. Pools of 5 fecal samples are recommended. Collection of environmental samples should be performed from high manure concentration areas. However, for the detection and removal of animals, individual fecal cultures must be done. It is important to note that detection and removal of the infected animals and management of the herd to prevent new infections are the only way to control this disease since no effective treatment or vaccines are available.

Science-at-a-Glance: Pet food associated renal disease in dogs and cats

by. Cathy Brown, VMD, PhD, DACVP

Last year, a large outbreak of toxic renal failure attributed to ingestion of melamine/cyanuric acid-containing pet foods occurred in dogs and cats from North America. Between February and June 2007, fourteen animal carcasses submitted to the University of Georgia Athens Veterinary Diagnostic Laboratory (AVDL) were confirmed as having melamine/cyanuric acid associated renal failure.

All 14 animals had clinical and laboratory evidence of uremia, including anorexia, vomiting, lethargy, polyuria, azotemia, and hyperphosphatemia. All animals died or were euthanized because of severe uremia. Histologically, all animals had characteristic lesions of mild distal renal tubular necrosis with distinctive intratubular crystals and variable inflammation and, in more chronic cases, fibrosis.

The first published scientific report describing the clinical, histologic, and toxicologic features of this pet food associated renal disease was based on the findings in these 14 animals (Brown CA, Jeong K-S, Poppenga RH, et al.: 2007, Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J Vet Diagn Invest 19:525–531.). In addition, this report established a link between the 2007 pet food associated nephrotoxicosis and a similar outbreak of renal failure occurring in Asia in 2004, when an estimated 6,000 dogs developed nephrotoxic renal failure. Reports of the 2004 outbreak were limited to a single publication from Korea, a single case from Taiwan (Armed Forces Institute of Pathology Slide Conference), and media reports within the affected countries. The nephrotoxicosis in Asia was originally attributed to mycotoxin contamination of raw materials in a manufacturing plant in Thailand. Through cooperation with pathologists in Korea, archived renal tissues from two Korean cases were reviewed by Dr. Brown in the AVDL, identifying the characteristic lesions of melamine/cyanuric acid toxicosis. Further confirmation was obtained by submitting fixed tissues to the California Animal Health and Food Safety toxicology laboratory for melamine analysis.

The toxic compounds in these outbreaks have been identified as melamine and cyanuric acid, which were present in wheat gluten, rice protein, and corn gluten imported from China and used as pet food ingredients. It is now generally accepted that melamine was intentionally added by suppliers in China to falsely elevate the measured protein content and, hence, the monetary value of these products. The reason for the presence of cyanuric acid is unknown, although it may also have been added intentionally or may have been a by-product of melamine synthesis. While melamine alone does not cause renal failure in toxicology studies in dogs and rodents, melamine and cyanuric acid in combination form insoluble crystals that obstruct and damage renal tubules, thereby causing renal failure.

The contaminated wheat gluten in the 2007 outbreak was a human food–grade product, raising questions about the potential danger to human health. In addition, contaminated gluten and scraps from the pet food industry were incorporated into the feed of food animals (pork, chicken, and fish) processed for human consumption. While the FDA/CFSAN Interim Melamine and Analogues Safety/Risk Assessment indicated that the scenario-driven consumption of meat products was "very unlikely to pose a human health risk," the assessment did not take into account the currently known potent synergistic toxicological effect of melamine with cyanuric acid. Another potential source of human exposure is vegetarian food preparation practices involving high levels of gluten.

The addition of melamine, cyanuric acid, or both to enhance apparent protein content of vegetable concentrates is reportedly commonplace in some regions, and has been occurring at least since 2004. Because chronic interstitial fibrosis is a self-perpetuating process and a common finding in animals with chronic kidney disease, sublethal melamine/cyanuric acid toxicosis could represent an important, previously unrecognized cause of chronic kidney disease in dogs and cats. However, as the characteristic crystals of melamine/cyanuric acid are likely only present for a limited period of time once ingestion of contaminated food ceases, establishing melamine/cyanuric acid as the cause of chronic renal disease would be impossible in most cases.

We are very interested in determining the residual effects of sublethal melamine/cyanuric acid toxicosis. If you treated animals that experienced renal failure in 2007 that were associated with the pet food toxicosis (either confirmed by a history of eating recalled food or the finding of characteristic crystals in urine) and which survived for more than four months after the food was withdrawn, we would appreciate the opportunity to further study these animals. Please contact Dr. Cathy Brown at cathybro@uga.edu or 706-542-5568.

Postscript: The problem of melamine (and cyanuric acid) in food products from China has not yet been resolved. According to a news article (www.usatoday.com/news/health/2008-09-11-tainted-formula_N.htm?csp=23&RM_Exclude=aol) released on 9/11/08, "Chinese newspapers report that some infant formula has been linked to kidney problems and kidney stones in babies in China because the formula contains melamine - the same industrial contaminant from China that poisoned and killed thousands of U.S. dogs and cats last year." There is additional concern that some of this contaminated formula may be distributed to Asian or ethnic markets in the U.S.

Science-at-a-Glance: MRSA in pets: a public health concern?

by Susan Sanchez, BSc, MSc, PhD, MIBiol, CBiol

Staphylococci are Gram-positive, facultative anaerobic cocci. The genus is subdivided into many different species and most of them are host adapted. For the most part, staphylococci are skin commensals of healthy individuals where they can be carried transiently or permanently and only cause disease when there is a breech of epithelial integrity. The most common clinical presentations are skin abscesses. The species of staphylococci most frequently isolated from both healthy and sick dogs and cats is *Staphylococcus intermedius* (SI). *Staphylococcus aureus* (SA), although isolated from animal infections, is not as common as SI. The susceptibility profiles of both staphylococci species is changing, with clear increases in the number of isolates that are multidrug resistant (MDR). Furthermore, the MDR profile of SA isolated from pets mirrors that of human methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. MRSA is on the rise among our pets here in Georgia (see Figure 1). SA has coevolved with people and is a well-adapted commensal of human skin. Hence, SA in pets is viewed by many veterinary epidemiologists as an anthropozoonosis, or a reverse zoonosis.

Community-acquired MRSA (CA-MRSA) in people is currently one of the most common and dreaded pathogens in outpatient care. These organisms are clinically and epidemiologically distinct from health care–associated MRSA (HA-MRSA). The number of people affected by CA-MRSA is increasing. It was estimated that 94,360 invasive MRSA cases occurred in the United States in 2005, with 18,650, or about 1 in 5, of those cases resulting in death. The GA Division of Public Health confirms that there were 13 deaths due to invasive MRSA in 2005, eight of them involving young, previously healthy individuals. Most interestingly, we have seen that this increase in MRSA among people in Georgia mirrored an increase in MRSA in the animal population.

Studies conducted at the Athens Veterinary Diagnostic Laboratory (AVDL) into the epidemiology of MRSA show that CA-MRSA and HA-MRSA are currently circulating among people in our local community with no ties to health care institutions.

These same strains seem to be also established among our pets in which they are causing recalcitrant infections. In parallel, we are seeing in our clinical cases an increase in the numbers of SI that are MDR, including methicillin-resistant (MRSI) and coagulase-negative SA that are also MDR. The AVDL is working in partnership with faculty members from the UGA Department of Genetics and the UGA College of Public Health to elucidate the epidemiology of antibiotic resistance as well as the co-host evolution of MRSA. Most of the resistance genes that are responsible for MDR are located in mobile elements that can be transferred from bacteria to bacteria. The bacteria-acquiring resistance genes in turn emerge as the dominant population with the use of antimicrobials. Intense selection for MRSA in human hospitals is known to occur when fluoroquinolones are used empirically for treatment of infections.

These findings bring up several important questions:

- Are pets reservoirs of MRSA and/or MRSI?
- Is there anything we can do to avoid the spread of MRSA and MRSI in pets?
- Are we aiding in the selection of these MDR strains?

Our continued research at the AVDL is aimed at answering these questions. If you have any questions, please contact Dr. Susan Sanchez at ssanchez@uga.edu.

Figure 1. Distribution of MRSA strains obtained from clinically sick companion animals at the Athens Veterinary Diagnostic Laboratory over the past 7 years.