

# The Georgia Veterinary Diagnostic Laboratories

The Georgia Veterinary Diagnostic Laboratory System (GVDLS) is composed of two world class laboratories, the Athens Veterinary Diagnostic Laboratory (AVDL) and the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL). The GVDLS is administered by the University of Georgia, College of Veterinary Medicine (UGA-CVM) through a contract between the Georgia Department of Agriculture and the UGA Board of Regents. The two laboratories, which occupy two buildings (one in Athens and one in Tifton) with a total of 53,500 sq. ft of laboratory space, are fully accredited by the American Association of Veterinary Laboratory Diagnosticians and are members of the National Animal Health Laboratory network (NAHLN).

The core mission of the two laboratories is to “render diagnostic services relative to the control, diagnosis, treatment, prevention and eradication of diseases for all domestic animals including cattle, sheep, goats, swine, equine, poultry, turkey, fowl, dogs, cats, and any wildlife or zoo animals” in the state of Georgia. Within the UGA-CVM, in addition to providing diagnostic services, the faculty and staff of the laboratories are also engaged in activities that support the research and teaching missions of the University.



## FACULTY AND STAFF

The faculty and staff of the GVDLS are highly educated, dedicated and motivated individuals who work as a team to provide the highest quality of service possible to our clients. Currently, the two laboratories employ 14 faculty and 55 staff. The faculty hold appointments of either Assistant, Associate or Full Professor in the departments of Pathology or Infectious Diseases at the UGA-CVM. The vast majority of our faculty are veterinarians, with PhD degrees in their area of expertise and/or are board certified in either the American College of Veterinary Pathologists or the American College of Veterinary Microbiologists. Individual pathologists have areas of specialization that include renal pathology, dermatopathology, reproductive pathology, laboratory animal pathology, and wildlife diseases. Virtually all of the technical staff at both laboratories hold either AS or BS degrees, and several also hold MS degrees. In addition, several of our technical staff are also certified animal health technicians, certified histotechnicians or certified medical technologists. The faculty and staff of the GVDLS are committed to providing our clients with the most accurate and expedient test results possible.

## QUALITY

Both laboratories are fully accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD), the gold standard for quality of veterinary diagnostic laboratories. Accreditation by AAVLD involves a rigorous on-site audit and evaluation of all aspects of the laboratory operation every 5 years. The AAVLD has adopted standards based on the International Organization of Standards (ISO) 17205 document as their guide to essential requirements for laboratory accreditation.

Both laboratories maintain an on-going quality management program that assures adequate troubleshooting and continuous improvement in the quality of test results. All diagnostic testing is performed using standardized test methods which are crucial to the accurate diagnosis of animal diseases. Test methods are continuously reviewed by our faculty and staff to ensure that our laboratories can provide clients with the most effective testing available. Employees are given internal and external opportunities for training and our highly-skilled laboratory technicians participate regularly in nationally recognized proficiency testing programs. All critical laboratory equipment is frequently checked by trained and experienced personnel to ensure peak performance.

The diagnostic laboratories are committed to providing our clients with timely and accurate test results. We realize that production and maintenance of healthy animals in trading nations worldwide is dependent upon the accurate diagnosis and reporting of animal diseases. We understand the importance of our role in the keeping of happy, healthy companion animals. Therefore, a robust quality management program is essential to providing our clients with the trusted information they need to ensure the well-being of all animals.



## SERVICE

The service mission of the laboratory is fulfilled through the provision of diagnostic testing to support veterinary practitioners and surveillance testing in support of state and national disease control and eradication efforts. The bulk of our work involves routine diagnosis whereby veterinarians statewide submit specimens for testing to help them apply appropriate treatments and preventive measures to animal diseases or provide adequate herd management advice to food animal producers. The major services routinely offered by the diagnostic laboratories include: anatomic pathology, bacteriology, clinical pathology, cytology, histopathology, molecular biology, mycology, serology, virology, electron microscopy and limited toxicology. Specimens received range from a few drops of animal fluids to tissue specimens to entire carcasses submitted for necropsy. The two laboratories combined receive about 85,000 requests for service and conduct

approximately 190,000 diagnostic tests per year. Combined, the two laboratories offer almost 500 different diagnostic tests and services to animal owners through veterinarians.

In recognition of veterinarians' need for timely results, the laboratories have recently developed and deployed approximately 100 nucleic acid (DNA or RNA) based tests including polymerase chain reaction (PCR) and *in-situ* hybridization for many bacterial, fungal, and virus pathogens. Many of these tests are offered as convenient syndrome-based panels and have a 24-hour turn-around time. The AVDL has also developed and deployed several laboratory animal and marine mammal diagnostic tests. We are only the second veterinary laboratory nationwide to offer a full-service laboratory animal diagnostic program and are the only laboratory that offers diagnostic services for several marine mammal infectious diseases (most notably morbilliviruses that have been linked to several recent mass mortality events). The TVDIL is a regional center for diagnostics and research on Johne's disease (ruminant paratuberculosis), a chronic insidious intestinal disease that affects all types of ruminants and has been suggested as a potential cause of Crohn's Disease in humans. The TVDIL also performs all of the GVDLS animal testing for West Nile virus, Eastern Equine Encephalitis virus, Western Equine Encephalitis virus and St. Louis Encephalitis virus. In addition, the TVDIL is one of the few laboratories in the United States that offers PCR testing of amphibian samples for *Ranavirus* and chytrid fungus, both of which are important causes of mortality in amphibians.

While providing routine diagnostic services to veterinary practitioners, our diagnostic laboratories play a major role in passive disease surveillance, essentially serving as "sentinels" for emerging and re-emerging diseases of importance to animal health and public health. Some infectious disease examples of this sentinel role in public health are our monitoring of methicillin resistant staphylococci (MRS) epidemiology as well that of *Salmonella* in both small and large animal populations. Other examples are leptospirosis, brucellosis, and rabies which may infect multiple species of animals and can be easily transmitted to humans.

## THE MELAMINE STORY

The surveillance role of the laboratories is not limited to infectious diseases. For example, in 2007, a large outbreak of toxic renal failure due to the ingestion of melamine/cyanuric acid-containing pet foods occurred in dogs and cats from North America. Based on findings from animals and tissues submitted to the University of Georgia Athens Veterinary Diagnostic Laboratory from Georgia practitioners and confirmed as having melamine/cyanuric acid-associated renal failure, Dr. Cathy Brown and other pathologists from the Athens laboratory published the first scientific report detailing the features of this toxicosis. In addition, this scientific 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. The toxic compounds in these outbreaks 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. In 2008, a similar outbreak of toxic renal disease occurred in an estimated 300,000 infants in China following the deliberate contamination of infant formula with melamine. Information gained by veterinary scientists in their investigations of pet food-associated melamine renal toxicity was used extensively by the human medical community in their treatment of renal disease in children due to consumption of melamine-contaminated infant formula.

In addition, the surveillance mission of the GVDLS laboratories is enhanced through our participation in state and federal government sponsored active surveillance programs. The major program diseases include: bovine spongiform encephalopathy (BSE; mad cow disease), scrapie, bird flu, swine flu and swine pseudorabies. Mad cow disease was first detected in the US in December 2003 and a surveillance program was established in 2004. The AVDL is currently one of only six national laboratories that conduct routine surveillance testing for BSE. AVDL participation in this program resulted in the detection of the last known US case of mad cow disease in March 2006. Surveillance testing for bird and swine flu is conducted at both labs under a fee-for-service contract with the USDA.

## RESEARCH

Research activities in the diagnostic laboratories involve applied research. Some recently completed and ongoing research projects involve leptospirosis, Johne's disease, canine parvovirus, MRSA, salmonellosis, bovine enterovirus, *Trypanosoma*, and Lyme disease.



Dr. Susan Sanchez

Dr. Susan Sanchez is involved in the monitoring and epidemiological observation of methicillin resistant *Staphylococcus aureus* (MRSA) in animals and how it relates to human health. This bacteria is a growing problem in people and other animals. MRSA in the US has shifted in the past 8 years from being an infrequent hospital-acquired infection to an infection that is now spreading through the community, being routinely encountered at gymnasiums and schools, disproportionately affecting our young, elderly, and the financially disadvantaged. Most interestingly, our investigations have shown that this increase in MRSA among people in Georgia was mirrored by an increase in MRSA in its animal population. Identifying differences beyond known virulence factors will allow better estimation of the evolutionary and epidemiological history of these strains, and may also uncover new virulence factors that act specifically in non-human hosts. *Salmonella* accounts for an estimated 1.4 million illnesses, resulting in 16,000 hospitalizations, and 582 deaths in the United States each year. The incidence of salmonellosis within the U.S. differs from state to state and within each state. These differences cannot be explained entirely by differences in population density, cultural/ethnic customs, or food-distribution networks. These regional differences in disease incidence are also reflected in *Salmonella* serovar distribution. We do not know or understand what might explain this geographic scattering of *Salmonella* infection in the US part of the answer lies in identifying alternate reservoirs, such as pets. A better understanding of the transmission dynamics of *Salmonella* in pets will help us understand the role of these unique companions, in the distribution dynamics of *Salmonella* and human illnesses.

Several of the TVDIL faculty led by Dr. Murray E. Hines II have two ongoing research projects on Johne's Disease totaling approximately \$600,000.00 in research funds obtained from the Johne's Disease Integrated Project (JDIP) and branches of the US Department of Agriculture (USDA-NIFA and USDA-APHIS). Johne's Disease is caused by the bacterium *Mycobacterium avium subsp. paratuberculosis* (*MAP*) and causes a chronic insidious intestinal disease of all ruminant species with no effective treatment currently available. Current Johne's disease diagnostic tests and vaccines lack sufficient efficacy, and improved diagnostic tests and vaccines are badly needed. One project involves the creation of a large well-characterized sample archive from dairy cattle containing both samples from confirmed infected and non-infected dairy cattle. This large sample archive will be used to compare currently available commercial and new experimental diagnostic tests including ELISA serological tests, multiple fecal culture methods and multiple PCR tests to determine the best and most economical tests for the diagnosis of Johne's disease in cattle. The second larger project is a two year Johne's disease vaccine efficacy study which is a part of a larger three phase project. Phases I and II of this larger project performed at other institutions involved the evaluation of over 20 experimental Johne's vaccines in a cell based system (Phase I) and in mice (Phase II) to determine the better performing vaccines in those systems. The TVDIL is performing the Phase III study where the 5 best performing experimental vaccines from Phases I and II will be evaluated in a goat challenge model. Goats and cattle are both commonly infected with *MAP*, but the smaller size and faster course of the disease in goats makes them the preferred animal model for Johne's disease vaccine and challenge studies. In this study, groups of goats will be vaccinated with the experimental and control vaccines, then later challenged with a wild-type strain of *MAP* and followed for up to 14 months using a wide variety of diagnostic tests, cultures and specimen evaluation to determine which vaccine(s) are best able to prevent or reduce the incidence and severity of Johne's Disease, and the associated economic losses to producers.



Dr. Murray E. Hines II

Dr. Sree Rajeev's primary research area at the TVDIL is on the diagnosis and prevention of leptospirosis in animals and humans. Leptospirosis is a major problem in the cattle industry due to its effect on reproductive performance. This is one of the top concerns for dairy producers as *Leptospira* infection is insidious and can cause cumulative economic loss to the industry. In other domestic animals and humans, leptospirosis can be a life threatening disease. In humans leptospirosis is an under-recognized, neglected and life threatening zoonotic disease with major global health impact. It has emerged as a major slum health problem in developing countries where one billion of the world's population lives. Dr. Rajeev has completed one project on "Leptospira infection and its role in infertility in dairy cows" and is working on another project on "Isolation and characterization of *Leptospira* strains infecting cattle" both funded by Southeastern Milk check off group. The long range goals of Dr. Rajeev's research are to develop strategies for early and accurate diagnosis of both human and animal leptospirosis, develop a suitable animal model for testing vaccines, and to develop preventive strategies so that complications, mortality and economic loss due to leptospirosis can be minimized.

Dr. Sheela Ramamoorthy at the TVDIL has funded research projects to develop improved diagnostics and vaccines for swine respiratory diseases. Porcine circovirus2 (PCV2) is an economically important swine disease. She has made important contributions to understanding the molecular pathogenesis and development of updated vaccines against PCV2 which are in the process of being commercialized. Current efforts are focused on addressing an urgent producer need for a vaccine that can differentiate between vaccinated and infected animals. Dr. Ramamoorthy has recently developed an advanced multiplex technology for the simultaneous detection of antibodies to more than one swine pathogen, resulting in higher testing efficiency and cost-saving. The 2009 pandemic H1N1 (pH1N1) virus (swine influenza) had a major impact on the swine industry health care systems and all over the world. Currently, only PCR based methods are available for the detection of the virus. Using sophisticated differential epitope analysis techniques, we are in the process of developing user-friendly and less expensive serological methods for the specific detection and differentiation of the pH1N1 virus from other circulating H1 influenza viruses.



Dr. Moges Woldemeskel

Dr. Moges Woldemeskel's research at the TVDIL focuses on the role of mast cells in tumor angiogenesis and gastrointestinal (GIT) infection in domestic animals. His work mainly focuses on tumor angiogenesis, progression and invasion mediated by mast cells. Furthermore, the research also strives to determine the role of mast cells in gastrointestinal inflammation. The results of his investigation to date have shown that mast cells are associated with angiogenesis in canine cutaneous hemangioma, hemangiosarcoma, mammary adenoma and adenocarcinoma, and suggests that mast cells play an important role in neovascularization and tumor progression in these neoplasms. Additionally, an important role played by mast cells in GIT inflammation in dogs has been documented. Dr. Woldemeskel's study is delineating the significance of mast cells in correlation with increased risk of metastasis and prognosis of associated neoplasms and their role in GIT inflammation, to ultimately develop treatment and control strategies which would target mast cells, their enzymes and associated cytokines.

Dr. Marcia Ilha with the assistance of other TVDIL faculty studied the occurrence of *Bovine viral diarrhea virus* (BVDV) in white-tailed deer (WTD) in the state of Georgia. Bovine Viral Diarrhea is a subclinical to fatal viral disease that causes marked economic losses to the cattle industry. Experimental studies indicated that BVDV can be transferred back-and-forth between cattle and WTD and amongst WTD. Surveys for BVDV in other states have showed extremely low prevalences of natural infection in WTD (less than 1%). From September to December of 2010, 367 samples of ear from hunter-harvested free ranging WTD from 37 counties in Georgia were tested for BVDV. Four samples resulted in suspect samples by either the antigen ELISA test (3 samples) or RT-PCR test (1 sample). However, none of these samples were positive in both tests and in other tests used (virus isolation and IHC). Even though a few of the samples resulted in suspect for BVDV, the presence of the virus within this deer population could not be further confirmed. Although the results of this preliminary study may not support the hypothesis that WTD could be a potential reservoir for BVDV in the state of Georgia, low prevalence of this disease in WTD in Georgia is still a possibility.

Dr. Blas-Machado's research at AVDL involves Bovine enterovirus (BEV), a picornavirus which consists of small (18–30 nm), non-enveloped viruses with an icosahedral capsid that encloses a single copy of positive-sense RNA genome. Bovine enterovirus is in the genus Enterovirus, along with poliovirus, human enterovirus, coxsackieviruses, swine vesicular disease virus, echovirus 11, and others. Despite the large volume of information available on other enteroviruses, very little documentation exists on the pathogenesis of BEV infections in cattle or on its prevalence in North America. Several case reports in the 1950s and 1970s document the isolation of BEV from feces and various tissues from apparently healthy animals or from animals with clinical signs that ranged from mild to moderate diarrhea to reproductive disease. However, these older reports are difficult to interpret as they relied solely on serological assays or had identified more than one infectious agent. Recently, in the first report of BEV in more than 20 years, BEV-1 was isolated from a 2 year-old pregnant Aberdeen Angus in Oklahoma, USA, with fatal enteric disease. Faculty members at the Athens Veterinary Diagnostic Laboratory conducting research on this virus isolate have recently published a report about BEV-1. This manuscript described the lesions associated with infection in animals experimentally infected with BEV-1 and postulated about its pathogenesis in cattle. Obtaining knowledge about the susceptibility of cattle to challenge, the pathology associated with infection, and the prevalence of BEV-1 infection in herds would be essential to the understanding of infection and disease in cattle.



Dr. Uriel Blas-Machado



Dr. Paula Krimer

Dr. Paula Krimer developed and characterized a canine model of Lyme disease for an international pharmaceutical company. Lyme disease is a tick-borne disease that incidentally affects both dogs and humans. The disease is caused by *Borrelia burgdorferi*, a small gram-negative spirochete bacterium that is transmitted by Ixodes ticks. The comprehensive study required the participation of all aspects of the AVDL services, from pathology to PCR to culture and clinical pathology, and resulted in two published research papers, including a paper on neuroborreliosis. This research model continues to be used in the development of vaccines to prevent canine Lyme disease.

Dr. Ellis of the AVDL participates in collaborative research projects whose goal is to elucidate the infection dynamics and potential reservoirs of *Trypanosoma cruzi*, which can cause a potentially fatal myocarditis in domestic animals and is the cause of Chagas disease in humans. Better understanding of the ecology of this disease could lead to better methods of prevention and control. In addition, Dr. Ellis provides pathological support for research projects involving the genetics of mammary and intestinal cancers in dogs. It is hoped that better characterization of these diseases on a molecular or genetic level will help to identify early events in carcinogenesis and/or targets for therapeutic intervention.

Dr. Saliki at the AVDL recently completed a Merial-sponsored study on canine parvovirus (CPV). In recent years, a new sub-type of CPV (type 2c) has arisen and become prevalent in many states. The goal of the project was to study the epidemiology of CPV-2c and to characterize the subtypes of CPV in current circulation with a view to identifying a type 2c virus that could serve as a candidate for the next generation of CPV vaccines. Indeed, given the rapid evolution of the virus, with new subtypes continuing to emerge, it is essential that CPV vaccine formulations are efficacious against prevalent CPV subtypes. The project identified a novel mutation in the newly emerged CPV-2c and also generated two virus strains that are potential candidates for future CPV vaccines.



Dr. Jeremiah Saliki

## A UNIQUE, SYNERGISTIC RELATIONSHIP WITH THE COLLEGE OF VETERINARY MEDICINE AND UGA

The relationship of the two diagnostic laboratories with the CVM is more than just administrative. Few state veterinary diagnostic laboratories in the United States share a relationship with a veterinary school and a major land grant university as close as the one we have in Georgia. The synergies related to this relationship are numerous. For example, the AVDL shares necropsy facilities, faculty and support laboratories with the CVM. This sharing of resources significantly lowers the cost of operations by reducing redundancies in facilities and staffing. It also provides DVM students, graduate students and pathology residents training by a greater variety of pathologists and exposure to a wider variety and larger number of animal species and disease conditions than they would have at most other veterinary colleges. At the AVDL, pathologists are directly involved in the training of anatomic pathology residents on the necropsy floor. They also serve the teaching mission of the CVM as instructors in anatomic and clinical pathology graduate courses, by active participation in seminar courses and journal clubs, and by organizing microscopic rounds on specific topics for pathology residents as well as other veterinary specialties such as dermatopathology and ocular pathology. The TVDIL serves as a base of operations for the CVM in South Georgia. A production animal veterinarian assigned to the laboratory works with clinical rotations of DVM students interested in large animal/production medicine and the laboratory has bunking facilities to support these rotations. Pathology residents, graduate students and DVM students profit from the experience and mentoring provided by the veterinary pathologists assigned to the two diagnostic labs, greatly enhancing the teaching mission of the CVM. The Diagnostic laboratories benefit from the sharing of resources with the CVM and the access to the in-depth expertise and cutting edge technology within the CVM and UGA.

## CHALLENGES

Historically, the two veterinary diagnostic laboratories were fully funded by the State of Georgia through a legislative line item in the Department of Agriculture's budget. However, state financial difficulties in the early 90s led to the institution of user fees to supplement operational budget shortfalls. The current economic crisis has resulted in drastic budget cuts totaling 21.5% in the last 3 fiscal years. These cuts, which exceed the state's average cut, coupled with the steady increase in the cost of laboratory supplies and equipment, have resulted in some service reductions and risk compromising the ability of the laboratories to continue playing their vital role in animal disease surveillance and contributing to the economic well-being and public health in Georgia. These budget reductions notwithstanding, the laboratories continue to offer a wide variety of services and continue generating timely and reliable test results, thanks to a highly trained cadre of workers, coupled with increased efficiency of operations.