MES 2011

Science in Service to Animals

166

The Georgia Veterinary Diagnostic Laboratories 35th Annual Report





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One of the important roles of the Georgia Veterinary Diagnostic Laboratory System is surveillance for emerging and reemerging diseases of importance to animal health. Among these diseases is leptospirosis, brucellosis, and rabies which may infect multiple species of animals and can be easily transmitted to humans. Our cover symbolically highlights the Laboratories' role as sentinel for one of these diseases, leptospirosis. Cover illustration by Brad Gilleland.

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The key to improved animal well-being is animal health. The key to improved animal health is veterinary research.





OVERVIEW, MISSION, & OBJECTIVES

The VMES mission is to coordinate research on animal disease problems of present and potential concern to Georgia's livestock and poultry industries.

The Veterinary Medical Experiment Station (VMES) was established as a budgetary entity by the state legislature in July 1976 following approval by the University of Georgia Board of Regents in 1973.

Specific VMES objectives are:

- To improve the health and productivity of domestic livestock, poultry, fish, and other income-producing animals and wildlife through research;
- To assist in preventing disease epidemics by providing laboratory resources and highly skilled scientific personnel;
- To assist in protecting human health through the control of animal diseases transmissible to man;
- To improve the health of companion animals, which serve to enrich the lives of humankind;
- To train new scientists in animal health research in order to provide continuity and growth in this vital area of veterinary medicine.

The Veterinary Medical Experiment Station is committed to enhancing animal production, profitability, and well-being by improving animal health.

All programs and activities of the Veterinary Medical Experiment Station are conducted without regard to race, color, national origin, age, sex, or handicap.



From the Director

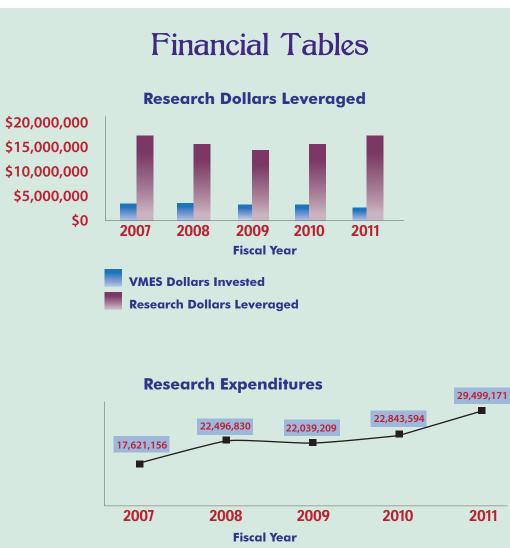
I often refer to the importance of basic and applied veterinary research to animal and human health in Georgia. I have also pointed out the challenges we have faced over the last three years resulting from severe budget cuts to state-supported research. Ironically, although the direct benefits of applied veterinary research to animals and the State's agricultural industries are most evident in the Georgia Veterinary Diagnostic Laboratory System (GVDLS), this unit has been one of the hardest hit by funding cuts (21.5% total reduction in operating budget over the last 3 years). The GVDLS provides a critical disease-surveillance and research service for all Georgia and U.S. citizens who are animal owners, producers, or consumers of animal-based agricultural products. The personnel and laboratory infrastructure of the GVDLS, which consists of the Tifton Veterinary Diagnostic and Investigational Laboratory

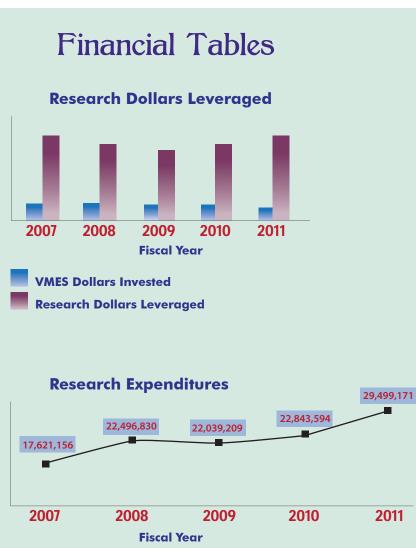
(TVDIL) and the Athens Veterinary Diagnostic Laboratory (AVDL), are supported by a unique synergistic budgetary arrangement with the University of Georgia College of Veterinary Medicine, the Veterinary Medical Experiment Station and the Georgia Department of Agriculture. The cover story of this year's annual report highlights the service and applied research provided by these laboratories. It is a compelling story, and one which I believe will leave the reader with a better understanding of the role of these laboratories and their importance to the State of Georgia and the nation. Moreover, the importance of applied veterinary research in fulfillment of their research, service, and education missions should be evident.

As in previous reports, the 35th VMES Annual Report provides an overview of peer-reviewed, competitive projects and new faculty start-up projects conducted during fiscal year 2011 (July 1, 2010 – June 30, 2011). Additional information on any of these projects can be requested by contacting the VMES office by phone, email or website, or directly from the investigators themselves. A list of publications is provided as well. These peer-reviewed papers represent a selection of VMES supported work and other scholarly research by faculty at the College of Veterinary Medicine, which includes those in the Georgia Veterinary Diagnostic Laboratory System.









Expenditures by Category

Budget Category	Amount	% of Budget
Personnel - Researchers/Techs/Research Staff	\$1,787,726	67.37%
Research Materials & Equipment	\$531,887	20.05%
Personnel – Research Administration & Accounting	\$327,240	12.33%
Travel	\$6,579	0.25%

\$178,995 or 6.7% of the overall VMES budget was expended in support of the Athens and Tifton Veterinary Diagnostic Laboratories in FY2011.

A summary of the College's research funding is provided above. Over the past year approximately six research dollars were leveraged for each VMES dollar invested. Expenditures are from all sources including State Appropriations, Extramural Research Funding, Donations - Includes all expenditures including personnel costs.

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. 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 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 helps us understand the role of these unique companions, in the distribution dynamics of Salmonella and human illnesses.

Susan Sanchez

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. paratubercuosis (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. 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. Murray E. Hines II

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'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



Dr. Uriel Blas-Machado

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. 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 Trypanasoma 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.

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.



Pathogen-Induced T and B Cell CDR3 Repertoires in Channel Catfish

Understanding the basic mechanisms of immunity following infection in fish is critical for development of efficacious vaccines for use against economically important pathogens. Because fish can acquire longterm immunity to infectious agents, vaccination represents the most cost-effective and efficient method for preventing outbreaks of disease in commercial aquaculture. Channel catfish comprise the largest market of commercially-reared fish in the United States, but the viability of the industry is threatened by increasing disease losses. We use infection of channel catfish with Ichthyophthirius multifiliis (Ich) to study B and T cell responses to infection. Ich is a highly virulent, protozoan parasite that infects the skin and gills of all freshwater fish. Our work focuses on understanding how anticipatory T cell and B cell repertoires in naïve fish are molded by infection to produce memory repertoires that provide long-term protection against re-infection.

The goal of our project is to determine the sequence diversity in the expressed repertoires of the antigenbinding domains of T and B cell receptors in naïve channel catfish and to test if infection with Ich alters these repertoires. We will use Illumina DNA sequencing technology to sequence in depth cDNA



Craig Findly

libraries constructed from RNA isolated from skin and head kidney of channel catfish. Diversity in T cell receptor beta (TCRB) and B cell immunoglobulin heavy chain (IgH) receptors is concentrated within domains known as the complementarity-determining region 3 (CDR3). CDR3 domains vary in sequence and size with an average length of 35-50 base pairs. This variation allows T and B cells to recognize and bind a diverse array of foreign antigens. We hypothesize that the anticipatory CDR3 repertoires will be similar in peripheral (skin) and central lymphoid (head kidney) tissues in naïve fish, but following infection will change in skin due to clonal expansion of effector and memory lymphocytes. DNA sequence will be obtained for the CDR3 domain and flanking regions for TCRB and IgH. The specific objectives of our proposed work are to determine sequence diversity in the expressed repertoires of channel catfish TCRB and IgH CDR3 domains in lymphocytes found in skin and head kidney of: 1) naïve, uninfected fish; 2) infected fish during the primary response to Ich infection; and 3) immune fish during a secondary response to Ich challenge infection. A clearer understanding of how immune repertoires are altered by infection through epithelial surfaces will help in the design and development of vaccines for aquaculture.

Principal Ivestigator: Dr. R. Craig Findly





A third project was to determine if use of an inactivated (dead) chicken infectious anemia virus (CIAV) would improve the protection in broiler offspring from the vaccinated breeders. CIAV is a disease that can severely affect a broiler chicken's immune system and make them more susceptible to other diseases.

The results of our work found that vaccination of breeders in the thigh muscle gave as good a protection as vaccination in the breast muscle and did not result in any lameness. This allows producers to vaccinate in a location that will not affect the value of the highest priced meat, the breast muscle. The work on evaluating MS isolates found that the new strains were more pathogenic and more easily transmitted from bird to bird than the previously isolated strains. The use of the CIAV inactivated vaccine in the breeders did not appear to provide enough additional protection to justify the added cost of this additional vaccine.

Principal Investigator: Dr. Charles Hofacre Co-Investigators: Dr. Steve Collett and Dr. Guillermo Zavala

Clinical Investigations of Poultry Diseases

The objectives of this project were to investigate the poultry health issues and determine solutions for poultry producers. The work was also done in conjunction with the training of the Master of Avian Medicine (MAM) students. There were several farms that local poultry companies designated as "problem farms." These were farms that did not have an obvious disease or husbandry issues but did not perform to the company standard. The clinician and two MAM students were able to identify the issues on these farms as primarily husbandry and help correct the situation. There were also research studies completed by the MAM students resulting in four presentations at state and national veterinary medical meetings. Some of the health issues that were addressed this past year were the pathogenicity of Mycoplasma synoviae isolates from outbreaks in broiler breeders. This Mycoplasma work done by Dr. Natalie Armour resulted in her receiving the 2010 Reed Rumsey Student Award from the American Association of Avian Pathologists (AAAP). Also, there was work to determine the effectiveness of a live vaccine for prevention of salmonella infections in laying chickens completed by the MAM candidate Dr. William D. Parker. Additionally, work was performed to determine the best location to inject inactivated (dead) vaccines in broiler breeder chickens to give maximum immunity with the least side effects. This work was prompted because the USDA inspectors were condemning a large number of end of lav breeders that had been injected in the breast muscle nearly one year previously.

Establishing the roles of local and systemic inflammation in reproductive failure in dairy cows



pregnancies that progress to calves. Studies of human infertility suggest that immune responses leading to persisting inflammation are often significant contributors to pregnancy failure. Some studies in dairy cows have demonstrated indicators of inflammation in the reproductive tract. Last year, we flushed the uterus of 32 cows with sterile saline and evaluated the flush fluid for the presence and number of neutrophils and other white blood cells, for the growth of coliform bacteria and for the growth of common pathogens, for the level of IgG and IgA antibodies, and for the level of prostaglandin E2. We found that cows had a wide distribution of these measurements in uterine flush fluid. They ranged from no white cells to many, low levels of antibody to very high, and low levels of prostaglandin to very high levels. We found a clear correlation among these values, in that those cows with high numbers of uterine white blood cells also had high levels of uterine IgG and IgA, and these same animals had the highest levels of prostaglandin E2. Many of these same cows had detectable numbers of bacteria that are associated with uterine infections. We have sampled, for a second time, a number of cows that did not become pregnant after several tries. All of these cows had high levels of IgG antibody, high levels of prostaglandin E2, yielded bacterial isolates consistent with pathogens, and most had white blood cells in the uterine flush sample.

During the current year we assessed the uterine flush fluid for evidence of IgG and IgA antibody that specifically bound common dairy cow pathogens or antigen prepared from 15 of the bacterial isolates collected from the flush fluid samples. Our serum testing demonstrated that all of the cows had been exposed to common production pathogens (bovine herpesvirus 1, bovine viral diarrhea virus, Staphylococcus aureus, and Streptococcus *uberis*) in the field or by vaccination. However, none of the flush fluid had either IgG or IgA antibody that bound to these antigens. We measured serum titers against a set of 15 isolates we recovered from uterine flush samples. IgG binding was evident for all 32 cows. Yet, only four of the 15 isolates demonstrated significant IgG binding from uterine flush fluid of at least 10 of the 32 cows, and only two flush fluid samples contained IgA that bound to eight of these isolates. The isolates that showed evidence of inducing uterine antibody will be further characterized. The results of these studies (that all animals had serum titers to all antigens, but only a small fraction of these antibodies were found in any of the uterine flush fluids) demonstrated that antibody (particularly IgG antibody) in the reproductive tract does not simply represent antibody that was transported from the circulation after systemic production. Rather, the reproductive tract antibody is either selectively transported to reproductive tissues from the circulation, or produced locally in the reproductive tract.

Our working model for how these immune and inflammatory factors impact pregnancy is that infectious agents colonize the uterus after birth and establish an ongoing inflammatory and immune response. This inflammatory response alters the environment of the uterus and blocks the development of productive pregnancies. It is possible that these pregnancies are blocked during critical events in the implantation process of embryos into the uterine tissue. We plan to complete the assessment of the properties of the isolates that appear to induce an immune response in the reproductive tract and examine their potential for providing a mechanism to modulate the problems in dairy cow fertility.

Principal Investigator: Dr. David J. Hurley

Co-Investigators: Dr. Michael W. Overton, Dr. Jakob Scherzer, Dr. Isaiah Smith, and Dr. Stephen Nickerson

One problem that the dairy industry faces is that cows stay in the milking herd for only 2 and 3 lactations on average. Two major factors contribute to that problem. First, cows develop significant mastitis problems that reduce their effective milk production period and marketable milk quantity. The second, and less clearly understood is that cows have difficulty becoming pregnant again after delivery. To make the dairy industry maximally productive and profitable, both of these problems must be solved.

In the studies we report here, we are examining the failure of cows to develop pregnancies. It appears from the assessment of current practices that we are able to provide good semen and that the cows will ovulate as expected in many cases and the dairy industry has been provided with protocols to enhance the predictability of ovulation and enhance the chances of fertilization. However, we still fail to achieve



During September to December of 2010, 367 samples of ear from free ranging WTD were collected from 37 counties in Georgia, mainly from south areas of the state (see map below). The samples were from 178 (48.5%) female deer, 187 (51%) male deer, and two (0.5%) unknown sex. The age varied from 6 months to 6.5years. The age was not recorded in 34 animals (9.3%). Within the animals with known ages, 42% of the deer sampled were under 2 years. Four ear samples resulted in suspect samples by either AgELISA (3 samples) or RT-PCR (1 sample). However, none of these samples were positive in both tests and other tests used (virus isolation and IHC).

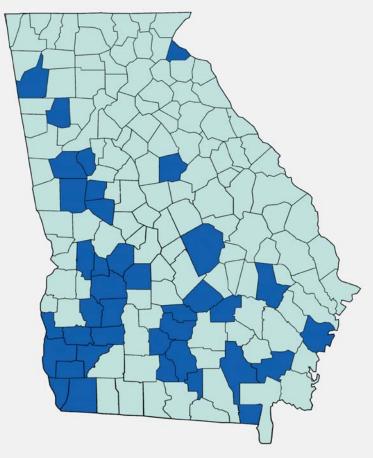
In conclusion, although a few of our samples resulted in suspect for the presence of BVDV, we could not further confirm the presence of the virus within the deer population studied. Although the results from this preliminary study in the population sampled do not support the hypothesis that WTD could be a potential reservoir for the BVDV in the state of Georgia. low prevalence of this disease in WTD is a possibility. Future studies targeting a larger population may be helpful to determine if BVDV is present in WTD in this region.

Principal Investigator: Dr. Marcia Ilha Co-Investigators: Dr. Sreekumari Rajeev

Study of the occurrence of Bovine Viral Diarrhea virus in hunter-harvested free ranging white-tailed deer in Georgia

Bovine virus diarrhea virus (BVDV) belongs to the genus Pestivirus of the family Flaviridae. Infection with BVDV in cattle can result in respiratory, gastrointestinal, and reproductive tract disease of varying severity, ranging from subclinical to fatal disease. There is little evidence that bovine virus diarrhea (BVD) occurs in free-ranging white-tailed deer (WTD) in North America. Several recent experimental studies have indicated that BVDV can be transferred back-and-forth between cattle and WTD and amongst WTD. To better recognize the role of pestiviruses in wild animal populations, surveys for BVDV in wild WTD have gradually been done in other states and very low prevalence have been found (less than 1%).

So far, to the best of our knowledge, no studies had been performed in WTD in the state of Georgia and the occurrence of BVDV in the population of free-ranging WTD in this region was unknown. A prevalence study was conducted to evaluate BVDV infection in free-ranging WTD in the state of Georgia using ear samples collected from hunter-harvested deer during the hunting season of 2010-2011. Ear notches were tested for BVDV by antigen capture enzyme-linked immunosorbent assay (AgELISA), immunohistochemistry (IHC), real time polymerase chain reaction (RT-PCR), and virus isolation.



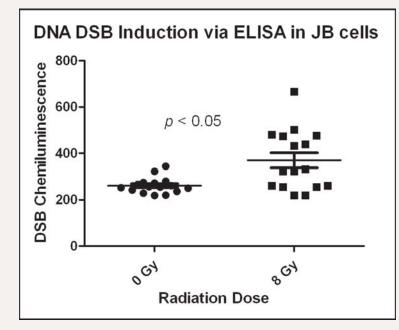
Map of the state of Georgia. Dark areas are counties from where deer samples were obtained.

In vitro investigation of the use of masitinib and radiation on feline injection site sarcoma cells

Injection site sarcoma (ISS) is a soft tissue tumor that can develop at the site where an inactivated vaccine has been administered, particularly rabies and feline leukemia virus vaccines. Time from vaccination to tumor development can be as short as 4 weeks or as long as 10 years. Radical surgery or a combination of surgery and radiation therapy offers cats the best chance for long-term control. Most cats treated aggressively remain cancer-free for 1-3 years; however, ISS is a difficult cancer to cure. New treatments are needed, as ISS will continue to be problematic as long as cats receive vaccinations.



Our research group has been evaluating the efficacy of a new drug, masitinib mesylate, which has recently been approved for use in dogs with mast cell tumors. Masitinib targets a pathway that can be over-active in mast cell tumors, but it also targets a pathway involving a receptor called platelet derived growth factor (PDGFR) that contributes to ISS growth in cats. Our group recently showed that masitinib inhibited PDGFR signaling in feline ISS in vitro, as it does in other species. Masitinib was also able to inhibit ISS cell growth in the laboratory in a dose-dependent manner.



As radiation is a common therapy used in the clinical management of ISS in cats, we investigated the effects of masitinib and radiation on ISS cells in the laboratory. Across a variety of masitinib and radiation doses, the two treatments interacted positively to decrease ISS cell growth beyond either treatment alone. Interestingly, we also found that prolonged exposure to very low doses of masitinib alone caused significant ISS cell growth inhibition, suggesting that chronic dosing regimens may be useful in the clinical setting.

To further investigate the interactions between masitinib and radiation, we have recently found that we can identify an increase in a DNA-associated protein called yH2AX, an indirect measure of radiation damage. Our current focus is on determining if an increase in yH2AX following treatment with masitinib and radiation is responsible for the increased cell kill over radiation alone. Based on work performed thus far, additional investigation is warranted which will hopefully lead to improvements in the treatment of cats with ISS

Principal Investigator: Dr. Jessica Lawrence Co-Investigators: Dr. Michelle Turek, Dr. Robert Gogal Jr., Dr. Corey Saba, Dr. Michel Vandenplas and Amanda Vance



including exposure to certain bacteria and viruses, inadequate protective immunity, and management practices such as shipping calves immediately after weaning, or mixing calves of different ages and sources. The importance of management in BRD has been demonstrated by the fact that BRD can be decreased significantly when calf management is modified.

While management modifications have been proven to decrease BRD in feedlot and dairy calf populations, almost nothing is known about how management practices are related to BRD in nursing beef calves. Thus, it is not possible for veterinarians to make science-based recommendations to cow-calf producers to prevent calf BRD. Ongoing research at the UGA College of Veterinary Medicine aims to address this issue. A questionnaire was sent to 2500 cow-calf producers in 3 Southeastern states (Georgia, Florida, West Virginia) and 3 Western states (Iowa, Nebraska, and Kansas) which requested information regarding the occurrence of calf BRD and management practices on the operations surveyed. Information from operations where calf BRD has occurred will be compared with information from operations where it has not occurred in order to identify factors associated with calf BRD. Additionally, a questionnaire will be sent to veterinarians who work with cow-calf producers in the same 6 states to gather information regarding how veterinarians identify, treat,



and attempt to prevent nursing calf BRD. This research represents the most extensive effort to date to determine causes of nursing calf BRD in U.S. cow-calf herds, and the results will improve the knowledge of producers and veterinarians regarding this problem. The research will also provide the basis for future studies to test treatments and preventative strategies to decrease the occurrence of nursing calf BRD.

Principal Investigator: Dr. Amelia Woolums Co-Investigators: Dr. Roy Berghaus, Dr. Roger Ellis and Dr. Lee Jones

A Survey of Risk Factors for Nursing Beef Calf Bovine Respiratory Disease

USDA surveys have shown that bovine respiratory disease (BRD) is the leading cause of death of U.S. feedlot cattle, weaned dairy heifers, and nursing beef calves 3 weeks of age and older. Calves with BRD (also called "pneumonia") may cough, breathe hard, have a snotty nose, and have a fever. Affected calves may get better, they may die, or they may survive but lose weight and look sick for weeks (become "chronic"). Thus BRD has a significant negative impact on the well-being of U.S. cattle; it also impacts the profitability of cattle operations through financial losses associated with decreased animal growth and survival, and the costs of treatment.

Research has shown that BRD in feedlot cattle and dairy calves is due to a combination of factors



Extramural Contracts & Grants

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