VMES 2003
Science in Service to Animals

AGROTERORISM

Veterinary Medical Experiment Station
College of Veterinary Medicine
The University of Georgia
Athens, Georgia
Our Cover

The everwatchful eyes of scientists monitor the threat of a bio-terror event to our nation’s food supply. Research in the detection, treatment, and preventive vaccines must be a state and national priority.
27th Annual Report

Enhancing animal production, profitability, and well-being by improving animal health.

This 27th Annual Report is published by the Veterinary Medical Experiment Station, The University of Georgia.

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The Veterinary Medical Experiment Station (VMES) supports a wide range of research that impacts on many aspects of our lives; the food we eat and the clothes we wear, our physical, emotional, and economic health, and the quality of our environment. VMES research includes efforts to improve the productivity and health of poultry and livestock, to better the quality of life for companion animals, and to improve public health through disease surveillance. This year’s research is profiled in our 2002 - 2003 VMES annual report.

VMES funds help support short-term applied research that directly benefits the health of animals and livestock in Georgia and are used to develop extramurally funded research programs at the College of Veterinary Medicine. Projects supported by VMES funds are evaluated for scientific merit, importance to animal health, consideration for experimental animal welfare, and their roles in meeting the research objectives of the VMES.

Our objectives are as follows:

- 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.
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*All programs and activities of the Veterinary Medical Experiment Station are conducted without regard to race, color, national origin, age, sex, or handicap.*
I am pleased to share with you the 27th Annual Report of the Veterinary Medical Experiment Station (VMES) in which we present a summary of the myriad research activities of the College of Veterinary Medicine. In the face of deepening fiscal restraints, we continue to effectively conduct research on animal health problems of present and future concern to our state’s livestock and poultry industries as well as its wildlife resources. Our mission will always remain critical to the people of Georgia. The food animal industries of the state are valued at well over $3 billion, and sales of livestock, poultry and their products account for more than half of Georgia’s annual farm income. A continued commitment at the state level to support research on animal health is a smart investment, particularly in view of the fact that there is limited federal and private funding targeted specifically for animal health research. Moreover, the interface between animal and human health is becoming blurred to indistinction, and basic research on animal diseases often leads to new knowledge that benefits both animals and humans.

The cover of this year’s Annual Report is focused on the threat of agroterrorism and the challenges it presents to the veterinary and agriculture communities. The accompanying article by Dr. Corrie Brown, an internationally respected veterinary researcher, discusses these issues and presents a compelling argument for the critical role of veterinary medicine in protection of our nation’s agriculture-based economies. Basic and applied research conducted by VMES researchers is important for the development of new diagnostics, therapeutics, and vaccines against pathogens of potential use as agents of bioterrorism.

The 27th Annual Report provides an overview of the VMES-supported projects during the fiscal year of 2003. Additional information on any of these projects can be obtained by contacting the VMES office by phone, email or website, or directly from the investigators themselves. A list of publications is provided. These peer-reviewed papers represent a selection of VMES supported work and other research originating at the College of Veterinary Medicine.

The following table reflects the level of research dollars from federal, state and private sources, the declining VMES budget from FY 2001-2003, and the projected state VMES budget for FY 2005.

**Research Funding**

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<th>Funding Source</th>
<th>FY2001</th>
<th>FY 2002</th>
<th>FY 2003</th>
<th>FY 2004 (Budgeted)</th>
<th>FY 2005 (Requested)</th>
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<td>VMES/VMAR Expenditures</td>
<td>$3,594,225</td>
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September 11, 2001, the anthrax events that followed, and subsequent bombings in far-flung segments of the globe, all underscore the vulnerability of our society to attacks from terrorists who are intent upon undermining a way of life. Huge efforts have been made to “harden” many of the more traditional targets, and anyone who has traveled by air, entered a federal building, or attended a major sporting event can appreciate the monumental changes that our society has undergone to improve public protection against terrorist activities. As these more conventional targets become less vulnerable to attack, it is certain that terrorists will begin to focus, and perhaps already are focusing, on other areas of interest and impact. Our national herds and flocks are very difficult to protect and so animal agriculture presents a “soft white underbelly” for terrorists. Attacks against animal or plant agriculture is referred to as agroterror.

Agriculture forms the cornerstone of the American economy. Responsible for 13% of the gross national product and 17% of all employment, the value of agriculture is predicated on our ability to export approximately 20% of all agricultural commodities. Not only would the introduction of a disease that made our agricultural products unpalatable to our trading partners devastate exports, but the non-exportable products spilling over into the domestic sector would create a glut that would cause the agricultural economy to implode. Basically, a terrorist event involving agriculture could destroy the American economy.

One need only look at foreign disease incursions around the world over the last few years to appreciate the significant damage caused by spread of infection to a new area. Foot-and-mouth disease (FMD) entered Taiwan in 1997, necessitating the destruction of 8 million pigs, costing the country over $25 billion and wiping out the entire hog industry. In the same year, classical swine fever was discovered in the Netherlands, and millions of pigs were killed to try to stem the spread of the disease. In 2000, FMD continued its global spread, entering previously disease-free zones of southern Brazil, Argentina, Uruguay, South Korea, Japan, and Russia. The Food and Agricultural Organization of the United Nations termed 2000 as the “year of the global pandemic of FMD.” Then in 2001, FMD made big headlines in our media, as Americans watched British farmers and farming communities deal with an outbreak there.

What are the possibilities that diseases could continue to spread globally? Whenever an unexpected disease enters the United States, both consumer and export markets are negatively impacted by increasing prices.

A bioterrorist event could change the disease status of our national herds and flocks in a precipitous way, with devastating results. There is documented evidence that the following agents have been prepared specifically for agroterror: foot-and-mouth disease, classical swine fever (hog cholera), African swine fever, rinderpest, sheep and goat pox viruses, and Chlamydia psittacci. Numerous other agents that have been mentioned as making excellent weapons could affect a wide range of species, either as the primary target or through collateral exposure, including captive animals, companion animals, wild animals, food animals, and endangered species.

Compared to bio-terror, agro-terror is appallingly easy. Animal diseases of greatest concern are those that, by nature, are very infectious and spread very rapidly through herds and flocks. Many of the animal diseases that are of greatest concern in terms of their ability to enter a new area and destroy trade are foot-and-mouth disease, classical swine fever, rinderpest, highly pathogenic avian influenza, and exotic Newcastle disease. These agents could be acquired in less developed countries where they are endemic.

What can we in veterinary medicine do to be prepared for either accidental or intentional introduction? First, the amount of economic damage will depend upon how quickly the disease is detected. If the earliest cases are recognized, and adequate control measures implemented immediately, we will likely circumvent severe economic consequences. Therefore, awareness and training are paramount. Second, basic research and a greater understanding of disease epidemiology is needed to develop improved diagnostics and novel vaccines.

Corrie Brown, DVM, PhD
The poultry industry is a significant economic force in the nation. According to USDA statistics, 84% of the total national broiler production is raised in the Southeast, where the state of Georgia produces over one billion of the 8.3 billion birds. An estimated 2.8 million chickens are condemned in Georgia’s processing plants because of E. coli respiratory disease. There are several antibiotics approved by the Food and Drug Administration for treatment of sick chickens but one group, the fluoroquinolones, has generated some concern about its impact on human health. The tetracyclines have been a commonly used antimicrobial in veterinary medicine; however, its efficacy is impacted by the frequent occurrence of resistant bacteria. Some people believe that the non-pathogenic intestinal bacteria of broiler flocks serves as a reservoir for drug resistance genes for pathogenic bacteria and that resistance is coupled to the usage of antibiotics in meat production. We hypothesized that the microbial ecology of the chicken intestine favors “silent” laryngotracheitis, which was first detected and described in our laboratories. Researchers are also focusing on the reduction of potential human pathogens on poultry products nationwide and on ways to prevent the development of resistance against antibiotics.

During FY03, avian medicine faculty were investigators or co-investigators in new extramural funding of $665,857 from 4 projects. This was primarily from USDA, U.S. Poultry and Egg Association, and private sources. Eight intramural projects totaling $235,350 were funded for FY03. Our faculty and graduate students have been active in presenting their research at national and international meetings. During FY02, there were 51 papers published in refereed journals, and 137 scientific and industry presentations.

Members of the Department of Avian Medicine, The University of Georgia, are involved in a wide range of both basic and applied research involving subjects in the area of poultry health. Many of the projects are designed to solve problems for local companies, but most have a broader application. This report points out a sample of these projects and the people involved.
Investigation of Natural Disease Outbreaks

This project is an ongoing proposal that provides diagnostic laboratory support for the poultry industry, source material for research, and teaching experiences for students in the Master of Avian Medicine (M.A.M.) program.

Field investigations by professional staff and students typically lead to significant changes in disease and farm management practices which bring solutions to difficult problems.

An example of field investigations includes scenarios such as: serological assessment of broiler operations which may be experiencing severe condemnations at processing due to respiratory disease. Serological testing showed significant titers against a specific strain of infectious bronchitis virus. Addition of the indicated strain of virus to the vaccination program ended the condemnations and the financial losses due to this virus.

Improvements in the lab database continue with functional and additional data search capabilities and simplified maintenance. Lab reports are being sent by email in “.pdf” (Adobe Acrobat) format directly from within the system without the need for an intermediate hard copy. Construction of an e-business web site where accessions and case reports can be managed electronically is being investigated.

The polymerase chain reaction (PCR) technique is an integral part of the diagnostic laboratory as seen by the consistent demand for these tests. PCR techniques for infectious bronchitis virus, Mycoplasma, Infectious bursal disease, Infectious laryngotracheitis virus, and Avian leukosis virus-J provide mostly same-day results. The PCR lab has been expanded for a new bacterial PCR expected to be put online by summer.

Diagnostic Services Laboratory activity is represented by 5,455 accessions, 39,249 bacterial procedures, 180 antimicrobial susceptibilities, 81,746 ELISA tests, 40,018 IBV-HI tests, 24,255 Mycoplasma plate agglutination tests, 2,382 agar gel precipitin tests, 2,038 diagnostic PCR tests, and 2,969 necropsies.

PI: Dr. Stephen G. Thayer (sthayer@uga.edu)

Co-PIs: Dr. S.H. Kleven, Dr. T.P. Brown, Dr. M. Garcia, Dr. J.R. Glisson, Dr. C.H. Hofacre, Dr. M.W. Jackwood, Dr. J.J. Maurer, Dr. G.N. Rowland, Dr. J.E. Sander, Dr. H.S. Sellers, Dr. S.A. Vezey, and Dr. P. Villegas

Clinical Investigation of Poultry Diseases

This project involves advanced clinical investigation and applied research on current field problems encountered by the PDRC clinicians and M.A.M. students. The studies involve research attempting to reproduce a naturally occurring disease or disease syndrome or field studies evaluating the effect of management/vaccinations. These studies conducted by the PDRC clinicians and M.A.M. students result in publications of case reports, research notes, and are often preliminary data for grant applications for other PDRC researchers.

This past year clinicians and students studied four problem broiler farms to determine the cause of poor performance. Also, studies were completed evaluating the heating of an oil emulsion Pasteurella multocida bacterin on tissue reaction/immunity in broiler breeders, the effect of feed restriction on hypoglycemia spiking mortality syndrome in broilers, the incidence of Salmonella in litter of North Georgia broiler farms, the prevalence of IBV during the downtime in broilers, and the effect of formaldehyde usage on in ovo injected eggs.

PDRC has also received and are rearing the 3 lines of the 1976 random bred broilers from Aviagen. These GGP broiler breeders will come into production in FY 2003, producing the GP generation moving PDRC closer toward a line of SPF broilers chickens.

PI: Dr. Charles Hofacre (chofacre@uga.edu)
CO-PI: Dr. J.R. Glisson and Dr. J. Sander

Detection of Foodborne Pathogens Using rRNA Signature Sequences and Macroarrays

Application of nested PCR to detection of Salmonella in poultry environment. Isolation of Salmonella from poultry environmental and processing plant samples requires sampling large numbers of...
areas within the poultry house or plant. This study examined the use of PCR to identify those secondary enrichments containing *Salmonella*. The unique *Salmonella* virulence gene *invA* was chosen as the target for development of a nested-PCR because of its uniform distribution among *Salmonella* serotypes. Using PCR as a screen of primary enrichments for presumptive *Salmonella* contamination, we improved our efficiency at isolating *Salmonella* upon secondary enrichment by 20% and no false negatives were observed. This method will not only validate the use of secondary enrichment procedures, but also reduce costs and manpower for surveillance of *Salmonella*.

**Detection of *Salmonella* and *Campylobacter* in poultry by PCR-ELISA.** Contamination of retail poultry by *Campylobacter* spp. and *Salmonella enterica* is a significant source of human diarrheal disease. Isolation and identification of these microorganisms requires a series of biochemical and serological tests. In this study, *Campylobacter* *ceuE* and *Salmonella* *invA* genes were used to design probes in PCR-ELISA, as an alternative to conventional bacteriological methodology, for the rapid detection of *Campylobacter jejuni*, *Campylobacter coli*, and *Salmonella enterica* from poultry samples. ELISA increased the sensitivity of the conventional PCR method by 100- to 1,000-fold.

*A restriction fragment length polymorphism based polymerase chain reaction as an alternative to serotyping for identifying *Salmonella* serotypes.* Twenty-four phase 1 flagellin and eight phase 2 flagellin genes could be differentiated among each other using restriction endonucleases in RFLP-PCR analysis. These genes comprise the major antigenic formulas for fifty-two serotypes of *Salmonella* sp., which include the common serotypes found in poultry and other important food animal species. With this knowledge, ninety percent of the *Salmonella* serotypes could be identified using this double restriction enzyme RFLP analysis.

These multiplex PCR assays for detecting specific O and H antigen gene alleles can become a rapid and cost-effective alternative approach to serotyping for the identification of common poultry *Salmonella* serotypes. This multiplex PCR has now become part of diagnostic services offered to poultry industry for identifying *Salmonella* serotypes.

**PI:** Dr. John J. Maurer (jmaurer@vet.uga.edu)  
**Co-PI:** Dr. M. D. Lee

**Avian Mycoplasmosis**

*Mycoplasma gallisepticum* strain K5054 has been further developed as a live vaccine strain. A patent has been applied for, and we are in negotiation with 3 companies for potential licensing. (Work in collaboration with Dr. Naola Ferguson).

Techniques for molecular epidemiology of avian mycoplasmas continue to improve. Primers for the *mgc2* gene of *M. gallisepticum* and *vlhA* of *M. synoviae* are about ready for field evaluation. PCR based on both of these genes are promising as diagnostic tests; the PCR products have potential value for preliminary identification of strains. Amplified fragment length polymorphism analysis shows promise as a definitive test for studying relationships among strains. (In collaboration with Maricarmen García, Yang Hong, Sharon Levisohn, David Yoge, and Dusan Bencina).

Diagnostic services included 5765 cultures, 13,451 *M. gallisepticum* HI tests, 13,606 *M. synoviae* HI tests, 163 *M. meleagridis* HI tests, for a total of 27,219 HI tests conducted. There were 106 cases involving *Mycoplasma* fingerprinting.

**PI:** Dr. S. H. Kleven (skleven@uga.edu)  
**Co-PIs:** Dr. W. D. Hall and Dr. V. Leiting

**Investigation into factors affecting hatch-ability and chick quality**

This project has been an ongoing study of disinfectant efficacy, and the effect of disinfectant use in the hatchery on hatchability and chick quality. During this period, studies were conducted using formaldehyde. This product has been studied in previous grants and found to be detrimental to respiratory epithelium at high levels. This study compared a constant rate infusion of a lower dose of formaldehyde to a higher dose given every 12 hours as was previously studied. This project also supported the continued work evaluating some problematic *Pseudo-
monas aeruginosa isolates that had caused severe chick quality problems as a result of hatchery contamination.

**PI:** Dr. Jean E. Sander (jsander@uga.edu)  
**Co-PIs:** Dr. J.L. Wilson and Dr. J.J. Maurer

Detection, Isolation, and Characterization of Avian Viruses

The objectives of this proposal are to provide diagnostic virology services for the U.S. poultry industry, conduct applied research on current avian disease isolates from the field, and improve detection and isolation methods for monitoring avian viruses. During FY03 we processed 327 accessions; 677 samples submitted for virus isolation; 296 virus isolations made from samples submitted; 381 negative samples (no virus isolated).

**PI:** Dr. Holly Sellers (hsellers@uga.edu)

Epidemiological Studies on Infectious Bursal Disease Virus Field Isolates in the Southeastern United States

Despite widespread vaccination, infectious bursal disease virus (IBDV) continues to cause economic losses to the poultry industry. Within serotype 1 there are classic, variant, and very virulent viruses. The U.S. poultry industry is most affected by the presence of antigenic variants that can be responsible for vaccination failures. The VP2 gene of IBDV has been the target for molecular classification of the virus since it is the major host protective antigen responsible for inducing serotype-neutralizing antibodies. Advancements in nucleic acid technology have led to the identification and classification of antigenic variants by RT-PCR/RFLP analysis. The objectives of this proposal are to conduct an epidemiological study of IBDV field isolates from the southeastern U.S. Field isolates will be chosen for the study based on unique RFLP patterns obtained using the current IBDV typing system at PDRC.

During FY03, full-length genome sequencing of IBDV field isolate 9109 and cell culture adapted Edgar was completed and phylogenetic analysis is currently being performed. Sequence analysis is being utilized to identify whether amino acid changes in locations outside of the hypervariable region of segment A might play a role in pathogenesis. The sequence data obtained will be compared to previously published full length sequences.

**PI:** Dr. Holly Sellers (hsellers@uga.edu)

Development and Characterization of Infectious Laryngotracheitis (ILT) Recombinant Virus

Differentiation of Infectious Laryngotracheitis Virus strains is one of the goals in our laboratory. We have developed a PCR-RFLP assay where the glycoprotein E was amplified by PCR and the amplification product was digested with restriction enzymes. This assay allowed discrimination of vaccines and vaccine subpopulations. A second generation of PCR-RFLP assays has been developed based on initial sequencing of glycoprotein I, and early genes ICP4 and ICP27. Amino acid substitutions specific to backyard flock isolates were detected in the glycoprotein I. These mutations were not present on any of the vaccines analyzed, or in outbreak related field isolates. Sequencing of the ICP4 and ICP27 genes allowed differentiation of two field isolates from vaccine strains. Therefore we have developed a system for ILTV strain differentiation by first using glycoprotein I to identify wild type strains: ICP27 to identify vaccine related isolates, and glycoprotein E to identify vaccine subpopulations.

Because vaccine strains are one source of outbreaks in the field, a second goal of this project was to compare the virulence and transmission of the chicken embryo origin (CEO) ILTV vaccine strain and CEO viral subpopulations. The objective behind this work was to determine if viral subpopulations within the vaccine are more attenuated. Clinical signs and transmission from inoculated to contact birds were recorded. Difference in the transmission and replication between CEO vaccine subpopulations was observed. Measured by the appearance of clinical signs during early stages of infection, one of the subpopulations transmits at a faster rate than the second subpopulation. From this data we have concluded that one of the vaccine subpopulations delays latency while the other enters latent infection faster. Ongoing studies from
this research will focus on determining which of these two subpopulations provides better protection and is more attenuated.

PI: Dr. Maricarmen García (mcgarcia@uga.edu)
Co-PI: Dr. S. Riblet

Advancements in the Isolation, Characterization, and Control of Avian Viruses

Research in the avian virology section has been concentrated on infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), and avian adenovirus. With IBV, several isolates used in commercial vaccines have undergone numerous passages in various systems (chicken embryos, chickens) in an attempt to attenuate their pathogenicity while maintaining their antigenicity. To date, the pathogenicity of one Arkansas-type strain has been compared with the original strain and no major differences have been found regarding their ability to multiply in tissues of the upper respiratory tract. Quantitative assays are being performed to establish differences between the two viruses.

Group I avian adenoviruses have been used to evaluate the effect of live and inactivated vaccines used to protect chickens against inclusion body hepatitis. Both vaccines provided protection to chickens challenged with homologous isolates obtained from the U.S. Live vaccines have the ability to spread very rapidly among the poultry populations.

The single-strand conformational polymorphism analysis (SSCP) was successfully used to differentiate variant, standard and very virulent strains of infectious disease virus.

PI: Dr. Pedro Villegas (pedrov@uga.edu)
Co-PI: J. El-Attrache

Control of Infectious Bronchitis Virus (IBV)

The main objective of this proposal is to control infectious bronchitis (IB). We propose to do this by continuing to study infectious bronchitis virus (IBV) isolates from the field and by developing and testing recombinant vaccines against IBV. The specific objectives are:

1. To study the molecular and serologic characteristics of new IBV isolates identified by our reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) serotype identification test.

2. To develop and test an IBV virus-like particle (VLP) for its utility as a vaccine against IBV.

3. To create an infectious clone for IBV.

Objective 1 is always ongoing in our laboratory; however, last year was a very quiet year for infectious bronchitis. Submissions to the laboratory resulted in typical viruses that have been previously characterized. We are continuing to monitor the heterogeneity of the population of IBV isolates circulating in the field.

The IBV VLP development (objective 2) is progressing, but without demonstration of VLP’s to IBV. We have cloned and again subcloned the spike and envelope proteins, which are necessary for VLP formation, and demonstrated expression of each protein in cell culture. Several attempts to visualize VLP’s by electron microscopy have been unsuccessful to date. We are looking at more sensitive detection methods and ways to increase expression of spike and envelope in cell culture.

To develop an IBV infectious clone, we cloned the Mass 41 genome into 5 overlapping segments, which represent the entire viral genome. The CMV and T7 promoters were ligated to the 5’ end clone, and the BHG poly A signal and a poly A tract onto the 3’ end clone. It has been difficult to cut the overlapping segments and ligate the 5 clones into one IBV infectious clone. We need sequence data for the entire viral genome so appropriate restriction enzymes can be identified and used to piece the clones together. Unfortunately we do not have the financial resources to do that at this time.

PI: Dr. Mark W. Jackwood, mjackwoo@uga.edu
Co-PI’s: D. Hilt, E. Wade, and S. Callison
Is Infectious Bursal Disease Virus the Cause of Broiler Proventriculitis?

Proventriculitis is a common naturally occurring disease of commercial broiler chickens that causes proventricular rupture, carcass contamination, and whole bird condemnation during routine processing. Infectious Bursal Disease Virus (IBDV) is implicated as a cause and vaccination for IBDV is marketed as a preventative. However, no direct cause and effect relationship has been established between IBDV and proventriculitis. Our original hypothesis was that immunosuppression caused by IBDV allowed a second opportunistic pathogen to directly produce proventriculitis. Our three-year project was designed to determine any acute or chronic role of Infectious Bursal Disease Virus (IBDV) in proventriculitis in broilers, and to look for other causative opportunistic agents. We have experimentally reproduced proventriculitis by oral exposure of broilers to proventricular homogenate from naturally affected chickens. We have shown IBDV does not localize in the proventriculus after experimental IBDV infection, and that naturally occurring cases of proventriculitis contain no proventricular IBDV. We have produced a proventricular homogenate that is free of IBDV but remains capable of reproducing proventriculitis. This homogenate will be inoculated into eggs, cell cultures, and proventricular organ cultures to isolate the proventriculotrophic virus that produces proventriculitis.

PI: Dr. Tom P. Brown  (tbrown@vet.uga.edu)  
Co-PIs: M. Pantin-Jackwood and M. Hamoud
Georgia’s aquaculture industry is steadily expanding, with its greatest increase occurring in channel catfish production. Pond acreage for catfish farming has continued to grow every year. Other species being developed for aquaculture include striped and largemouth bass, yellow perch, and tilapia. In addition to Georgia’s developing food-fish industry, there is an increasing interest in ornamental fish production, particularly koi, and cultured shellfish. It is estimated that aquaculture production in all countries will have to expand at least twofold to meet world demand for fisheries products over the next 25 years.

Continued commercial aquaculture success will depend on increased efficiency in resource use, innovative farming methods, and a quality end product. Fish health is an essential issue at every level of fish production. As Georgia’s aquaculture industries continue to grow, research aimed at improving the health of aquatic animal species will help growers reduce production costs and improve profits.

DNA Receptors and Innate Immunity in Catfish (II)

A novel approach to amplify adaptive immunity in teleosts consists of the use of oligodeoxynucleotide (ODN) adjuvants. This model suggests that injections of bacterial DNA in conjunction with specific immunogen may significantly amplify adaptive immune responses. We have previously identified molecular pattern ligands in the form of CpG, GpC and single base oligodeoxynucleosine 20-mers that bind to nonspecific cytotoxic cells (NCC). These ligands represent the experimental homologue of bacterial DNA and they specifically bind to DNA binding proteins (DBPs) on NCC. In the present VMES grant we identified three different molecular weight species (i.e. 14, 18 and 29 kDa) of DBPs on NCC. The 14 kDa protein was partially sequenced from tilapia NCC and is similar to histone core proteins. The 18 kDa molecule (from catfish NCC) is histone-1 and the 29 kDa molecule is a novel protein that is similar (but not identical) to histone linker proteins. The 29 kDa molecule was expressed as a recombinant protein and studies were carried out to determine ODN binding, antimicrobial activity and a polyclonal was generated against this molecule. This recombinant (referred to as NCC antimicrobial protein-1/ncamp-1) bound ODN; lysed Gram positive and Gram negative bacteria; and polyclonal anti-ncamp-1 bound NCC by Flow Cytometry analysis. These studies demonstrated that NCC express membrane DNA binding proteins and that in soluble form (ncamp-1) kills bacteria. This indicated that NCC may directly participate in amplification of innate immune responses to bacteria by recognition of DNA and elaboration of an antimicrobial protein.

PI: Donald Evans (devans@vet.uga.edu)

Identifying Virulence Mechanisms of Mycobacterium shottsii: An Emerging Disease of Fish

Striped bass (Morone saxatilis) represent an important commercial and recreational fish with significant economic benefits to the boating and tourism industries. In recent years there has been heightened concern regarding the health of striped bass populations in eastern coastal waters of the United States. An epizootic of mycobacteriosis was reported in the Chesapeake Bay which was characterized by lesion prevalence as high as 30-50%. Skin lesions were focal to multi-focal and ranged in severity from small grayish-white depressions to large reddened, hyper-pigmented shallow lesions rendering the fish unattractive and unpalatable. Histological examination of skin lesions and internal organs revealed granulomatous inflammatory responses accompanied by the presence of acid-fast bacilli. A higher prevalence of granulomatous lesions in visceral samples than in skin indicated that many stripers are asymptomatic. Subsequent bacteriological studies revealed that infections were associated with a variety of mycobacteria but were dominated by one unique mycobacterial type (designated as M175), which had not been previously described. Based on prior studies and field studies in progress by Virginia Institute of Marine Science (VIMS) investigators,
mycobacteria have been aseptically isolated from the spleens of >70% of the striped bass, and >70% of these isolates have been type M175. It has been proposed that M175 isolates be designated as a new species, \textit{Mycobacterium shottsii} sp. nov. and thus we have used this name for M175 in this proposal. The significance of heavy mycobacterial infections in native striped bass from coastal waters of the eastern U.S. is currently unknown.

Based on sequence analyses of 16S rRNA gene and phenotypic characteristics, \textit{M. shottsii} is closely related to \textit{M. marinum} and \textit{M. ulcerans}. The former is considered one of the primary etiologic agents of fish mycobacteriosis associated with tubercle granulomas in aquarium, cultured, and wild fish populations. \textit{M. marinum} is also capable of producing disease in humans with the primary clinical syndromes including skin and soft tissue infections, cervical lymphadenitis, and pulmonary disease. Disseminated infections due to \textit{M. marinum} are often limited to immunocompromised persons. \textit{M. ulcerans} produces necrotic skin lesions (Buruli ulcers) in humans and is considered the third most prevalent mycobacterial disease in humans. The high prevalence of \textit{M. shottsii} infections in striped bass could potentially cause human infection in people that handle these infected fish. This proposed research will define the virulence mechanisms associated with this new and emerging pathogen. In association with ongoing epidemiological studies that will define the extent of the spread of this agent along the eastern U.S. coast, the work described here may indicate a direction towards appropriate treatment and prevention strategies for afflicted fish and potentially human populations.

The primary mission of this laboratory is to identify virulence factors from pathogenic mycobacteria, and with this information, devise methods for treatment and disease prevention. To these ends, virulence studies using mammalian cells and pathogenic species of \textit{Mycobacterium} are routinely performed. These studies will now be extended to include fish-derived cell culture lines and the newly discovered pathogen, \textit{M. shottsii}. We expect these studies to provide new information that will further define the pathogenesis of this emerging species and lead to better control strategies. The specific objectives, using fish monocyte/macrophage, epithelial and fibroblast cell monolayers, will be to: 1) measure intracellular or extracellular bacterial growth, and 2) perform a necrosis/apoptosis analysis of culture filtrate from \textit{M. shottsii}.

At the completion of this study we will have: 1) determined if \textit{M. shottsii} is pathogenic for fish cells and if the organism possesses “intracellular” or “extracellular” virulence traits, and 2) determined if \textit{M. shottsii} causes cellular destruction through necrotic or apoptotic mechanisms.

These findings will be made available to ecologists, fish health specialists, and human public health officials for further investigation of the threat to commercial fish by this putative pathogen.

\textit{PI: Dr. Frederick D. Quinn (fquinn@vet.uga.edu)}
Ca\textsuperscript{2+} entry mechanisms regulating the tone of bovine small laminar arteries

Laminitis is a major disease in cattle. The available treatments are largely ineffective due to our lack of knowledge of the processes causing the dysfunction of small laminar arteries, which underlies this condition. Elucidation of the processes that cause laminitis has been hampered by a lack of techniques to study the functional aspects of the small laminar arteries that control local blood flow. We have directly addressed this issue by developing techniques by which small laminar arteries can be routinely isolated and their function examined in vitro. Calcium plays a fundamental role in the regulation of vascular function. The hypothesis driving this project is that abnormal calcium influx plays a vital role in the expression of microvascular dysfunction in laminitis. The main objective of this project is to determine the importance of calcium entry mechanisms in normal laminar arteries with the view of providing key background information for future studies on small laminar arteries from laminitic cattle. The specific aim of the project is to determine the relative role of voltage-gated, receptor-operated and store-operated calcium entry in regulating the active tone of bovine small laminar arteries. In these studies, small laminar arteries (100-300 µm internal diameter, 1-2 mm in length) are being mounted on small vessel myographs and functional pharmacological studies performed. These studies will define the calcium-dependent mechanisms regulating the tone of vascular smooth muscle in bovine small laminar arteries. The results of this project will provide the basis for comparative studies on cattle with acute laminitis and for the development of novel strategies to treat laminitis.

PI: Stephen J. Lewis (slewis@vet.uga.edu)
In the past few decades, horses have reemerged as a very important animal species in Georgia. In ages past, horses were concentrated on farms in rural parts of the state and were used primarily as work animals. Today horses assume many roles, ranging from companions to pleasure animals to show animals. They are used for pleasure riding, jumping, dressage, showing, cutting, and barrel racing. Because of the increasing financial and emotional impact of the horse industry on the state, VMES researchers are focusing on the mechanisms responsible for some of the most important diseases that affect horses.

Diagnosis Of Equine Fungal Keratitis Using Polymerase Chain Reaction

Equine fungal keratitis is a common sight-threatening disorder of horses. The number of cases is increasing in temperate regions, such as the southeastern United States. Even with appropriate therapy, 44-45% of the cases of equine fungal keratitis result in blindness. Early diagnosis and treatment is necessary in order to have a successful outcome. The purpose of this study was to evaluate the use of polymerase chain reaction (PCR) as a rapid and accurate tool for early diagnosis of equine fungal keratitis.

Corneal samples for PCR were obtained from equine cases evaluated for fungal keratitis, ulcerative keratitis, and stromal abscess formation. Standard PCR was carried out using universal fungal primers and gel electrophoresis. The PCR results were compared to cytology, fungal culture, and histopathology for the presence of fungal organisms. Fungal PCR (n=22), corneal cytology (n= 22), fungal cultures (n= 22), and histopathology (n=16) were performed in 22 cases of equine keratitis. PCR results were positive for universal fungal primers in 50% (n=11/22). Corneal cytology was positive for fungal hyphae in 59% (n=13/22). Fungal cultures were positive in 50% (n=11/22). Histopathology confirmed the presence of fungi in 44% (n=7/16). Of the 14 samples positive for fungal organisms by cytology, fungal cultures, or histopathology; 43% (n=6/14) were positive by PCR. Of the 8 samples negative for fungal organisms by cytology, fungal cultures, and histopathology; 63% (n=5/8) were positive by PCR. Of these five cases, four were clinically agreeable with fungal keratitis, stromal abscess (n=3) and deep progressive corneal ulceration (n=1).

Our results support the conclusion that PCR is a fast and sensitive diagnostic tool to aid in the clinical diagnosis of equine fungal keratitis.

PI: Dr. Phillip Anthony Moore (pamoore@vet.uga.edu)
Co-PIs: Dr. A. Neary, Dr. M. Chandler, Dr. C.B. Mosunic, Dr. K.P. Carmichael, Dr. U. Dietrich, and Dr. S. Sanchez
Companion animals reside in 55 million U.S. homes. These animals include an estimated 66 million cats, 58 million dogs, 88 million fish, 40 million birds, 13 million small animals (rabbits, hamsters, and gerbils), and 8 million reptiles. The increasing recognition of the close bond between people and their pets has magnified the importance of insuring the quality of our pets’ lives. Because of medical advances, companion animals are living longer than their predecessors. Longer life, however, means more age-related diseases and ailments, such as cancer, neural degeneration, kidney dysfunction, poor circulation, and decreased respiratory and cardiac capacity.

However, unlike other research areas, there are no federal funds and only limited state funds to support projects specifically for companion animals. The VMES has been useful in assisting new clinical faculty in their initial research projects, but the vast majority of funding has come from foundations and private industry. Industrial monies have been awarded based upon the potential knowledge gained from studying companion animals with diseases comparable to diseases found in humans. Examples of externally funded projects include urinary incontinence, diabetes mellitus, renal disease, pain relief for arthritis, transdermal fentanyl patches for pain relief, feline baronellosis and herpes virus, and minimally invasive surgery.

Efficacy of recombinant feline omega interferon on feline herpesvirus 1 (FHV-1) replication in vitro

The use of high dose recombinant human alpha interferon for the treatment of ocular herpes simplex keratitis (HSV-1) in humans is considered an effective topical treatment, if alpha interferons are given in adequate doses before or shortly after infection.

The objectives of this study are to evaluate the efficacy of feline recombinant omega interferon (rFeIFN-ω) on feline herpesvirus 1 (FHV-1)-replication in cell culture and to determine the optimal concentration of this drug that could be eventually used for the treatment of ocular herpesvirus infection in cats.

FHV-1 (strain C-27) will be grown on Crandell-Reese feline kidney cells and virus titers established after multiple passages. Virulence and homogeneity of FHV-1 will be verified by transmission electron microscopy, indirect immunofluorescent antibody testing and a plaque forming assay. Five different dilutions of rFeIFN-ω will be dissolved in culture medium. 10-fold concentrations ranging from 1x10^2 to 1x10^6 U/ml will be used. Antiviral assay will be conducted as a plaque reduction test, and run in triplicates to test the reproducibility of the assay. Feline omega interferon will be added to the cell culture before and after virus challenge and cultured in maintenance medium for 2-3 days. Plaques will be counted and expressed in percent of counts obtained from untreated control cultures.

FHV-1-induced ocular disease is widespread among the cat population. Antiviral treatment is only effective during virus shedding in the active phase of infection; the latent stage of the disease cannot be influenced by conventional antiviral therapy. Interferons might be used for prophylaxis and therapy of FHV-1-related ocular infections in cats.

The following steps of this research project have been completed since start time in April 2003:

- Crandell-Reese feline kidney cells were propagated and cultivated in cell culture medium. The seventh passage was harvested and stored and will serve as our working cell stock.
- FHV-1 virus was grown in cell culture from the originally established virus stock and cytopathogenic effect on cell culture monolayer was readily observed. Virus was verified by electron microscopy. Virus titration for TCDI 50 was started in 4 x 24 well plates.
- We are currently working on a plaque assay in order to determine virus infectivity. Mean viral titers will be expressed as plaque forming units (PFU)/0.1 ml.
- Antiviral assay using recombinant feline interferon omega will be performed from mid to end of September 2003. First results of this assay should be expected by the end of September/beginning of October 2003.

PI: Dr. Ursula Dietrich (dietrich@vet.uga.edu)
Co-PI: Dr. N. Siebeck, Dr. M. Garcia, and Dr. C. Greene
Comparative biomedicine investigates how a particular disease affects one species versus another; that is, how a disease manifests itself for example in a mouse versus a human or cow. Researchers can compare diseases between species because different species often share substantial genetic information. Scientists study data such as symptoms, disease progression, treatments, mortality, and so on. Thus, one species serves as a disease model for another. And interestingly, both species may benefit. For example, researchers study cardiomyopathy in dogs and humans and both have benefited in the short- and long-term.

Evaluation of Chalcone Derivatives in a Murine Model of Canine Neoplasia

Therapeutic inhibition of angiogenesis as a treatment for cancer has gained much interest in the last 30 years because of the potential for broad-spectrum efficacy, lack of acquired resistance, and low incidence of associated adverse effects. Chalcone is a biologically active flavonoid compound that is widely distributed in edible plants. Chalcone and its derivatives have been identified as anti-proliferative agents and their anti-angiogenic activity has been demonstrated in vitro. The purpose of this study was to evaluate chalcone derivatives in vivo, using a mouse model of canine cancer, the anti-angiogenic, anti-tumor, and anti-metastatic activity of synthetic chalcone derivatives designed and synthesized by the University of Georgia, Department of Chemistry.

One goal of this study was to standardize our production of reliable, predictably behaving models of both canine prostatic carcinoma and osteosarcoma. Using BALB/c-\(n u/n u\) (athymic) mice. In the first year of the study, we developed the ability to reliably produce transplanted canine prostatic carcinomas with aggressive, metastatic behavior. The canine osteosarcoma cell line was not reliably tumorigenic in the athymic mice so this cell line was not included in the study.

In preliminary studies, we encountered difficulty in solubilizing the test compounds into a form that would be safe and reliable for administration. Although we were able to administer the compounds orally, poor water solubility and the potential for ineffective drug delivery was a concern. The Department of Chemistry then developed several water-soluble chalcone derivatives that could be safely administered subcutaneously. Based on in vitro testing of anti-angiogenic activity at Emory University, the most effective water-soluble agent was selected for evaluation in our murine model of prostatic cancer. Unfortunately, this agent was uniformly hepatotoxic, resulting in the death of the mice, even when reformulated and dosed at \(\frac{1}{2}\) and \(\frac{1}{4}\) of the original dose. It is unclear why a compound that should be safe was so toxic. Consequently, further development of chalcone derivatives has been put on hold pending recruitment of a pharmacologist to this collaborative effort.

Using the expertise in creating mouse models of cancer that was acquired in this study, we have recently initiated a novel study to investigate molecular mechanisms controlling the biologic behavior of injection site sarcomas in cats. This is a particularly aggressive type of cancer that is very invasive locally and will metastasize in approximately 25% of cats. Understanding the mechanisms responsible for the behavior of injection site sarcomas will help to predict the behavior of this tumor in individual cats and allow the development of more specific targeted therapies to prevent or control this cancer.

PI: Dr. Nicole Northrup (northrup@vet.uga.edu)
Co-PI: Dr. Karen Cornell and Dr. Nancy Stedman
VMES — Working For Georgia
Cover Illustrations and Lead Articles
1999 to 2002

Emerging Diseases
1999

Genomics
2000

West Nile Virus
2001

Food Animal Health and Management Program
2002
Baldwin, Charles. Diagnostic services relative to the control, diagnosis, treatment prevention, and eradication of livestock diseases. Ga Dept. of Agriculture. $2,093,866

Brackett, Benjamin. Marker-assisted selection of bovine blastocysts. Tulane University. $43,440

Brown, Corrie. Preparing veterinarians to deal with global issues in animal health, trade and food security. FIPSE - U.S. Dept. Education. $52,584

Brown, Corrie. Veterinary curriculum and the future: Preparing veterinarians to deal with global issues in animal health, trade and food security. FIPSE - U.S. Dept. Education. $52,420

Brown, Corrie. Emergency management of agricultural bio-terrorism training curriculum. GA Tech Research Institute. $58,880

Brown, Corrie. Veterinary curriculum issues in the next millennium: Emerging diseases, food safety, bioterrorism and food security. Texas A&M Research Foundation. $47,103

Budsberg, Steven. Effect of topical diclofenac on an experimental subcutaneous model of inflammation in horses. Blue Ridge Pharmaceuticals. $26,311


Cole, Dana. Quantitative risk assessment of the potential for secondary spread of an agricultural bioterror agent in a rural community. UGA - Faculty Research Grants. $9,101

Davidson, William. Human ehrlichiosis surveillance and epidemiology. National Institutes of Health. $137,560

Dickerson, Harry. A research training experience for veterinary medical students. Merck Company Foundation. $20,000

Edwards, Gaylen. Metabolic regulation of growth and development. Pennington Biomedical Res Ctr. $38,213

Edwards, Gaylen. Catecholamine controls of alcohol intake. National Institutes of Health. $72,400

Ferguson, Duncan. Molecular genetic approach to development of a feline thyroid stimulating hormone. Morris Animal Foundation. $64,823

Fischer, John. Development of scientific information on animal tracts for selected wild vertebrate species by providing necropsy data on injuries associated with use of animal restraint devices. USDA. $12,650

Fischer, John. Coop. agreement for developing and evaluation of data relative to disease relationships that may involve wildlife, domestic livestock and poultry. USDA. $350,000

Fischer, John. Cooperative agreement for developing and evaluation of data relative to disease relationships that may involve wildlife, domestic livestock & poultry. USDA-APHIS. $150,000

Fischer, John. Federal assistance to support the distribution of pseudorabies virus and Brucella suis in feral swine populations in Georgia. USDA-APHIS. $50,000

Fu, Zhen. Regulation of rabies virus transcription and replication. National Institutes of Health. $253,400

Fu, Zhen. Development of recombinant rabies virus vaccines for animals. Fort Dodge Animal Health. $40,000

Glisson, John. Surveillance for West Nile Virus Encephalitis (WNVE) and other arboviral pathogens. GA Dept. Natural Resources. $13,400

Graves, Jonathan. Effect of wellness and performance formula on the deleterious effects of a high cholesterol diet. Platinum Research Foundation. $40,000

Graves, Jonathan. Menopause, lipids and changes in cardiovascular structure and function. UGA - Faculty Research Grants. $7,685


Hoenig, Margarethe. Lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activity in muscle and fat of lean and obese cats. Ralston Purina. $7,770

Hoenig, Margarethe. The effect of obesity on the feline immune system. Ralston Purina. $32,014

Hoenig, Margarethe. Insulin sensitivity and glucose and fat metabolism in cats. Nestle Purina. $243

Hofacre, Charles. Task Order for “Research Support”. USDA-APHIS. $18,887

Hurley, David. Comparison of the capacity of in vitro tools to assess the immune response of cattle to inactivated and modified-live vaccines. Merial Limited. $75,000

Hurley, David. Comparison of the capacity of in vitro tools to assess the immune response of cattle to inactivated and modified live vaccines … Georgia Research Alliance. $30,000

Jaso-Friedman, Liliana. The effect of obesity on the feline immune system. Ralston Purina. $36,108

Kaplan, Ray. Rotation of pastures with crops to achieve productivity and environmental quality. USDA-ARS. $6,384

Kleven, Stanley. Development and validation of a rapid diagnostic test for Mycoplasmosis infectious bronchitis and Infectious Laryngotraehitis. USDA-ARS. $640,000


Lewis, Stephen. Effects of wellness and performance formula on cardiovascular function, metabolism and longevity in a model of type 2 diabetes. Platinum Research Foundation. $37,500

Lewis, Stephen. Ischemia-reperfusion injury in equine laminar arteries. Grayson-Jockey Club Research Foundation. $29,000

Maki, Joanne. Ictalurus punctatus: A model to study mucosal immunity. NIH. $107,887

Maurer, John. Task Order. USDA-ARS. $2,970
Maurer, John. 2002-2003 ARS Funds For Molecular Biological Techniques. USDA. $2,970

McCall, John. Supply of Brugia infective larvae. National Institutes of Health. $24,973

McCall, John. Filariasis research reagent resource center. National Institutes of Health. $409,390

McCall, John. Antifilarial drug screening in dogs. World Health Organization. $46,866

McCall, John. Furnish Brugia malayi adult worms and/or B. malayi infective larvae. National Institutes of Health. $126,077

Mead, Daniel. West Nile Virus surveillance in West Virginia. West Virginia Dept. Health and Human Services. $24,800

Miller, Doris. Diagnostic services relative to the control, diagnosis, treatment, prevention, and eradication of livestock disease 2003 - Athens lab. GA Dept. of Agriculture. $1,308,581


Moore, James. LPS-Binding Protein and the Major LPS Receptor in Horses with Colic. Morris Animal Foundation. $26,213

Moore, Julie. T-cell memory and protection against placental malaria. National Institutes of Health. $324,043

Murray, Thomas. Neurotoxins from marine algae and cyanobacteria. Oregon State University. $126,028

Murray, Thomas. Dynorphin analogs as kappa opioid receptor antagonists. Univ. of Maryland. $10,500

Murray, Thomas. The equine adenosine A2A and A3 receptors: Potential therapeutic targets for endotoxemia. Morris Animal Foundation. $7,500

Murray, Thomas. Hypoxia and the control of fetal breathing movements. Univ. of California at Los Angeles. $25,733

Murray, Thomas. Affinity labels for opioid receptors. Univ. of Kansas. $65,619


Okinaga, Tatsuyuki. Assessment of recombinant swine PSP proteins on swine monocyte and macrophage function, and replication of porcine reproduction and respiratory syndrome virus in vitro. UGA - Faculty Research Grants. $5,982


Palmarini, Massimo. Distinguished Cancer Clinicians and Scientists Program. Georgia Cancer Coalition. $75,000


Prasse, Keith. Section 1433 Animal Disease and Health Formula Funds. USDA-CSREES. $110,062

Quinn, Fred. Characterization of the SigE regulon of Mycobacterium tuberculosis. National Institutes of Health. $48,148

Ritchie, Branson. Research Associate In Exotic/Zoo Infectious Disease And Pathology. Postgraduate program. Zoo Atlanta/Riverbanks Zoo. $13,000

Sanderson, Sherry. Comparison of two dietary approaches to managing canine chronic renal failure. Iams Company. $24,151

Sellers, Holly. Detection of infectious laryngotracheitis virus utilizing a DNA probe and in situ hybridization. UGA - Faculty Research Grants. $4,000

Stallknecht, David. Wildlife reservoirs for the H5 and H7 avian influenza viruses. USDA-ARS. $79,950


Stallknecht, David. Peridomestic avian species as amplifying hosts and sentinels of WN and SLE viruses in Georgia. Centers for Disease Control. $185,613

Stallknecht, David. West Nile surveillance in wild birds. GA Dept. of Human Resources. $178,340

Stallknecht, David. Determine infectious rate and distribution of avian pathogens in wild birds of midwestern and southeastern U.S. for Homeland Security surveillance. USDA-ARS. $100,000
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<td>Crowell-Davis, Sharon L., DVM, PhD, Professor, Anatomy and Radiology, (706) 542-8343</td>
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<td>Dzimianski, Michael T., DVM, Research Associate, Medical Microbiology and Parasitology, (706) 542-8449</td>
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<td>Edwards, Gaylen L., DVM, MS, PhD, Professor, Physiology and Pharmacology, (706) 542-5854</td>
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<td>Egger, Christine M., DVM, Assistant Professor, Small Animal Medicine, (706) 542-6369</td>
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<td>Eggleston, Randall, DVM, Clinical Assistant Professor, Large Animal Medicine, (706) 542-6320</td>
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<td>ENSLEY, Doug, DVM, Asst. Prof., Large Animal Medicine, (706) 542-6326</td>
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<td>Evans, Donald L., MS, PhD, Professor, Medical Microbiology and Parasitology, (706) 542-5796</td>
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<td>FAYNER-HOSKEN, Richard, BVSc, PhD, MRCVS, Professor, Large Animal Medicine, (706) 542-6451</td>
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<td>Ferguson, Duncan C., VMD, PhD, Professor, Physiology and Pharmacology, (706) 542-5864</td>
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<td>Fischer, John R., DVM, PhD, Associate Professor and Director, Wildlife Disease Study, (706) 542-1741</td>
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<td>Flattland, Bente, DVM, Assistant Professor, Small Animal Medicine, (706) 542-6376</td>
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<td>Frazier, Kendall S., DVM, PhD, Assistant Professor, Tifton Diagnostic Laboratory, (229) 836-3340</td>
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Selected Publications


