VMES 2000

Science In Service To Animals

Genomics

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

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July 1, 1999 to June 30, 2000

24th Annual Report

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

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VMES Objectives

The Veterinary Medical Experiment Station (VMES) supports a wide range of research that impacts on almost all aspects of our lives, from the food we eat and the clothes we wear, to our physical, emotional, and economic health, to 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 1999-2000 VMES annual report.

VMES funds are intended to help develop extramurally funded research programs at the College of Veterinary Medicine. In addition, VMES funds are used to support short-term applied research that directly benefits the health of animals and livestock in Georgia. 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 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.

Report of the Director

It is with pleasure that I introduce the 24th Annual Report of the Veterinary Medical Experiment Station (VMES). It has been a good year for the Experiment Station. Among other accomplishments, we have begun to build the College's Food Animal Health Program with the hire of Dr. Mel Pence, a food-animal veterinarian based in Tifton, GA. Through an additional enhancement to the VMES budget provided by the 2000 Georgia General Assembly, we plan to hire in fiscal year 2001 a research scientist based in Athens. This individual will conduct basic and/or applied research that will be of direct benefit to production animal health.

The VMES has been instrumental in attracting to the College productive new researchers in animal health by providing critical start-up funds for research. Over the last four years, the College of Veterinary Medicine has hired 32 new faculty members to replace individuals who have left the University through retirement or for other reasons. Through VMES support (and additional support from the University of Georgia Office of the Vice-President for Research), we have been able to attract and hire some of the best and the brightest veterinary researchers in the country. These individuals provide the foundation for the continued success of animal health and biomedical research at the University of Georgia, as well as the creative base for animal biotechnology industry in Georgia. To emphasize the importance of this aspect of our mission, new faculty projects supported by VMES are highlighted in red in this 24th Annual Report.

Our mission remains critical to the people of Georgia. The VMES plays a primary role in 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 food animal industries are valued at well over $3 billion. 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.

Station researchers, using a science-based approach, addressed many challenging animal health problems this year in areas ranging from the emergence of antimicrobial resistance in veterinary pathogens to studies on the human-animal bond. The 24th Annual Report provides a brief overview of many of the VMES-supported projects during the fiscal year of 1999-2000. Additional information on any of these projects can be obtained by contacting the VMES office by phone, e-mail, 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.

Beginning with the 1998 VMES Annual Report, we have highlighted in each subsequent year an area of science that is sure to have a critical impact on animal health. This year's cover and the accompanying article by Dr. Al McGraw are focused on genomics. As you will read, genomics is the inscription and annotation of the entire genetic complement (DNA sequence) of an organism. Bioinformatics is the process by which this incredibly complex information is digitally transcribed and analyzed through the use of powerful computer programs and models. The biologic information provided by genomics is revolutionizing biology and medicine. Veterinary researchers are poised to apply and synthesize this new information for the benefit of animal and human health.

Finally, it is with some sadness that I announce the retirement of Ms. Mary Hooper at the end of August 2000. Ms. Hooper has served over the last 18 years as Managing Editor of the VMES Annual Report and as Administrative Assistant to the Director. Her special financial expertise, critical administrative insights, and warm personal skills will be missed. We wish her all the best in her retirement.

Harry W. Dickerson, BVSc, PhD

Genomics

In recent years, we have all heard a lot about "the human genome project," and most of us know that this effort has resulted in a complete DNA sequence for all of the human chromosomes. We understand that this accomplishment is likely to have profound effects on human medicine, both in terms of diagnosis and therapy. But what, if any, are the implications for veterinary medicine?

First of all, it should be understood that genome projects are currently underway in many species, including plants, animals, and bacteria. The projects are being carried out in university and government laboratories and in the private sector. The essential goal in each case is to completely characterize the DNA of the species, with the idea that having this information will allow us to understand and influence life's processes - growth, development, reproduction, pathogenesis.

Species vary greatly in the sizes of their genomes, that is, in the amount and complexity of DNA contained in each cell. The genome of a typical bacterium contains a few million base pairs; the genome of a typical mammal contains several billion base pairs. The justification for sequencing the genome of a particular species depends on the size of the job and on the perceived benefit of carrying it out.

Historically, funding for veterinary medical research has been severely limited as compared with that for human medicine. Even so, we can expect that genomic studies will have a major impact on veterinary medicine in the years to come. One reason for this is that much of what is learned from the human genome will be applicable in veterinary species. If we know that a mutation in a certain gene causes a particular disease in humans, then it is likely that a similar disease in another species is caused by a mutation in a corresponding gene. There is sufficient similarity between such genes to allow us to examine them directly even in the absence of a complete genome sequence for the species of interest.

Perhaps a more important reason to anticipate that genomics will have a major impact on veterinary medicine is the fact that genomics technology is applicable across species. Technology that took decades to develop for deciphering the human genome can now be applied directly in other species in only a few years and at a fraction of the cost. This is reflected in the current situation with regard to genome maps.

Construction of a genome map is typically the first step in a genome project. Such maps consist of a set of hundreds to a few thousands of DNA markers spaced along the genome. Maps serve as a framework for more detailed genomic analysis, but in themselves are quite useful - they can help to localize genes that are responsible for any genetic trait of interest. In humans, of course, we are interested primarily in identifying disease-related genes. These can provide a basis for diagnosis, risk assessment, and rational therapy. Although health management is also clearly an issue in veterinary species, maps in livestock also offer the opportunity to identify genes affecting quality and production traits, opening the door to improved breeding practices through marker-assisted selection.

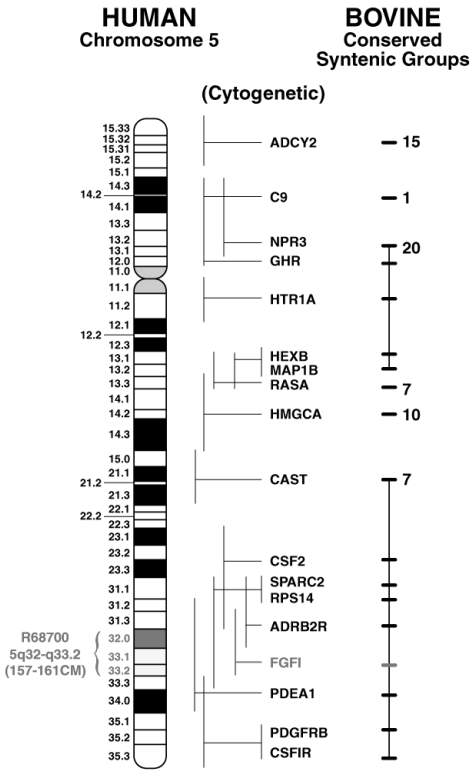
Efforts to map the human genome began more than twenty years ago, and the technology to build and read maps has improved dramatically during this time. Maps exist now for many veterinary species, even though most of these efforts began only a few years ago. Among the best developed maps at this time are those for cattle and sheep, but maps also exist for pigs, horses, sheep, goats, dogs, cats, and chickens. Even maps for catfish and shrimp are under development.

Similarly, technology for large-scale sequence analysis has advanced exponentially in recent years. Complete genome sequences already exist for scores of pathogens and for a handful of more complex model organisms including the fruit fly and the nematode worm *Caenorhabditis elegans.* Not so long ago, the idea of completely sequencing the human genome seemed almost unattainable, yet it is now in our hands. Although it may be unrealistic to think that complete genome sequences will be produced for all of our livestock and companion animal species any time soon, such projects are feasible now and may well become a reality in the not-so-distant future.

Within a species, the DNA of every cell is the same. Differential gene expression gives each specific cell type its unique properties. Likewise, the response of a cell to a stimulus, whether part of a normal or pathological process, is often mediated through activation or repression of specific sets of genes. Genome-scale studies have led to the recent development of "microarrays" and "DNA chips," which make it possible to monitor the expression levels of tens of thousands genes in a single experiment, using a device no larger than a standard microscope slide. Such experiments offer an unprecedented view of the complex inner workings of the cell and will lead to a profoundly improved understanding of physiological and pathological processes. In a different format, high-density DNA chips can be also used to monitor the inheritance patterns of thousands of genes in a particular cross. This latter application holds particular promise for animal breeding programs.

Is there a role for genomics in veterinary medicine? Yes, absolutely. And sooner than you might think.

- Dr. Royal A. McGraw



Human and bovine chromosome map comparison.

Poultry

Georgia's poultry industry dominated the state's animal agricultural dollars with nearly $2.7 billion in annual revenue in 1999. The state's poultry industry is continuing to expand as broiler production in Georgia increased from 23.1 million per week in 1998 to 23.8 million in 1999. The urbanization of northern Georgia is causing the broiler expansion to occur primarily in the state's southern section. Because of the intensive management system, poultry producers are emphasizing disease prevention. VMES scientists have responded to industry demands by developing vaccines to prevent infectious diseases. Scientists are also helping to improve poultry health by developing inexpensive, rapid, and accurate methods for disease diagnosis. Although the primary poultry health concerns are respiratory diseases, recent efforts have been initiated to control type J avian leukosis virus, a major cause of the tumor, myeloblastosis. Researchers are also focusing on the reduction of potential human pathogens on poultry products nationwide.

The effects in broilers of avian leukosis virus-subgroup J (ALV-J) infections

The purpose of this study was to document the effects of ALV-J in broiler chickens and to attempt to mitigate these effects through determination of age resistance and through use of heterologous in ovo injected antibody, and/or with homologous maternal antibody. There were no significant effects on hen/day/egg production but hen-housed production was decreased 5% to 8% because of increased mortality (30% vs. 14%). Hatch rate was depressed 10% to 30%. Individual hen ALV-J status was stable over time with no spread from hen to hen. Progeny had lateral transmission with 100% infection rate by 6 weeks of age.

Positive broilers weighed less than 65% of negative chicks from 1 to 8 weeks of age.

Bone marrow had necrosis, hypoplasia, lymphoid nodule formation, and myeloid hyperplasia and neoplasia. Lymphocytes had no differences in mitogen responses, but there were decreased circulating T lymphocytes and altered CD4+:CD8+ ratios. Heterophils had no change in resting numbers, had incorporated provirus, had decreased phagocytic/bacteriocidal activity, and had suppressed reactivity to *Staphylococcus aureus* inoculation *in vivo*. These heterophil results may explain the increased incidence of secondary infections seen in birds with ALV-J infection. Macrophages contained provirus, but their numbers and *in vivo* functions were not altered. To examine the effects on macrophages *in vivo*, studies examining reactivity to killed commercial vaccines are in progress.

Specific pathogen free (SPF) chickens injected with cloned ALV-J developed neutralizing polyclonal antibody J that was not detectable with the commercial ALV-J antibody enzyme-linked immunoadsorbent assay (ELISA) system. This means using the ELISA antibody test as an eradication tool will lead to false negative results with some ALV-J isolates. Antibodies were used to detect ALV-J in tissue sections by immunoperoxidase staining. Studies on the ability of developed neutralizing antibodies (injected *in ovo*) to increase resistance of chicks are in progress. Results to date show ALV-J spreads easily among broilers after hatching, suppressing heterophil reactions to infections. Studies in progress will show if maternal/injected antibody will protect against exposure in the hatchery and broiler house and increase chick resistance to exposure. Future studies will examine the response in ALV-J positive broilers to commercial vaccines that are macrophage dependent.

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Differential diagnosis of infectious laryngotracheitis virus (ILTV) by polymerase chain reaction (PCR)

Infectious laryngotracheitis (ILT) is a severe acute respiratory disease of chickens and is caused by ILTV. Despite efforts to control the disease through vaccination and implementation of biosecurity measures, outbreaks of ILT are still a threat to the poultry industry. To better understand the epidemiology of the disease, a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay has been developed. Restriction fragment length polymorphism (RFLP) of glycoprotein E gene with enzymes *DdeI* and *EaI* was observed among vaccine strains and field isolates. Two different patterns were observed with enzyme *EaeI* that allowed differentiating tissue culture origin (TCO) vaccine from chicken embryo origin (CEO) vaccine. All field isolates from nonvaccinated flocks were characterized as CEO-like, suggesting that CEO vaccine-derived strains may be the source of field isolates. Three RFLP patterns were observed with *DdeI*, two single patterns designated A and B, and mixed pattern C, a combination of patterns A and B. Pattern A was observed for the TCO vaccine. Pattern C was observed for five of the six commercially available CEO vaccines. Subpopulations of

viruses with patterns A and B were separated from a CEO vaccine by PCR-RFLP analysis of plaque purified CEO-derived viruses. The specific sequence producing the mixed RFLP patterns in CEO vaccine preparations was identified as either a guanine (G) or adenosine (A) at position 485 of the gE gene open reading frame. Furthermore, the viral subpopulation with RFLP pattern A appears to be the minor population within the vaccine preparation. RFLP analysis of field isolates obtained from outbreak areas showed that isolates with RFLP pattern A predominate in the field. The above suggests that selection of virulent subpopulations in the field persists in naïve bird populations. The identification of subpopulations of viruses within currently used ILT vaccines has allowed for the development of improved molecular epidemiological tools to track vaccine-derived strains and to precisely identify the source of poorly attenuated strains in the field.
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Clinical investigation of poultry diseases
Local poultry companies use the clinical teaching program to critically evaluate management and disease issues on farms, which have been designated as problem farms. Problem farms typically have a history of poor performance without immediately obvious reasons. This past year, we investigated three such farms for Harrison Poultry. All three investigations used methods to evaluate water, litter, air, temperature, and management factors. In addition, disease challenge and resulting pathology were evaluated. In all cases, constructive recommendations were made and implemented.

This project also contributes to the support of the Master of Avian Medicine (MAM) student projects. Naola Ferguson's project involves the serial passage and attenuation of the GA 98 infectious bronchitis virus (IBV). Miguel Ruano has also worked with GA 98 IBV to develop hemagglutination-inhibition (HI) antigens. The antigens are being optimized and will be used in the Poultry Diagnostic and Research Center (PDRC) diagnostic laboratory. Bill Stanley has studied the use of ultraviolet light to visualize defects in the shells of broiler breeder hatching eggs. Eggs are categorized and grouped according to appearance in ultraviolet light and set for hatching. The project is also evaluating the use of ultraviolet light to quickly differentiate high-quality hatching eggs from those with defects.
John R. Glisson, Charles L. Hofacre, and Jean E. Sander
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Bactericidal efficacy of avian β-defensins against food-borne pathogens in poultry
Antimicrobial peptides are important components of innate disease resistance in all plants and animals. β-defensins are a class of antimicrobial peptides that are widely distributed in mucosal tissues of mammals and in leukocytes of some animals. Recently, the cDNA for two turkey and two chicken leukocyte β-defensins was sequenced. Using reverse transcriptase–polymerase chain reaction (RT-PCR) and northern blot analysis, mucosal β-defensins were identified in the conjunctiva, bursa, and lungs of broiler chickens. Using immunohistochemistry, we are currently investigating the cellular distribution of this β-defensin in chicken tissues. We are developing an *in situ* hybridization technique to evaluate increased β-defensin expression in inflamed or diseased tissues. A complete evaluation of the distribution of β-defensins in chicken tissues and the changes in expression associated with diseases will clarify their role in innate disease resistance in poultry. In addition, we are in the process of expressing a recombinant β-defensin to evaluate the spectrum of antimicrobial activity against food-borne human pathogens found in poultry products as well as against primary poultry pathogens. Once the tissue distribution and spectrum of antimicrobial activity of β-defensins are known, the feasibility of improving β-defensin expression in poultry can be evaluated as a means of enhancing overall disease resistance.
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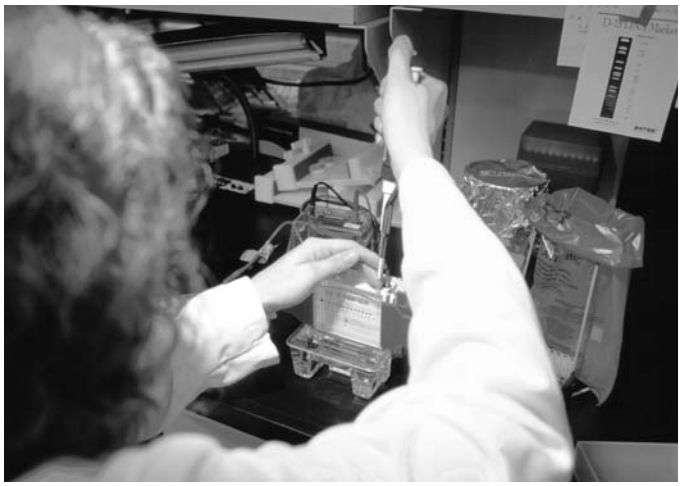
Impact of competitive exclusion on reducing the level of antibiotic resistant *Escherichia coli* in poultry and their environment
The use of antibiotics in both humans and animals contributes to the development of resistance, with a concern that there can be antibiotic cross-resistance in animal pathogens transferred to humans via consumption of animal meat. Veterinary drugs are a critical component of food animal production. For example, the leading loss to the poultry industry is *Escherichia coli* infections. Many *E. coli* isolates are resistant to the antibiotics available for use in poultry. Therefore, finding a solution to reducing the level of antibiotic resistant *E. coli* on poultry farms can help make the poultry *E. coli* sensitive again to the available antibiotics and also may aid in reducing the potential exposure of humans to any antibiotic resistant bacteria from consuming poultry meat.



A graduate student in Dr. Maricarmen Garcia's laboratory loads an electrophoretic gel apparatus during an experiment to characterize the genome of avian viruses.

This research uses the knowledge that providing the bird with the intestinal bacteria of a healthy adult chicken to a 1-day-of-age chick helps that chick resist intestinal colonization by potential pathogens (competitive exclusion), such as *Salmonella* sp., *Clostridium* sp., and *E. coli*. It was found that one dose of a commercial competitive exclusion (CE) product at 1 day of age significantly reduced the colonization of the small intestine, large intestine, and ceca by a highly antibiotic-resistant, poultry pathogenic *E. coli* (078:K80) at 7 and 14 days after challenge.The overall mean $\log_{10}$ reductions were 3.0 for the large intestine, 2.0 for the small intestine, and 1.75 for the cecum.

In a second trial in floor pens more closely simulating commercial poultry conditions, the CE given at 1 day reduced the level of antibiotic resistant *E. coli* by 2 $\log_{10}$ by 7 days after challenge.

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Control of infectious bronchitis virus (IBV)

In our ongoing efforts to type new infectious bronchitis virus (IBV) variant viruses, we examined and typed 30 of 51 samples obtained between 1997 and 1999 from Mexico. Of those isolates, eight were Mass, six were Mexican variant 97-8147 (BL56), five were 97-8123, five were a new variant (#1), four were a different new variant (#2), one was Ark, and one was Conn. After modification of our reverse transcriptase–polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) test to detect the DE072 type last year, we began to observe variant RFLP patterns. In addtion, based on sequence data, virus-neutralization testing, and protection studies, we identified a new serotype of IBV designated Georgia 98. In our efforts to control IBV, we have tested a DNA vaccine and are working on *in vitro* expression of spike protein. Some serotype cross-protection was observed when a DNA vaccine and modified live viruses were used to vaccinate chickens. *In vitro* expression of spike has been difficult. We have obtained a new expression vector and plan to use a more sensitive detection test to aid our efforts to access the suitability of *in vitro* expressed spike as a vaccine for IBV.

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Avian mycoplasmosis

The avian mycoplasmas *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis* (MM), and *Mycoplasma iowae* (MI) are egg-transmitted infections causing respiratory, reproductive, and joint and tendon disease in chickens and turkeys. The study's objectives were to improve detection and control measures for avian Mycoplasma infection, to study their pathogenesis, and to determine the incidence of avian mycoplasmas by DNA fingerprinting.

Live MG vaccines are an effective control measure in commercial layers, but escape of the vaccine strain to neighboring poultry flocks is a concern. DNA fingerprinting has enabled us to readily identify such occurrences. A vaccine strain isolated from commercial egg layers was shown to be somewhat more pathogenic than the original vaccine strain, suggesting that the vaccine strains may become more pathogenic when allowed to spread from bird to bird. DNA fingerprinting has identified a commercial egg flock infected with a *M. gallisepticum* strain, which is very similar to the strain found in wild house finches. Fingerprinting methods were improved; it is no longer necessary to isolate the organism in pure culture before fingerprinting.

Chickens were challenged with a strain of *Mycoplasma pullorum*, which had been associated with clinical respiratory disease in Cuba. The strain appears to be only marginally pathogenic.

These results improve our ability to detect and control MG and other mycoplasmas in commercial poultry.

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Rapid identification and epidemiological typing of food-borne pathogens in nondomestic birds by polymerase chain reaction (PCR)

Salmonella has been implicated in large-scale die-offs of wild birds in the United States. Although we know quite a bit about the epidemiology of *Salmonella* among domestic fowl, we know little about the incidence, epidemiology, and genetic relatedness of *Salmonella* in nondomestic birds. To gain further insight on *Salmonella* in these hosts, 22

Dr. Charles Hofacre performs a necropsy on a chicken, the necessary first step in determining the presence or absence of potentially pathogenic strains of E. coli.

Salmonella isolated from diseased, nondomestic birds were screened for the presence of virulence and antibiotic resistance-associated genes as well compared genetically using pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD). Of *Salmonella* examined (n=22), 15 isolates contained virulence genes commonly associated with pathogenic *Salmonella* including the virulence plasmid and invasion genes. Most *Salmonella* were generally sensitive to antibiotics. However, two *Salmonella* isolates from pet birds were identified as the multi-drug resistant *Salmonella typhimurium* DT104. Despite the general susceptibility of these *Salmonella* to most antimicrobial agents, antibiotic resistance-associated genes were identified in several of these isolates. Five distinct *Xba*I and nine distinct *Bln*I DNA patterns were observed for the 22 *Salmonella* isolates typed by PFGE. PFGE analysis determined that *Salmonella* isolates from passerines in Georgia and Wyoming were genetically related.

According to genotype and antibiotic resistance pattern, the *Salmonella* associated with captive and free-ranging, nondomestic birds represent a potential threat to humans. Most *Salmonella* isolates possessed the 90 kb virulence plasmid, enabling the organism to colonize and spread in its avian or nonavian host. The other troubling news is the identification of the multi-drug resistant *S. typhimurium* DT104 from pet birds. As far as we are aware, this is the first reported case of *Salmonella* DT104 from exotic birds in the United States. Nondomestic birds can obviously serve as a reservoir for transmission of *Salmonella* to pet owners and bird watchers. Bird feeders are popular in the United States, and determining the incidence of *Salmonella* at these feeders is important in order to assess their health risk to the general population.
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Adaptive response(s) of avian tendon to injury
Spontaneous tendon failure in heavy meat-type birds is poorly understood. This proposal will correlate the effects of altered load in the *in vitro* and *in vivo* expression of matrix proteins; fibrillar procollagen I and collagen III; aggrecan and decorin; and expression of regulatory proteins TGF, CTGF, and HSP 47. Specific aims of this study are first, to determine the roles of TGF and HSP 47 and their interaction in the expression of embryonic and posthatch fibrillar collagens; second, to define the biomechanical parameters of gastrocnemius tendons undergoing stress-related structural changes; and third, to determine structural changes in collagen(s) and proteoglycans in gastrocnemius tendons subjected to altered load. Exposure of female broiler chickens to immobilization and treadmill pacing resulted in significant changes in body weight, shank length, and shank width caused by immobilization. Overall tibia bone mineral content (BMC) and bone mineral density (BMD) were not significant with treatments; however, normalization of BMC and BMD by tibia length showed a significant effect of immobilization at 5 and 6 weeks. Maximal shear load in treadmill-paced birds was significantly decreased in comparison to control. Preliminary findings regarding exposure of broiler chickens to heat and 3-Nitro show a correlation between reduced shear strength, increased BMC, and 3-Nitro. Heat and, to a degree, TGF increased the expression of HSP 47 in cultured chicken embryonic tenocytes. This increase was time and concentration dependent. Addition of enrofloxacin to chicken embryonic tenocytes maintained in serum-supplemented medium led to a significant decrease in synthesis of decorin. In serum-free cultures, decorin synthesis increased.
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**Biological Agricultural Engineering*

Investigation into factors affecting hatchability and chick quality
Commonly used disinfectants can have an adverse effect on hatchability and chick quality if used in high doses. Increasing the activity of these disinfectants would allow the use of a lower concentration and result in fewer potentially toxic effects on the hatching egg and resulting chick, while maintaining a low level of bacteria in the air within the incubators. This study evaluated the effectiveness of a quaternary ammonium compound (Biosentry 904 and Timsen) misted into egg incubators according to commercial guidelines against an *Escherichia coli* bacteria known to be pathogenic to chickens. These products were also misted into a second incubator at a reduced rate either unaltered or potentiated with EDTA-Tris in a ratio shown to increase the activity of the disinfectant against this particular bacteria in the laboratory. These combinations were compared with distilled water to determine if the addition of these disinfectants in the environment had a significant difference on egg shell permeability and egg weight or moisture loss during the incubation period. Aerosol bacterial counts were measured inside the incubators during incubation. Fertility and hatchability of the eggs exposed to the various treatments was evaluated. The egg of embryonic mortality was determined. Chick weights were measured at 1 day of age as was yolk sac weights and incidence of contamination. Two-week livability was evaluated.
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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 instructional experiences for students in the Master of Avian Medicine (MAM) and other graduate programs.

Improvements continue to be made to the new lab database with completion anticipated for summer 2000. Laboratory reports are being provided by e-mail, fax, and phone, which has significantly

reduced reporting time. Other automation is being investigated, and specialized in-house reports are being designed to streamline lab management.

The polymerase chain reaction (PCR) technique has become an integral part of the diagnostic laboratory as seen in the continuing increase in the demand for these tests. PCR techniques for infectious bronchitis virus have been significantly improved to provide same-day results with virtually no down time. A new mycoplasma PCR technique implemented last year has been refined further to improve turnaround time and reliability. Other techniques for performing large volume processing of PCR samples are being pursued and will provide even better turnaround for large samplings. Research continues and new PCR tests will be applied to diagnostics as applications are developed and as other ways to improve service to poultry industry clients are being investigated.

Diagnostic Services Laboratory activity is represented by 6,090 accessions, 26,300 bacterial procedures, 300 antimicrobial susceptibilities, 60,485 enzyme-linked immunoadsorbent assay (ELISA), 26,400 infectious bronchitis virus-hemagglutination inhibition (IBV-HI) tests, 30,300 histopathology slides, 2,600 diagnostic PCR tests, 3,042 mycoplasma cultures, 26,191 mycoplasma plate and hemagglutination inhibition (HI) tests, and 1,452 necropsies.

Stephan G. Thayer, Thomas P. Brown, Stanley H. Kleven, Mark W. Jackwood, Maricarmen Garcia, John R Glisson, George N. Rowland, Pedro Villegas, Jean E. Sander, John J. Maurer, and Stanley A. Vezey
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Methods for isolation, identification, and control of avian viruses

Avian viral populations that present a continuous threat to both the U.S. and Georgia commercial poultry industry are continuously monitored by our laboratory. More than 800 virus isolation cases were submitted to our laboratory last year. The basis of our research is to improve and advance the methods used for rapid diagnosis and as a result control viral diseases that affect the poultry industry worldwide. Specific projects involving infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), and avian leukosis virus subgroup J (ALV-J) are the focus of our laboratory's research.

Variant strains of IBV, which are common to the poultry industry worldwide, are persistently isolated from flocks exhibiting clinical respiratory disease. Protection provided by commercial vaccines is often inadequate when a variant strain of IBV infects a poultry flock. As a result of these inadequacies, our laboratory has studied the recombination potential of IBV in embryos and in chickens. These recombinant viruses, along with isolated variant IBV viruses, are evaluated by challenge studies in order to develop products that provide better protection against IBV variants.

IBDV, which can cause clinical disease and mortality along with immunosuppresion in chickens, has been characterized in our laboratory by both molecular and clinical analysis. Molecular techniques have been used to identify variants of IBDV. Like IBV, variant strains of IBDV are problematic to the poultry industry because of their ability to circumvent application of vaccines and maternal antibod-

Dr. Carlos Estevez uses the UVP Biodoc-IT System for rapid and accurate analysis of DNA migration patterns in an agarose gel following electrophoresis.

ies. Our laboratory has characterized unusual variant IBDV isolates using both molecular methods and pathological trials in commercial broilers.

Condemnations at processing plants, mortality with or without tumors, decrease in egg size and production, and depletion of chick uniformity are some of the fatalistic effects caused by ALV-J. Classical virological assays for ALV-J identification and detections have been established in our laboratory and are currently being compared with modern molecular assays. Possible interactions between very virulent Marek's Disease Virus (vvMDV) and ALV-J were studied in meat-type chickens. Dual infection produced with ALV-J and vvMDV appeared to prolong ALV-J induced viremia in birds that were not vaccinated against MDV. Molecular studies have demonstrated the similarities of some of the U.S. ALV-J isolates with U.K. strains.

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Beta carotene modulation of Marek's disease virus infection rate and tumor development in commercial broilers

Marek's disease (MD) is still the most common lymphoproliferative disorder affecting young chickens because of the continuous emergence of new, more virulent strains, resulting in increased rates of MD condemnations. Prevention through vaccination has promoted a sustained immune selection pressure that periodically has produced an emergence of strains with increasing virulence. The recently described very virulent plus (vv+) strains can promote a severe early immunosuppression, most likely caused by an increased replication rate of MDV. This higher replication rate produces a more severe early cytolytic infection, resulting in a dramatic lymphoid depletion that leads to an early mortality syndrome. This high replication rate can either suppress the immune system so severely that infection results in early mortality syndrome or sufficiently suppress the immune system to induce tumor formation by vvMDV strains.

Because conventional vaccines cannot prevent serious outbreaks, alternative methods to control MD should be considered. Immunomodulation systems that hamper the early viral replication process may be useful. β-carotene is considered a good candidate for immunomodulation because it consistently upregulates natural killer (NK) cell function in both *in vivo* and *in vitro* studies. Innate immunity by NK cells has been documented to be important in the decrease of tumor formation. In fact, long-term β-carotene supplementation in elderly humans helped to decrease the occurrence of tumors, more likely via NK cell stimulation. Moreover, viral infections, particularly those caused by herpesviruses, are known to be quite susceptible to the control by NK cells.

In our studies, the downregulation of MD infection rate in spleen cells was evaluated by using β-carotene, which has proved to modulate chicken cell-mediated immunity. The daily oral administration of the immunomodulator in male broilers effectively reduced the infection rate, with significant differences between treated and nontreated birds at 8, 12, and 16 days postinoculation. The tumor observations significantly decreased in β-carotene treated birds, and the results of splenocyte immunophenotyping showed that the proportion of cells expressing MHC-II molecules between nonmodulated and modulated MD virus infected birds was similar, but the proportion of activated CD4 cells was significantly lower in the birds receiving β-carotene.

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The goal of the Poultry Diagnostic and Research Center (PDRC) is to provide Georgia poultry growers with healthy and productive flocks.

Fish

Georgia's aquaculture industry is steadily expanding, with its greatest increase occurring in channel catfish production. Over the last five years, pond acreage for catfish farming has grown from 6,000 to approximately 8,000 acres. In addition to Georgia's developing food fish industry, there is an increasing interest in ornamental fish production. 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 and productivity on the farm. Fish health is a key issue for increased productivity. 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.

Immunoregulation of streptococcal infections in tilapia: Apoptosis versus necrosis

In the present study, the *in vivo* responses of tilapia nonspecific cytotoxic cells (NCC) following injection with different isolates of intact killed *Streptococcus iniae* was investigated. Tilapia injected intravenously with killed *S. iniae* produced different cytotoxicity responses compared with fish injected intraperitoneally. Within minutes following IV injection, NCC cytotoxicity from the PBL increased 100% compared with naïve controls. *S. iniae* strain #173 produced activation of cytotoxicity compared with isolates #164 and ATCC. Evidence for soluble factor involvement in increased cytotoxicity was obtained by passive activation of NCC with serum from #173 injected fish. Studies were also performed to study the mechanisms of passive activation. Flow cytometric analysis revealed that NCC from spleen, anterior kidney, and PBL constitutively expressed cytosolic (not membrane) Fas ligand (FasL). Stress serum treated NCC obtained from the PBL produced an increase in the expression of of FasL, CAS, and FADD by Western blot examination. In other experiments, soluble FasL (sFasL) was secreted by NCC following ligation with tumor cells. Inhibition of killing of Fas receptor positive HL-60 target cells was observed following treatment with either anti-FasL or anti-FasR monoclonal antibodies. These studies demonstrated that for catfish and tilapia, initial target cell conjugate formation was required; however, the terminal killing mechanism depended on at least two different pathways of cytotoxicity. One pathway was by release of preformed sFasL (i.e., tilapia), and a second pathway has yet to be determined.

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Molecular analysis of the NCCRP-1 gene from tilapia

Nonspecific cytotoxic cells (NCC) are the best characterized effectors of natural immunity in teleost fish, and they are believed to be the evolutionary precursor of mammalian natural killer (NK) cells. Activation of NCC occurs by triggering a membrane receptor protein, NCCRP-1, which is necessary for innate immune functions of NCC. We have direct evidence that NK cells in other animals express an NCCRP-1 homologue. We have sequenced the complete cDNA from catfish NCCRP-1, and we now have USDA funds to characterize the gene in the same species. The central hypothesis of this grant proposal is that characterization of the NCCRP-1 gene from tilapia will provide invaluable insights into the function of this molecule in tilapia NCC and will also provide important phylogenetic information about the level of conservation of such an immunologically relevant protein. Our goals are to understand how NCC and NCCRP-1 provide innate immunity against protozoan parasites, pathogenic bacteria, virus-infected cells, and immune surveillance against tumor cell outgrowth in teleost fish. As our next objective in pursuit of our research goals, we propose in this application to clone and sequence the NCCRP-1 gene from tilapia. Information gathered from these studies will be crucial to determine the molecular features that are necessarily conserved between a phylogenetically older (catfish) and a more recent (tilapia) species, features that allow for maintenance of the functional characteristics of the receptor. These clues will increase the present body of knowledge in tilapia immunity: there are no genetic sequences of immunity-related genes in this species. Furthermore, sequence information will be used to find the most conserved regions to search for the mammalian homologue of NCCRP-1.

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Development of poly- and monoclonal antibodies against *Piscirickettsia salmonis*

Piscirickettsia salmonis is the first of the previously unrecognized obligate intracellular group of fish pathogens isolated, characterized, and demonstrated to be the etiologic agent of an epizootic disease. *P. salmonis* produces an epizootic disease of fish called piscirickettsiosis. The mortality attributed to piscirickettsiosis in salmonids ranges from a high (up to 90%) among coho in Chile to a low of 0.06% in Canada and Norway. All species of salmonids

(coho, masu, Atlantic, chinook, and rainbow trout) cultured in Chile are affected by this disease, but the greatest mortality occurs in coho salmon cultured in salt water. We hypothesize that the *P. salmonis* isolates from various geographical locations will present different protein repertoires. This variation in protein expression may represent the differences in virulence.

To begin the process of assessing the diversity and exploring the pathogenesis, the overall goal of this proposal is to develop immunologic tools that can be used in the identification, characterization, and cloning of immunoreactive *P. salmonis* proteins. Once these proteins have been identified, their use as diagnostics and vaccine candidates will be determined. This will be accomplished by the following: 1) produce rabbit polyclonal antibodies against *P. salmonis* to be used as a diagnostic tool and in the future to screen a genomic library for immunoreactive proteins; 2) develop mouse monoclonal antibodies against *P. salmonis* to be used for diagnostics, especially in differentiating isolates. These monoclonals will be used for *in situ* experiments to begin exploring the cellular pathogenesis of this bacterium and in screening genomic libraries.

During the last year, protocols have been developed to produce sufficient quantities of the intracellular bacterium – *P. salmonis* – to be used in producing polyclonal antibodies in rabbits, monoclonal antibodies in mice, and serve as the antigen in an antibody detecting screening enzyme-linked immunoadsorbent assay (ELISA). *Piscirickettsia salmonis* is an obligate intracellular bacteria and tends to stick to the host membranes. A major obstacle was to remove host-cell antigens from the bacterial preparation. A differential centrifugation, membrane filtration, and buoyant density centrifugation protocol has been developed and produces viable bacteria free of host-cell antigens. Although the recovered bacterial sample is free of host-cell antigens the protocol results in the recovery of only 0.6 to 3.0% of the initial tissue culture infectious dose titer. The low recovery necessitates the production of a large amount of infected cell cultures to produce sufficient antigen. Currently, sufficient bacterial antigen has been produced and shipped to the UGA monoclonal antibody facility and monoclonals are in development. A cyclophosphamide treatment to reduce the mouse response to host-cell antigens will be followed to enhance the production of *P. salmonis* specific monoclonal antibodies and to reduce the screening effort.

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Dr. Liliana Friedmann dilutes bacteria with media for a cloning experiment with the NCCRP-1 gene from tilapia.

Cattle and Other Ruminants_

Cattle, sheep, and goats are three of Georgia's important food-animal ruminants. They are considered ruminants because their four-chambered stomach enables them to digest copious roughage, which is inedible for direct human consumption. These three industries have gone through recent dynamic changes. The beef and dairy industries have liquidated their herds because of a relatively large cattle supply and low milk and beef prices. Today's cattle producers are working with narrow profit margins and must watch their expenses more closely than ever. Consequently, biomedical researchers are providing these industries with ways to maintain healthy animals, which will help reduce production costs. Mastitis, Johne's disease, pasteurellosis, pneumonia, infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), parainfluenza-3 (PI-3), and leptospirosis continue to challenge the immune systems of Georgia's cattle herds. Ruminant herd health as it pertains to food safety is also a major concern to consumers and producers. Scientists need to investigate pathogenic *Escherichia coli, Salmonella, Campylobacter*, and other food-borne organisms as to their origin, transmission, and prevalence.

Photochemical elimination of viruses from bovine embryos produced *in vitro*

A major problem recognized by the bovine embryo transfer industry is the tenacity with which infectious viruses adhere to *in vitro* produced (IVP) embryos. In contrast to *in vivo* produced embryos (i.e., those flushed from reproductive tracts of donor cows), the current International Embryo Transfer Society (IETS) recommended that washing and trypsin treatment is ineffective for rendering IVP embryos virus-free. Two approaches to viral elimination have been investigated and involve treatments of IVP zygotes (1) with a more potent enzyme and (2) with photosensitive chemical agents in combination with light. Exposure of IVP zygotes to a nonspecific protease from *Streptomyces griseus* led to the finding that more than 45 seconds irreversibly damaged the embryos. Exposure of oocytes to epizootic hemorrhagic disease virus serotype 2 (EHDV-2) during *in vitro* maturation was followed by inhibited cumulus cell expansion and markedly reduced embryonic development ($p<0.05$). There were no differences in development among virus-exposed groups of IVP zygotes receiving no treatment or treatment with trypsin or protease. However, proportions of infected embryos were reduced after protease treatment versus positive (infected) controls and trypsin-treated (infected) embryos. In other experiments, developmental potential of zygotes and cell numbers of resulting hatched blastocysts were assessed after exposure to helium neon laser light, photosensitive chemicals hematoporphyrin (HP) and hypericin (HY), and a combination of light with each chemical treatment. Conditions were found that did not compromise development. Then zygotes were exposed to EHDV-2 and subsequently exposed to HP or HY and light. HP plus 3 minutes of light exposure was effective at EHDV-2 inactivation (not different from negative control), whereas HY + 3 minutes of light was less effective in elimination of viral infectivity.

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Multidisciplinary evaluation of fatal feedlot ARDS

For cattle producers in Georgia and across the United States, respiratory disease is an important cause of expense because of treatment costs, production loss, and mortality. In feedlots a unique form of respiratory disease, the acute respiratory distress syndrome (ARDS), causes severe disease that often ends in death. Feedlot ARDS is particularly devastating because it tends to affect animals that have been on feed for more than 45 days and that are otherwise apparently very healthy. Thus, feedlot owners lose animals in which they have already invested a significant amount of time and money. Although feedlot ARDS has been recognized for at least two decades, the cause is still unknown. Dietary components, environmental dust, and bacteria and viruses, which cause other types of respiratory disease, have all been implicated in feedlot ARDS.

VMES funded research carried out by Dr. Larry Hawkins and Dr. Amelia Woolums will be aimed at determining the cause of feedlot ARDS. Dr. Hawkins and Dr. Woolums will conduct a case-control study of risk factors for feedlot ARDS. Data regarding management and environmental factors collected from feedlots where ARDS deaths occur will be used in the analysis. The role of bacterial and viral pathogens will be also be evaluated. Lung tissue from animals with signs of fatal feedlot ARDS will be tested for common respiratory pathogens. The results will be compared with those from animals without signs of ARDS to determine which pathogens are associated with ARDS. The results of these studies will answer long-posed questions regarding the role of infections and environmental factors in feedlot ARDS and will decrease losses to producers by clarifying factors amenable to control to minimize morbidity and mortality in cattle.

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Emergence of florfenicol resistance in veterinary pathogens

Florfenicol is the newest antibiotic in the arsenal available to food-animal veterinarians. It was approved by the FDA for use in beef cattle in 1996, and resistance to this drug has already emerged in *Salmonella* and *Escherichia coli* isolated from diseased animals in Georgia. We have determined that these bacteria contain the same gene, which confers resistance to florfenicol, suggesting that the gene is transmissible to different types of bacteria. We have also determined that the incidence of florfenicol resistance is low except in situations of prolonged and frequent use of the antibiotic. Florfenicol has a broad spectrum of effectiveness, and because it is not used in humans, it is an attractive drug for food-animal use. Prudent use of this antibiotic may extend the life of this drug as an effective antimicrobial for food-animal medicine.

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Natural and experimental *Ehrlichia* infection in small ruminants

Cowdria ruminantium is the causative agent of heartwater. Although currently an exotic disease, heartwater is endemic on some Caribbean islands and has great potential for introduction into the Southeast. Serologic tests for *C. ruminantium* have been shown to result in false-positives when ruminants are naturally exposed to antigenically related *Ehrlichia,* including *Ehrlichia chaffeensis.*

To evaluate the susceptibility of goats to infection with an antigenically related *Ehrlichia* endemic in deer in Georgia, naïve goats were experimentally infected with *E. chaffeensis* and then monitored for development of antibodies to *E. chaffeensis* and *C. ruminantium* and for evidence of active infection by molecular diagnostic assays and cell culture. In a parallel field survey, blood samples were collected from goat and sheep herds and tested for evidence of natural exposure to or infection with *E. chaffeensis*. We found clear serologic, molecular, and cell culture evidence of natural infection with *E. chaffeensis* in goats, but not sheep, kept on pasture. However, our experimental infection trial failed to produce active infection with this agent in goats. Results of serologic assay for *C. ruminantium* on samples from the experimental infection trial and from the field are not yet available. Subsequent studies will be needed to resolve the apparent discrepancy between evidence of natural infection with *E. chaffeensis* in goats and the failure of our experimental trial to produce evidence of active infection.

The data gathered from this study helped characterize the susceptibility of small ruminants to infection with endemic *Ehrlichia*, enabling more accurate interpretation of *C. ruminantium* serology. In addition, the data from this study may eventually lead to identification of the organism(s) responsible for false-positive heartwater test results, facilitating development of more specific diagnostic tests. Improved control measures will ultimately result.

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Cytokine and T-cell responses to mucosal BRSV vaccination

In Georgia and across the United States, respiratory disease is a leading cause of sickness and death in cattle, particularly in calves. Bovine respiratory syncytial virus (BRSV) is a common cause of this problem. Although BRSV vaccines exist, their efficacy is debatable; moreover, vaccination has at times appeared to enhance disease. Research aimed at the development of safe and effective BRSV vaccination strategies could decrease the incidence of pneumonia in cattle. BRSV research will also improve our understanding of the closely related human respiratory syncytial virus (RSV), a leading cause of colds and pneumonia in children. Development of an effective RSV vaccine has been particularly problematic, and no RSV vaccine is currently available.

VMES-funded research conducted by Dr. Amelia Woolums and Dr. Corrie Brown will focus on a little-used means of vaccination to protect calves against BRSV infection. Rather than a conventional intramuscular vaccine, calves will receive intranasal vaccination before BRSV infection. Intranasal vaccination should provide superior protection by stimulating a strong response at the surface of the respiratory tract where the virus first attaches.

The immune response is controlled in part through the release of proteins called cytokines by T lymphocytes and other cells. Preliminary research suggests that vaccines can be tailor-made to induce T-cell and cytokine responses that are helpful and not harmful. Dr. Woolums and Dr. Brown will measure levels of cytokines in calves receiving intranasal vaccination to determine how this approach affects cytokine balance. Their research will thus determine whether a new approach to BRSV vaccination can lead to improved protection of cattle; it will also form the basis for future studies of vaccines uniquely designed to prevent and not enhance disease.

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Dr. Amelia Woolums examines a calf as part of a study to protect them from acute respiratory distress syndrome (ARDS).

Horses

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. The list goes on. 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. To dissect these mechanisms, these researchers are using state-of-the-art techniques to get at the very heart of the problems. This year's VMES-funded studies on diseases of horses take this approach. One study deals with the identification and characterization of genes in equine intestinal parasites that control the parasite's ability to resist the effects of anthelmintic compounds (dewormers). A second study focuses on the molecular mechanisms responsible for the life-threatening effects of bacterial endotoxins And another study characterizes the mechanisms by which new solutions prevent the formation of intra-abdominal adhesions. By approaching clinically important problems from a mechanistic point of view, these researchers are making strides that will help control or eliminate these important problems.

Antibiotic-induced tumor necrosis factor activity in septicemic foals

Gram-negative bacterial septicemia is the most common cause of death in newborn foals. The presence of gram-negative bacteria in the blood of foals is significantly correlated with mortality, and at least 50% of septicemic foals are endotoxemic. Endotoxin, a lipopolysaccharide constituent of gram-negative bacteria, is released as bacteria rapidly grow or die. Once liberated, endotoxin activates white blood cells in the circulation to release a variety of inflammatory mediators, such as tumor necrosis factor (TNF). The net effect of endotoxin-induced mediator release is cardiovascular collapse, multi-system organ failure, and death.

The working hypotheses of this proposal were that 1) the amount of endotoxin released from gram-negative bacteria during treatment of septicemia is dependent on the type of antibiotic used and, 2) antibiotic-induced release of endotoxin contributes to the synthesis of TNF. The goal of this research was to determine which antibiotics used to treat the most common pathogen (*Escherichia coli)* involved in equine neonatal septicemia induce the least amount of TNF activity while maintaining bactericidal effect.

Mononuclear cells were isolated from 50 ml of blood collected from five healthy foals by density gradient centrifugation. Mononuclear cells (3 x 106) were suspended in 1 ml of RPMI-1640 media and placed into sterile polypropylene culture tubes. 1 x 105 colony forming units of *E. coli*, isolated from a septicemic foal was added to each tube. Either 2 times or 20 times the minimal inhibitory concentration (MIC) of each of the following antibiotics was added: amikacin with ampicillin, ampicillin, amikacin, ceftiofur, or imipenem. Saline was added to tubes as a control for the antibiotics. Mononuclear cells were incubated for 6 hours at 37°C. At the end of incubation, supernatant was collected and tested for TNF, using a WEHI bioassay.

Compared with control tubes (saline and bacteria), treatment of blood containing *E. coli* with ampicillin, ceftiofur, or imipenem significantly increased TNF activity. Tubes containing *E. coli* and amikacin or amikacin with ampicillin had less TNF activity, compared with tubes containing *E. coli* and saline and significantly less TNF activity compared with tubes containing *E. coli* and ampicillin, ceftiofur, or imipenem. Tumor necrosis factor activity was not dependent on the concentration of antibiotic. Analysis of endotoxin data is pending.

Although antibiotics are necessary for the treatment of *E. coli* septicemia, antibiotic therapy induces TNF activity. The results of this study indicate that amikacin is least likely to promote TNF activity during the treatment of *E. coli* neonatal septicemia. If ampicillin, ceftiofur, or imipenem are used for treatment of septicemia, their use should be accompanied by concurrent treatment for endotoxemia.

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Characterization of anthelmintic resistance-associated genes in equine cyathostomes

Control of gastrointestinal parasites remains one of the most important health-related concerns facing the equine industry. This problem recently has been magnified by the increased prevalence of anthelmintic resistance, which is recognized globally as one of the greatest health threats to grazing livestock. Extensive reliance on chemical control for equine cyathostomes (small strongyles) has led to the development of resistance to all classes of available anthelmintics except the avermectin/milbemycins (ivermectin, moxidectin; AM). However, most parasitologists agree that it is a question of when, not if, resistance to this drug class will appear. When resistance to the AM drugs does develop in the cyathostomes, the frequent and widespread movement of horses will lead to rapid dispersion of resistant parasites, causing the incidence of morbidity and mortality from parasitic disease to rise dramatically.

Our long-range goal is to prevent or manage the development of anthelmintic resistance in parasites, thereby ensuring that chemotherapeutic control will remain possible. Over the past year, we have made significant progress in achieving this goal. First, we have started a selection protocol using moxidectin in a group of nine ponies for the purpose of producing moxidectin-resistant cyathostomes. Over time, these worms will provide a characterized population in which we can study the genetics of AM resistance. We have also cloned and sequenced a full-length glutamate-gated chloride channel gene from *Cylicocyclus nassatus* (Cyathostominae). This gene codes for the protein that serves as the target of AM drugs and has been demonstrated to be strongly associated with ivermectin resistance in *Caenorhabditis elegans*.

We are also in the process of characterizing specific mutations in the β-Tubulin gene, which is the gene that codes for the protein target of benzimidazole (BZ) anthelmintics. We have cloned this gene from numerous individual worms of four different cyathostome species that were collected from three different worm populations with differing sensitivities to BZ drugs. In those clones sequenced so far, we have identified two separate mutations located in the β-Tubulin-BZ binding sites that have been associated with BZ resistance in other organisms. One of these mutations has not been previously reported in Strongyle parasites. We are continuing to obtain more sequences from these clones and should soon be able to correlate specific mutations with BZ resistance. Collectively, these outcomes will help to provide the framework necessary to establish the genotypic basis for resistance to AM and BZ drugs that will enable the future development of molecular assays to detect the emergence of resistant worms. This is expected to have significant positive effects on equine health, because it will enable the detection of anthelmintic resistant cyathostomes on individual horse farms or in individual horses, providing a means to prevent and control their spread.

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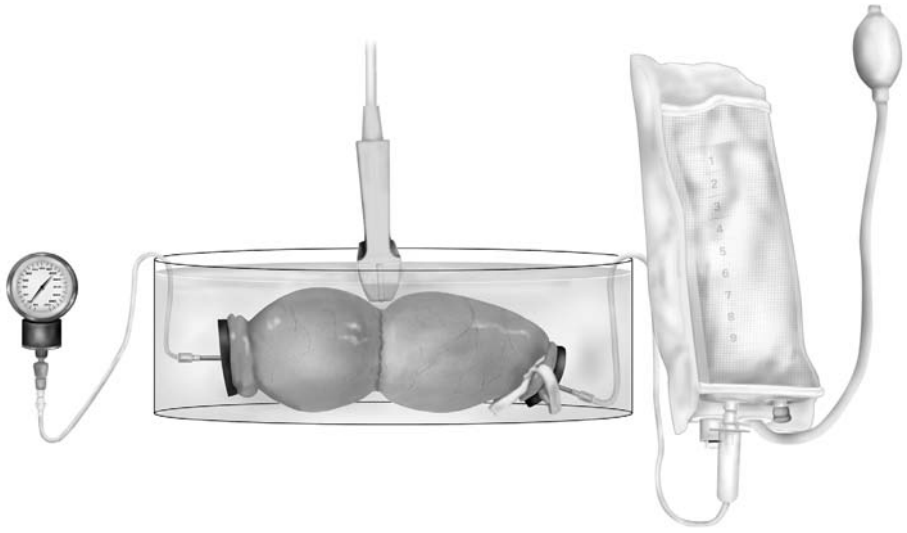

Diagram of the method used to evaluate stomal diameters at the anastomosis in isolated segments of equine small intestine. Ultrasonographic images were acquired in the transverse plane, and the stomal diameters and circumferences were obtained and averaged.

Effect of intra-peritoneal solutions on adhesions and jejunal anastomoses in horses

Small intestinal strangulations are one of the most serious causes of abdominal pain in horses. Treatment often involves resection and anastomosis of the affected segments of intestine. Despite surgical intervention, mortality rates are unacceptably high. In many cases, this poor prognosis for survival is caused by the postoperative development of intra-abdominal adhesions. This study compared the use of sodium carboxymethylcellulose (SCMC) or sodium hyaluronate solution (HA) with three hand-sutured jejunal anastomoses techniques with respect to adhesion formation, intestinal bursting tension, and histologic quality of healing. We hypothesize that a single-layer, appositional anastomosis coated with SCMC or HA would provide a reliable method of small intestinal anastomosis in horses. A ventral midline celiotomy and a jejunal resection and end-to-end anastomoses were performed in 18 horses. The anastomoses were closed with either a two-layer inverting pattern (group 1, n=6), single-layer appositional pattern coated with SCMC (group 2, n=6), or single-layer appositional pattern coated with HA (group 3, n=6). All horses were euthanatized 21 days after surgery. The abdominal cavity was evaluated for adhesions and anastomotic healing. Bursting tension measurements were performed on all anastomoses. Histopathology was performed to evaluate anastomotic healing. All anastomoses healed normally. Fibrous adhesions were associated with the anastomoses in 3/6 horses from group 1, 2/6 horses from group 2, and 2/6 horses from group 3. The mean bursting tension of the anastomoses for group 1, 2, and 3 horses were 890 ± 143, 1,373 ± 262, 1,152 ± 372, respectively. All intestinal segments failed at a point distant to the anastomoses. The results of this study suggest a single-layer, appositional anastomosis coated with SCMC or HA may provide a safe and reliable method of small intestinal anastomosis in horses.

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Cloning of equine toll-like receptors involved in mediating endotoxin responses

Toll-like receptors 2 and 4 (TLR-2 and TLR-4) have been implicated as the transmembrane receptors responsible for activation of mononuclear cells by endotoxin, a component of gram-negative bacterial cell walls. Physiological concentrations of endotoxin cause cytokine synthesis by monocytes through TLR-4, whereas evidence in other species indicates that TLR-2 may play a pivotal role in the response of mononuclear cells to toxic components of gram-positive bacteria and mycobacteria.

To provide a basis for defining the role of TLR-2 and TLR-4 in mediating the proinflammatory response to bacterial products in horses, we have cloned and sequenced equine TLR-2 and TLR-4 from an equine monocyte cDNA library. Our 3.3 kb equine TLR-4 cDNA has an open reading frame corresponding to 843 amino acids and 113 and 654 bp of 5' and 3' untranslated region, respectively. The sequence of the equine TLR-4 protein is 76%, 66%, 69%, and 66% similar to sequences for the human, mouse, hamster, and rat TLR-4 proteins, respectively. Our 2.8 kb equine TLR-2A cDNA clone contains an open reading frame corresponding to 748 amino acids and a 541 bp untranslated region and 19 bp poly A tail at the 3' end. This clone lacks a 5' untranslated region, ATG start codon, and the region encoding the first 26 amino acids of the protein relative to that of other species. A second round of library screening resulted in the isolation of a second equine TLR-2 clone. This clone contains a 1,576 bp insert, is 106 bp shorter than the first clone at the 5'end, and contains a 1,129 bp deletion towards the 3' end relative to that of the first clone. However, the remaining sequence of both clones are identical. Finding the 1,129 bp deletion in an equine TLR-2 cDNA clone was unexpected. The horse from which the cDNA library was originally constructed had chronic obstructive pulmonary disease (COPD), a condition characterized by a chronic inflammatory response in the lungs. Because it has been proposed that horses with COPD may have a genetic predisposition to develop the condition, we are currently screening healthy and COPD horses for the presence of this deletion mutation.

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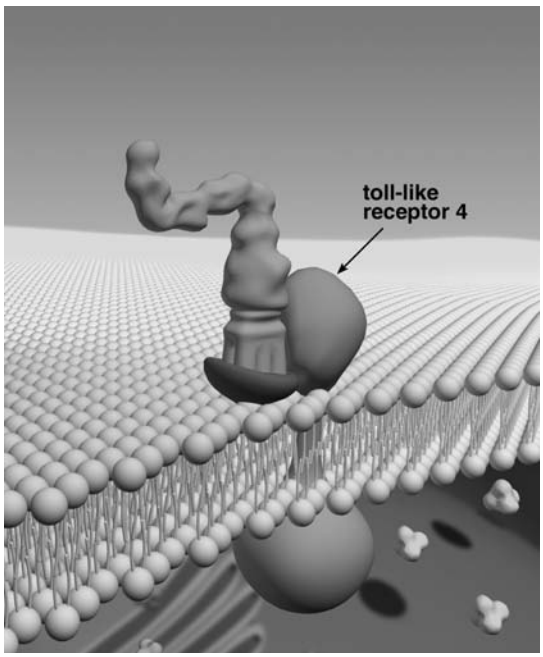

An artist's depiction of the interaction between bacterial endotoxin and the extracellular component of toll-like receptor 4 on the surface of an equine mononuclear phagocyte. As a result of this interaction, a signal is transmitted to the interior of the cell where proinflammatory cytokines are synthesized.

Swine

The productivity of swine herds continues to improve nationally. Much of this improvement is because of new technology such as environmentally controlled housing, artificial insemination, and high health strategies such as multiple-site production. This innovative technology along with macroeconomic pressures continues to reshape the structure of the swine industry.

In spite of these economic changes, however, there are significant pig health concerns that continue to challenge producers. Additionally, food-safety challenges continue to increase in importance. Georgia scientists are focusing on pig health concerns such as porcine reproductive and respiratory syndrome (PRRS) and Psueudorabies. Food-safety concerns including farm-level control of *Salmonella*, *Campylobacter*, and Mycobacteriosis are being studied. Epidemiology studies into the relative risks associated with use of antimicrobials used in food animals are also being examined.

Evaluation of hydrated lime in reducing *Mycobacterium avium* contamination of wood

Mycobacterium avium is a common soil organism that prefers an acid environment (pH 5-6.5) for optimal growth. Typical wood-shaving litter contaminated with swine feces and urine provides an acid and nutrient-rich environment favorable for mycobacterial growth that results in a higher incidence of swine mycobacteriosis (tuberculosis) and increased carcass condemnations at slaughter. Addition of hydrated lime will increase the pH of the litter creating a basic environment that is unfavorable for mycobacterial growth. Hydrated lime has disinfectant qualities for numerous bacterial pathogens and has been used safely and successfully as a general disinfectant in animal facilities. Study objectives are to evaluate the efficacy of hydrated lime in reducing contamination of wood shaving litter with *M. avium* and determine the optimum level of lime necessary to inhibit proliferation. Wood shavings without added hydrated lime will be compared with shavings with added hydrated lime adjusted to pH values of approximately 9, 10, and 11 to evaluate its effect on mycobacterial growth. Specimens will be inoculated with two different concentrations of *M. avium* serovar 2 followed by weekly additions of a slurry of swine feces and urine. All specimens will be cultured initially and then every 2 weeks for a total of 12 weeks. Mycobacterial colony counts will be compared to determine the efficacy of hydrated lime in reducing mycobacterial contamination.

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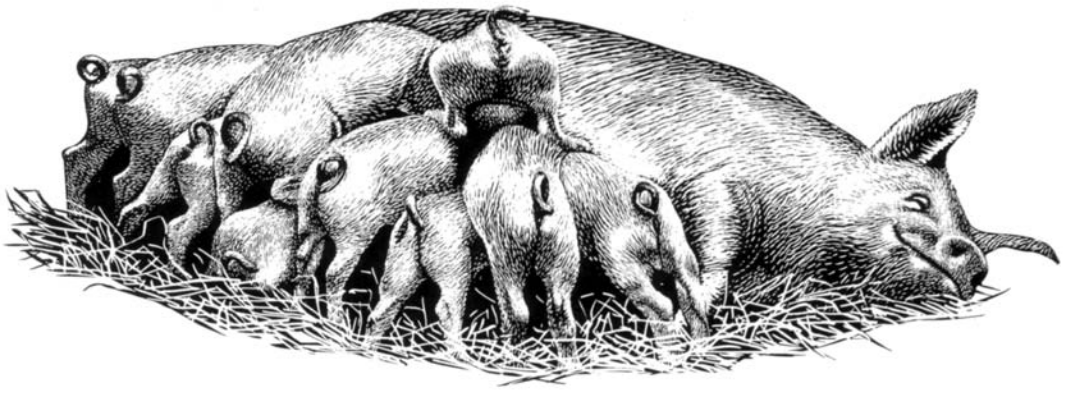
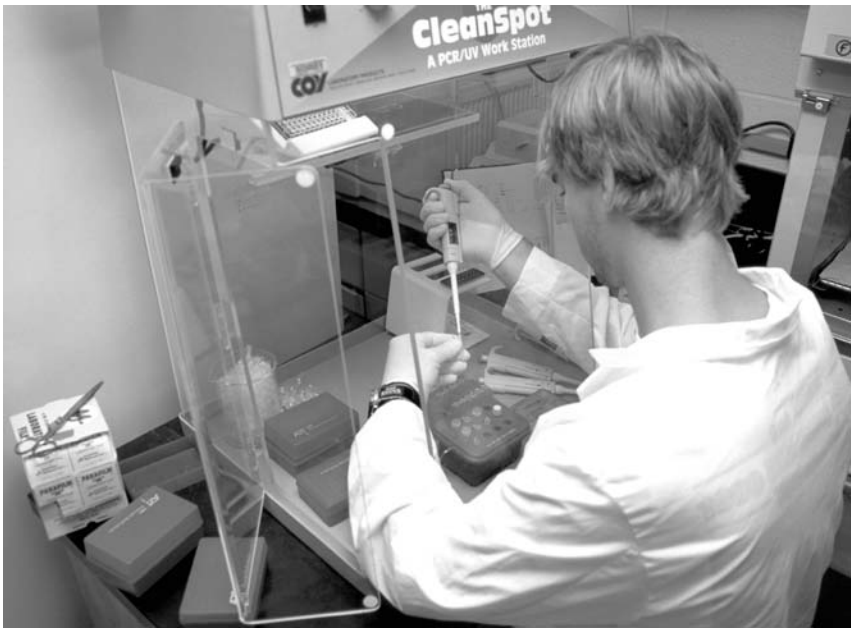


Companion Animals

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. Companion animals' popularity can be attributed to aging baby boomers looking to pets for companionship after their children leave home. 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 capacity.

Unlike other research areas, however, 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. Much industrial money has 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 external skeletal fixation of fractures, urinary incontinence, renal disease, motion analysis, pain relief of joint disease, transdermal fentanyl patches for pain relief, feline bartonellosis and herpesvirus (cutaneous infections), prostatic disease and treatment, pacemakers, and minimally invasive surgery.



Mr. Mason Florence performing a polymerase chain reaction (PCR) to determine the presence of antibiotic-resistant genes in bacteria isolated from fecal samples.

Feline leukemia virus-associated myelopathy in cats

Feline leukemia virus (FeLV) is a retrovirus with a complex life cycle associated with proliferative and degenerative diseases in cats. We have recently identified a neurological syndrome in long-term FeLV-infected cats consisting of abnormal vocalization, hyperesthesia, and paresis that progressed to paralysis. Affected cats were FeLV antigenemic for more than three years but did not have tumors or hematological abnormalities. Cats were invariably euthanized because of progressive neurological dysfunction. Necropsy of eight affected cats did not identify gross central nervous system abnormalities; however, microscopically there were profound lesions in the spinal cord and brain stem. Lesions consisting of diffuse white-matter degeneration characterized by dilated myelin sheaths and swollen axons were present in the absence of mononuclear cell infiltrates. Immunohistochemical examination revealed expression of FeLV antigens in neurons and glial cells in spinal cords of affected animals. Furthermore, proviral DNA was amplifiable from sections of spinal cord as well as intestine, spleen, and lymph nodes. These findings suggest that in a proportion of chronically FeLV-infected cats, a virus has evolved with an expanded host-cell range enabling infection of neurons and glial cells and resulting in axonal degeneration.

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Attachment to owners and separation anxiety in pet dogs

Behavioral problems are one of the primary reasons that healthy dogs and cats are euthanized and relinquished by their owners. Understanding the behavioral phenomena underlying such problems will aid in their prevention, diagnosis, and treatment. Separation anxiety is a common behavioral problem seen in pet dogs. Dogs with separation anxiety commonly eliminate in the house, destroy objects or the house itself, and/or vocalize excessively, all in their owners' absence. These same dogs generally do not show these behaviors in their owners' presence. Dogs with separation anxiety often cause substantial monetary damage to houses and draw complaints from neighbors. Gaining more knowledge of separation anxiety will help prevent this problem in many dogs and assist in (1) predicting which factors predispose dogs to this problem, and (2) treating dogs that would otherwise suffer from severe separation anxiety.

The goal of this project is to determine what combinations of attachment behaviors dogs with and without separation anxiety show in a laboratory attachment test. Attachment behaviors shown by dogs include maintaining proximity to their owners and showing distress (e.g., whining and scratching the door) when separated from their owners. The test simulates conditions of low stress (a stranger entering the test room) and high stress (the owner leaving the room); both of these conditions simulate attachment behaviors to different degrees. By analyzing the durations of attachment behaviors shown by the different dogs, we will be able to determine whether dogs with and without separation anxiety experience different types of attachment to their owners. In addition to the attachment test done in the lab, the dogs will be videotaped in their homes after their owners have left. The behavior on the tapes will be compared with the behavior during the attachment test for each dog to determine how closely the test reflects the home situation.

The results of this project will provide us with a greater understanding of how dogs' attachment to their owners is related to separation anxiety. In addition, it can provide a way of diagnosing separation anxiety in the clinical setting and a way to possibly identify dogs that are in the preliminary stages of developing severe separation anxiety.

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Companion animals as reservoirs for antibiotic resistant zoonosis

The emergence of multi-drug resistant *Salmonella*, *Campylobacter,* and *Escherichia coli* has made antibiotic resistance an important issue in medicine. Once antibiotic pressure is introduced into an environment, antimicrobial resistance quickly develops, spreads, and persists, even without continuing selective pressure from antibiotics. The current dogma is that the transfer of resistance takes place bi-directionally, between pathogenic bacteria and the benign resident microflora. However, there is little information on this genetic exchange of antibiotic resistance among the microflora and even less data of the genetic exchange between pathogenic bacteria and commensal microorganisms. Epidemiological studies suggest an association between the use of antibiotics in animals and the emergence of drug resistant bacteria, thus presenting a risk to public health from multiple-resistant zoonotic infections (e.g., *Salmonella* and *Campylobacter*). There is quite limited information on the role of companion animals as a source of resistant bacteria. Cross-contamination of resistant organisms between pets and human beings is a concern because of their close and prolonged contact. If this cross-mixing of bacterial elements is present, then resistance mechanisms expressed by bacteria in human, dog, and cat populations should have common features. Determining if there is exchange of bacterial resistance between the normal flora and the pathogens in companion animals and if this bacteria can be shared with their owners will help to determine if cats and dogs are a potential source of resistant bacteria for humans. Our preliminary results do in fact show shared resistance patterns between humans and pets in the same household. Further, results from this study will shed more light into the complex interaction of animals, humans, and their shared environment. Results from this study can help clinicians select antibiotic treatment regimes that are not likely to encounter resistance and that do not promote the dissemination of antibiotic resistant zoonoses to companion-animal owners.

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Dr. Sharon Crowell-Davis is studying the different ways that dogs relate to their owners.

Pilot study evaluating two doses of pivalic acid on carnitine levels in dogs

Dilated cardiomyopathy (DCM) is one of the most common acquired heart diseases in dogs and the most common primary heart disease in people. The cause remains unknown in most dogs and people; however, nutritional deficiencies, such as carnitine deficiency, have been shown to cause DCM in hamsters, humans, and dogs. Carnitine, an amino acid derivative, is essential for generating energy in the heart from fat. Pivalic acid, a branched-chain fatty acid used in human medicine to enhance intestinal absorption of drugs, has been shown to induce carnitine deficiency in people, rats, and dogs. The purpose of this study was to determine a safe, effective dose of pivalic acid necessary to reduce plasma carnitine concentrations in two groups of dogs consuming diets containing differing quantities of carnitine.

Results from both the high (n=6) and low (n=7) carnitine diet groups revealed a statistically significant ($p<0.05$) decrease in plasma carnitine by the second week of study, while dogs were receiving the lower dose of pivalic acid. This decrease persisted through the fourth week of study. Plasma carnitine results from the second month of the study, while dogs were receiving the higher dose of pivalic acid, are still pending because of a temporary shortage of the carnitine standard for the assay. However, based on results from the first month of study, it is likely that plasma carnitine will be even lower during the second month of study.

Echocardiography results from the high carnitine diet group revealed no statistically significant changes; however, one of six dogs developed decreased contractility. Results from the low carnitine diet group revealed a statistically significant difference in contractility during the first two months of study. In addition, two of seven dogs developed a dilated left ventricle. Reduced cardiac contractility and dilation of the left ventricle are consistent with the onset of early DCM.

Based on this pilot study, administering pivalic acid resulted in decreased plasma carnitine concentrations. In addition, in the group of dogs consuming the low carnitine diet, a statistically significant reduction in cardiac contractility occurred. These results suggest the potential of using pivalic acid-induced carnitine deficiency as a mechanism for creating a model of DCM in the dog.

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Pharmacokinetics and tissue distribution of nucleoside analogs in cats

Feline immunodeficiency virus (FIV) is similar to human immunodeficiency virus type 1 (HIV-1) in replication, genomic organization, and clinical disease manifestations. FIV has been used as an animal model for studying various aspects of HIV-1 infection, such as transmission from mother to offspring, effects on the immune system, and vaccine development. The FIV model has been underutilized for studying the penetration of drugs into the brain and deep lymph tissues where the virus hides. Nucleoside analogs, drugs that inhibit the reverse transcriptase enzyme responsible for retroviral replication, are used commonly to treat people infected with HIV-1. These drugs have not been thoroughly studied in cats. Current treatment of FIV-infected cats with nucleoside analogs is empiric rather than based on sound pharmacokinetic studies. The purpose of this project is to determine the plasma pharmacokinetics of the nucleoside analogs, AZT and 3TC, in normal cats so that we can intelligently treat naturally and experimentally infected cats. In addition, we plan to measure drug levels in various regions of the brain and in deep lymph tissues, studies that cannot be done in people and that will have important implications for the treatment of human AIDS.

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**Pharmaceutical and Biomedical Sciences*

The effects of dietary fatty acid modulation in tumor bearing athymic mice

Prostate cancer is the most commonly diagnosed neoplasm of men and the second leading cause of male death in the United States. Epidemiological studies implicate dietary fat as a causal factor for prostate cancer. A high correlation has been reported between prostate cancer deaths and total fat consumption. Consumption of a diet high in omega-3 fatty acids (e.g., fish and seafood) has been associated with a low risk of prostate cancer. Recent studies suggest that the specific types of fat and the balance of these fats may be more important in the development of prostate cancer than the total dietary fat.

This study is designed to evaluate the ability of four diets varying in fatty acid composition (e.g., high omega-6, high omega-3, and diets containing conjugated linoleic acid) to modulate tumor growth in mice inoculated with canine prostate cancer cells. Prostate cancer in the dog has been identified as an appropriate model for human prostate cancer. Prostate cancer in both dogs and men is strongly associated with a putative precursor lesion, prostatic intraepithelial neoplasia, has a high propensity for skeletal metastasis, and is strongly influenced by aging.

We have completed our study and are currently analyzing data to evaluate differences among dietary groups. Important factors to be evaluated include differences in tumor volume, prevalence of metastases, and the number of tumor cells undergoing programmed cell death.

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Financial Highlights

Research Funding

Funding Source*	Fiscal Year 1999	Fiscal Year 2000
VMES Budget	$3,174,529	$3,334,563
Federal Grants and Contracts	1,619,584	2,226,794
State Grants and Contracts	179,825	129,825
Private Grants and Contracts	2,347,355	3,041,722

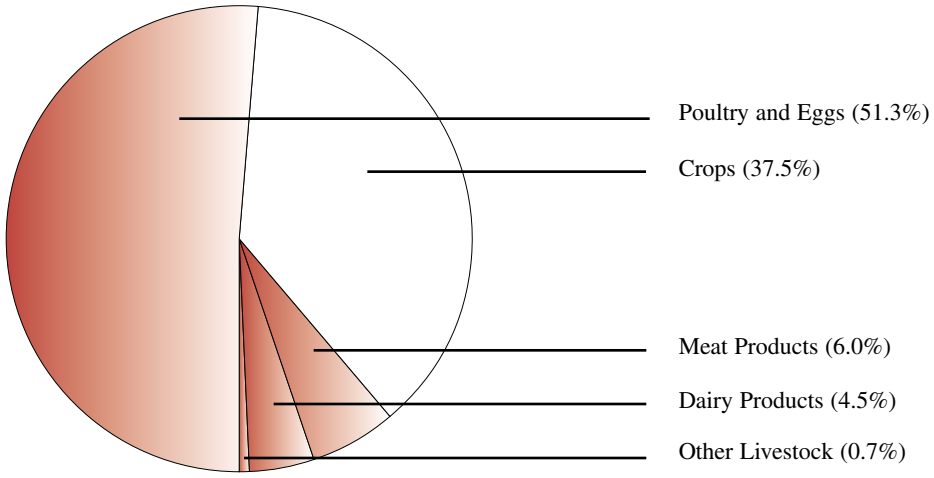
*Excluding carryover funds

Georgia Livestock and Poultry: Inventories and Values[a]

Species		Number on Farms and/or Produced	Production Value
Cattle	Beef	1,204,000	$565,600,000
	Dairy	96,000	346,950,000[b]
Hogs		1,640,000	209,200,000
Poultry	Broilers	1,239,700,000	2,293,445,000
	Non-broilers	21,039,000	12,943,000
	Eggs	5,172,000,000	378,849,000
Horses and Ponies		251,000	125,500,000

[a]Based in part on information published by the Georgia Agricultural Statistics Service, Athens, Georgia
[b]Includes value to dairy cattle and milk produced

Georgia Farm Cash Receipts



Research Contracts and Grants

Brackett, B.G. Ovarian cortex transplantation in the SCID mouse. Reproductive Biology Associates, $15,137.

Brown, C.C. Immunopathogenesis of a small round virus associated with poult enteritis. U.S. Department of Agriculture, $63,600.
Investigation into pathogenicity and virulence classification of heterogeneous Newcastle disease virus strains. U.S. Poultry and Egg Association, $53,935.

Brown, S.A. Efficacy evaluation of amlodipine in cats with partial renal ablation. Pfizer, Inc., $1,037,739.
Exploratory efficacy evaluation of amlodipine beseylate in modifying arterial blood pressure and proteinuria in dogs with partial renal ablation. Pfizer, Inc., $102,924.
Spirapril in cats with hypertension and chronic renal insufficiency. Schering-Plough Animal Health, $74,307.

Budsberg, S.C. Effects of glucosamine on osteoarthritis in dogs. Ralston Purina Company, $32,777.
Evaluation of the ability of flupirtine to modulate ground reaction forces in a chronic canine stifle osteoarthritis model. Bayer Corporation, $39,171.

Coffield, J.A. Neuromuscular targets of botulinum toxin. National Institutes of Health, $176,175.

Davidson, W.R. Level of exposure to lead among wild birds and mammals at the Federal Law Enforcement Training Center. Federal Law Enforcement Training Center, $16,594.

Dickerson, H.W. Research training experience for veterinary medical students. Merck Company Foundation, $30,000.

Edward, G.L. Role of dorsomedial medulla oblongata in amylin action on gastrointestinal activity and food intake. American Diabetes Association, $72,356.

Fayrer-Hosken, R.A. Development of synthetic sterilant vaccine for dogs and cats. Georgia Research Alliance, $50,000.
Elephant project. U.S. Humane Society, $21,655.
Wild horse fertility control. Medical College of Ohio, $29,100.

Finco, D.R. Validation of iohexol clearance as a metric of glomerular filtration rate in the cat and dog. Pfizer, Inc., $13,166.

Fischer, J.R. Epidemiologic investigation of coot and eagle brain lesion syndrome. Arkansas Game and Fish Commission, $60,000.

Garcia, M. Protection studies on DNA vaccination against infectious laryngotracheitis virus (ILTV). U.S. Poultry and Egg Association, $16,100.

Hoenig, M. Effect of diet on glucose tolerance and lipid metabolism in the cat. Ralston Purina Company, $187,450.
Evaluation of conjugated linoleic acid for obese cats. IAMS Company, $21,727.

Howerth, E.W. Pathogenesis of vesicular stomatitis in horses. Grayson Foundation, $28,703.

Jackwood, M.W. Development of an extremely rapid and more precise method of identification of infectious bursal virus isolates. U.S. Poultry and Egg Association. $64,799.

Jacobs, G.J. Possible role of *Bartonella* infection in cardiomyopathies in cats. Morris Animal Foundation, $9,626.

Little, S.E. Efficacy of Heardgard-30 chewables when administered to dogs 120 days after challenge with infective *Dirofilaria immitis* larvae. Merial Limited, $78,294.
Goat model of *Ehrlichia chaffeensis* reservoir infection. National Institutes of Health, $139,131.

Lowder, M.Q. Dental abnormalities in horses: Is there an association with colic? Academy of Veterinary Dentistry, $5,000.

Lukert, P.D. Bovine enteric diseases challenge model. Merial Limited, $80,209.
Development of experimental challenge models for bovine enteric diseases in newborn calves. Georgia Research Alliance, $79,825.

Maki, J.L. *Ictalurus punctatus*: A model to study mucosal immunity. National Institutes of Health, $79,380.

Maurer, J.J. Characterization of multiple fluoroquinolone resistance among bacterial pathogens. U.S. Department of Health and Human Services, $61,258.
Following resistant salmonella through the food chain. U.S. Department of Agriculture, $814,564.

McCall, J.W. Antifilarial drug evaluation in dogs. World Health Organization, $60,334.
Experimental chemotherapy of filariasis and screening of filaricides. World Health Organization, $108,148.
Supply of *Brugia* infective larvae. National Institutes of Health, $93,200.

Moore, J.N. Molecular mechanisms of enteric LPS in horses. U.S. Department of Agriculture, $190,000.

Murray, T.F. Affinity labels for opioid receptors. University of Maryland, $39,670.
Drug discovery and biodiversity among the Maya of Mexico. National Institutes of Health, $99,700.
Dynorphin analogues as kappa opioid receptor antagonists. University of Maryland, $46,804.
Reactor for generation of compound derivative libraries. Bend Research, Inc., $57,160.

Nettles, V.F. Alternate baits for delivery of V-RG rabies vaccine to gray and red foxes. Merial Limited, $20,000.
Oral vaccine delivery to raccoons. U.S. Department of Agriculture, $5,000.
Southeastern cooperative wildlife disease study. Southeastern States, $196,420.

Peterson, D.S. Characterization of DBL-domain proteins in *Plasmodium falciparum*. National Institutes of Health, $141,416,

Poet, S.E Use of nucleic acid vaccines in working Navy marine mammals. U.S. Department of the Navy, $10,000.

Quist, C.F. Scientific information on the use of animal traps for selected wild vertebrates. U.S. Department of Agriculture, $52,800.

Rawlings, C.A. Balloon catheter for radiation delivery in the caprine model. Proxima Therapeutics, Inc., $47,422.
In vivo study of biodegradable urethral stents in the canine cystotomy. J & J Corporation Biomaterial Center, $32,316.

Ritchie, B.W. Postgraduate program. Zoo Atlanta and Riverbanks Zoo, $38,000.

Sharma, R.P. Tumor necrosis factor involvement in fumonisin toxicity. National Institutes of Health, $156,497.

Stallknecht, D.E. Wildlife reservoirs for the H5 and H7 avian influenza viruses. U.S. Department of Agriculture, $84,634.

Villegas, P. Marek's disease virus pathogenicity study. Pfizer, Inc., $49,370.

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