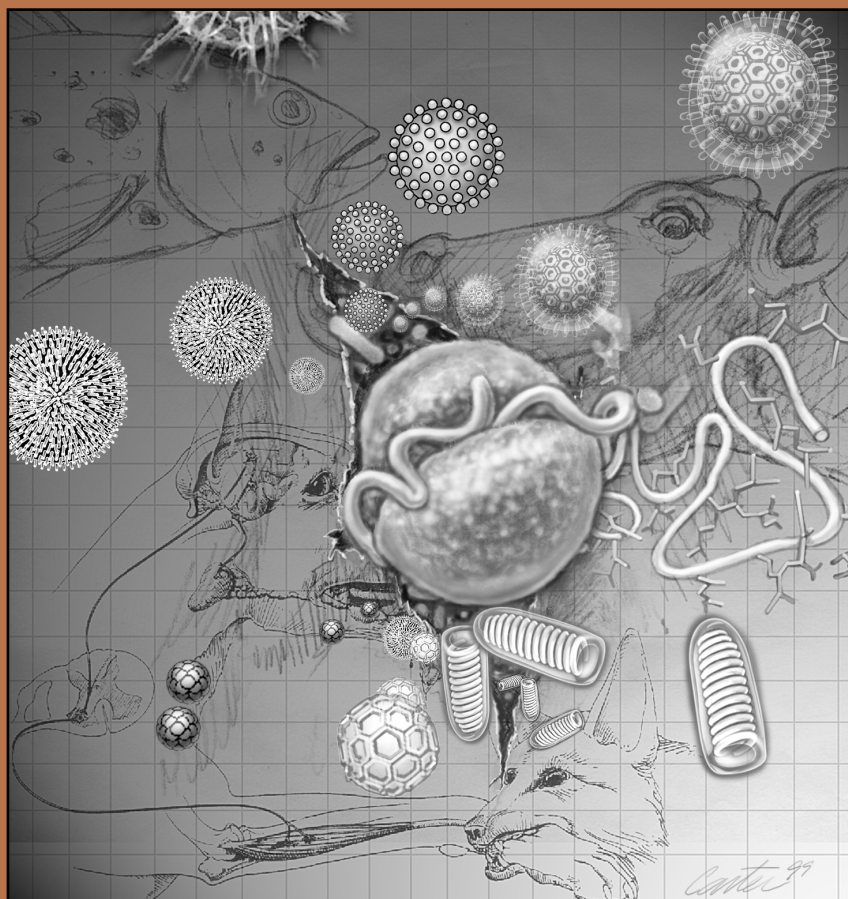


VME S '99

Science In Service To Animals



Emerging Diseases

VETERINARY MEDICAL EXPERIMENT STATION
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA

VME S '99

Veterinary Medical Experiment Station
College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602
July 1, 1998 to June 30, 1999

23rd Annual Report

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

This 23rd Annual Report is published by the Veterinary Medical Experiment Station, The University of Georgia.

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Cover Design: Lari Cowgill and Kip Carter
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This report may be viewed in Adobe Acrobat form at www.vet.uga.edu/testbed/html/vmes99.pdf

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 1998-1999 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.

These 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



I am pleased to present the 23rd Annual Report of the Veterinary Medical Experiment Station (VMES). This year I am especially gratified to announce the completion of the Animal Health Research Center, which will be dedicated on August 12, 1999. The Animal Health Research Center, referred to as the AHRC, (an acronym pronounced ark) is a major new research center focused on improving animal health through both basic and applied science. It is the first multispecies biosafety level 3 building associated with a veterinary college in the Southeast. As such, it provides the specialized, critically needed facilities required for safely studying current and emerging animal diseases.

This year the cover of our Report, and the accompanying article on page 5 authored by Dr. Corrie Brown, highlight the significance of emerging diseases, and the vital role in controlling them played by facilities such as the AHRC. To emphasize this important area, the College of Veterinary Medicine is presenting a conference titled, "Emerging Diseases: Veterinary Medicine, Agriculture, and Human Health." This symposium will be held on August 13-14, 1999 in conjunction with the dedication of the AHRC.

The dedication of the AHRC marks the completion of a planning and construction process initiated in 1978 by my predecessor, Dr. John Bowen. The approximate construction cost of \$19 million includes \$8 million contributed by the federal government and \$11 million paid by the state of Georgia. The AHRC's construction is indeed a major milestone for the College and the VMES. Its existence is the direct result of the vision and support of university faculty, administrators, and the political leaders of Georgia.

Further planning and construction has begun on facilities located on the second floor of the AHRC. These include an innovative vaccine production laboratory and fully equipped research laboratories. The University of Georgia and the Georgia Research Alliance provided funding for this phase of the project. These state-of-the-art laboratories will provide the foundation for a new animal vaccine group led by an eminent scholar in vaccine research; a new endowed position created through the efforts of the Georgia Research Alliance. A search is currently in progress to identify a world-class researcher for this position.

Needless to say, research conducted in the AHRC is expected to have a major impact on the prevention of animal disease, the relief of animal suffering, and the advancement of medical knowledge. In addition, research here will provide the training of new scientists and create innovative vaccine technologies, which in turn will boost the state's animal and biotechnology industries.

In 1999, the VMES made significant progress in establishing a new nonpoultry food animal program integrating veterinary outreach, applied research, and the state's nonpoultry food animal industries (primarily beef, dairy, and swine). This program, based in the College of Veterinary Medicine, will serve as a center for applied and basic research related to food safety and disease diagnosis and prevention. A major focus will be to improve marketability of Georgia's animal products through prudent attention to animal health, food safety, and public health. In support of this program, the 1999 Georgia legislature provided an enhancement to the VMES budget allowing the hire in fiscal year 2000 of a field veterinarian dedicated to this new initiative.

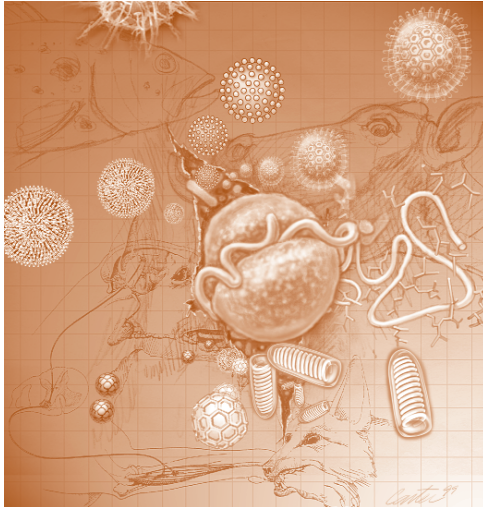
College and VMES researchers address many challenging animal health problems in the areas of infectious diseases, disease diagnosis, and disease prevention and treatment. Our Annual Report provides concise summaries of College research and VMES-supported projects undertaken or completed during the fiscal year of 1998-99. Additional information on these projects can be obtained directly from the investigators or by contacting the VMES office. A list of publications resulting from these studies is included. This Annual Report is accessible on-line at www.vet.uga.edu/testbed/html/vmes99.pdf

As always, I look forward to your comments and suggestions.

Harry W. Dickerson, BVSc, PhD

Emerging Diseases

Emerging diseases are defined as those infections that have recently appeared in a population or are extending their geographic range. The last decade has witnessed an explosion of emerging diseases. Numerous factors, inherent in our postmodern society, have been operating synergistically to create these diseases.



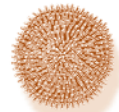
Movement of a disease to a new susceptible population is occurring with increasing frequency. Raccoon rabies leapfrogged from Florida to the northeastern United States in transported raccoons, causing major public health problems. Just within the last two years, foot-and-mouth disease has moved into Taiwan, and classical swine fever to the Netherlands, both with devastating economic and environmental consequences. The deliberate introduction of a new disease needs to be considered, such as the illegal importation and spread of viral hemorrhagic disease in New Zealand. The specter of agricultural terrorism looms large as well.

Crossing species boundaries is a time-honored way of creating new disease, with such recent well-known examples as AIDS and Lyme disease. Equine morbillivirus, a brand new agent of fatal equine and human disease, emerged in Australia in 1995, and it is now known that it came from the uterine secretions of fruit bats. Conjunctivitis in house finches, an increasingly prominent problem around bird feeders, is caused by a mycoplasma that came from domestic poultry. A pandemic influenza scare in Hong Kong underscores the ease with which the world could suffer tremendously because of cross-species disease transfer.

Environmental disruption as a factor causing emerging disease has been documented numerous times, including many of the Rift Valley fever and Venezuelan equine encephalitis outbreaks that happened subsequent to irrigation projects or climatic events. In recent years, epizootics of avian cholera, viscerotropic velogenic Newcastle disease, and duck plague have been associated with massive die offs in wild waterfowl, engendered as a result of crowding caused by shrinking wetlands.

Husbandry changes have been known to cause new disease, with perhaps the best example being bovine spongiform encephalopathy, which emerged as a result of changes in the rendering process of dairy cattle feed. Tuberculosis in captive cervids and emergence of toxigenic strains of *Pfiesteria piscicida* are two other diseases that are related to changes in the way in which we raise our animals, specifically, increasing animal concentrations for more efficient production.

Given the exponential growth of the human population and all the attendant implications, including population mobility, the ecological disruption that accompanies the overall increase, and the necessity of exploring new agricultural technologies to feed a burgeoning population, it is certain that emerging diseases will continue to arise. Our only defense is to maintain the ability to study each new disease as it arises, such that we can develop effective control strategies. To do this, it is essential to perform these studies in a facility that protects other animals, the general public, and the environment.



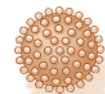
Rift Valley fever
Bunyavirus



Viral Hemorrhagic Disease
Calicivirus



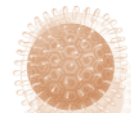
Hoof & Mouth Disease
Picornavirus



AIDS
Retrovirus



Raccoon Rabies
Rhabdovirus



Duck Plague
Herpesvirus



Pfiesteriosis
Pfiesteria



Avian Cholera
Rod Shaped Bacterium



Mad Cow Disease
Prion

Poultry



Georgia's poultry industry dominated the state's animal agricultural dollars with more than \$2.6 million in annual revenue in 1997. The state's poultry industry is continuing to expand as broiler production in Georgia increased from 20.6 million per week in 1995 to 22.7 million in 1997. 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.

Purification and culture of chicken NK-like cells

Infectious diseases have an important economic impact on the poultry industry. The natural killer (NK) cell capability of eliminating a broad variety of infectious agents and transformed cells without a previous sensitization is well known. NK cell activity potentially can be modulated and positively selected in certain chicken lines. Avian NK cell activity has been described as an important trait in the resistance against viruses and protozoa such as rotavirus, Marek's disease virus, and coccidia.

Unfortunately, the incomplete characterization of NK cells has delayed the study of their protective role in several infectious diseases. Obtaining a relatively pure population of cells with NK activity will provide the first step in the production of specific monoclonal antibodies (Mabs), opening the possibility of pursuing more detailed characterization, cloning, and more specific studies on immunity of infections.

The overall objective of the present study was to purify an embryonic NK cell splenic population in order to culture NK cells *in vitro*. Biomagnetic beads were successfully used to deplete chicken mononuclear splenocyte cell preps of CD3⁺ and Ia⁺ cells as determined by immunofluorescence flow cytometry. However, we were unable to practically harvest sufficient cell numbers to perform functional assays or extract surface proteins. Long-term bone marrow cell cultures were established from white leghorn chicken embryos to selectively cultivate NK-like cell populations. A series of cultures were stimulated with Con A, prokaryotic and

eukaryotic mixture of chicken IL-2, or hrIL2. Flasks showed a significant shift from granulocytic cells that were predominant in the control flasks to agranular lymphoid-like cells. Unfortunately, the cellular yield was still low. In our future experiments, we hope to increase the cellular yield.

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

Avian leukosis virus-subgroup J (ALV-J) is a serious economic threat to continued production of poultry meat in needed quantities because of decreased production present in infected chickens. This project was designed to quantify these effects and to investigate methods to control or eliminate them. Our first objective was to determine the rates of egg production, hatch, and horizontal spread of ALV-J infection in commercial chickens. There were no significant effects on egg production per hen, but doubling the mortality of egg-laying hens decreased overall egg production by 5% to 8%. Hatch was depressed 10% to 30% in ALV-J viremic hens. Individual hen ALV-J status and that of progeny produced was stable over time with no spread from hen to hen. The second objective was to determine the effects of ALV-J on broiler growth. ALV-J positive broilers weighed less than 65% of negative chicks from one to eight weeks of age. The third objective was to examine the effects of ALV-J on resistance to other infections. ALV-J infection damages bone marrow and produces myeloid neoplasia, and decreases required T-

lymphocytes. Heterophils incorporated proviral DNA and had decreased ability to protect against bacterial infections. Studies of macrophages are in progress. These heterophil and macrophage results may explain the increased incidence of secondary infections. Our last objective was to produce antibodies to use as potential therapies and to detect the virus in tissue samples. These studies are currently in progress.

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Differential diagnosis of infectious laryngotracheitis virus by PCR

Infectious laryngotracheitis virus (ILTV) is a severe acute respiratory disease of chickens. ILT outbreaks cause severe financial losses to the poultry industry; however, the direct source of these outbreaks is quite difficult to determine. The wide use of attenuated vaccines and the homogenous antigenicity of ILTV strains have made it difficult to understand the epidemiology of the disease. We have developed a polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assay of the glycoprotein E to easily characterize field isolates of ILTV. RFLP analysis of the gE gene showed three different patterns among vaccine strains and field isolates with enzymes DdeI and PstI. Pattern A was observed for the tissue culture origin (TCO) vaccine as well as field isolates, whereas pattern B was observed uniquely for field isolates. However, chicken embryo origin (CEO) vaccines showed RFLP patterns including bands corresponding to both A and B. This was observed for three commercially available CEO vaccines and five field isolates. The above may indicate that a mixed population of viruses may be present in some of these vaccine strains. To determine the potential of gE PCR-RFLP assay to trace ILTV sources outbreaks, 25 field isolates were analyzed. RFLP analysis with DdeI enzyme showed that 21/25 were TCO-like or A pattern, 1/25 was CEO-like or A+B pattern, whereas 3/25 isolates have a unique or B pattern. RFLP analysis with PstI showed 15/25 isolates with a TCO-like or A pattern, 5/25 isolates were CEO-like or A+B pattern, and 5/25 isolates had a unique or B pattern. Although most of the tested viruses were isolated from unvaccinated flocks, 88% of the field isolates were either TCO-like or CEO-like.

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Clinical investigation of poultry diseases

The clinical teaching program is used by local poultry companies as a means to critically evaluate management and disease issues on farms that 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, one each for three different broiler integrators. All three investigations used methods to evaluate water, litter, air, temperature, and management factors. Moreover, disease challenge and resulting pathology were evaluated. In all cases, constructive recommendations were made and implemented.

One particular broiler integrator has been struggling to cope with an apparently significant issue of immunosuppression in broilers. The evidence for immunosuppression is a high incidence of inclusion body hepatitis, gangrenous dermatitis, and vaccine-induced respiratory disease in broilers. This conclusion is supported by the observation of lymphoid organ atrophy at an early age in broilers. We hypothesized that infectious bursal disease virus (IBDV) was infecting broilers early and that the offending virus must be antigenically unusual to accomplish an early infection. In cooperation with the broiler company, Dr. Daral Jackwood, and Dr. Pedro Villegas, we placed sentinel birds on 25 broiler farms. IBDVs were isolated from each farm and



Modern tissue culture techniques are used to study avian viruses in the laboratory.



Victoria Leiting is reading hemagglutination-inhibition (HI) tests for antibodies to *Mycoplasma gallisepticum*, an important infectious disease of poultry.

characterized by reverse transcriptase-polymerase chain reaction (RT-PCR). Five unusual viruses were characterized by RT-PCR. Dr. Villegas's lab has further characterized the viruses in challenge studies and serologically. Current data indicate that the hypothesis is true. The integrator is now pursuing the commercial production of an autogenous inactivated IBDV vaccine for their broiler breeder program.

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Antimicrobial peptides in broiler chickens

Antimicrobial peptides are important components of innate disease resistance in vertebrate animals. These peptides are present in phagocytic leukocytes and mucosal epithelial cells in a variety of mammalian tissues including respiratory, reproductive, and intestinal epithelial cells. We have discovered two chicken and two turkey antimicrobial peptides (β -defensins) in the granules of leukocytes. We have sequenced the cDNA for these peptides and have now constructed recombinant baculoviruses for expression of recombinant β -defensins. In addition, we are characterizing the distribution of these peptides in the chicken tissues. An analysis of the microbicidal spectrum of recombinant β -defensins and an understanding of their tissue distribution will allow us to determine the feasibility of improving the innate disease resistance of chickens by manipulating the expression of β -defensins. Benefits may

include improving resistance to enteric diseases such as coccidiosis and reducing the carriage of food-borne pathogens such as *Salmonella* sp. and *Campylobacter* sp.
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Control of infectious bronchitis virus (IBV)

The main goal of this proposal is to control infectious bronchitis. The specific objectives are 1) to study the molecular and serologic characteristics of variant field infectious bronchitis virus (IBV) isolates identified by our reverse transcriptase-polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) serotype identification test; 2) to further develop and test a nucleic acid vaccine for IBV; and 3) to test a subunit vaccine against Ark IBV expressed in avian cell culture. Eleven foreign IBV samples were analyzed. Seven novel viruses were detected among nine foreign IBV samples. To date none of the foreign IBV isolates have been detected in the United States. Because of difficulties in detecting the DE072 strain of IBV, we further characterized that strain and redesigned our test so that the DE072 strain as well as the other serotypes of the virus could be detected. The gene sequence of that virus indicates that the DE072 strain has undergone a recombination event as well as extensive antigenic variation. For objective 2, *in situ* hybridization and immunohistology were used to identify tissues infected when IBV is injected into the egg. It appears that IBV initially infects lung tissue then migrates to and infects cells of the bursa. To understand the effect of DNA vaccination in eggs on cell-mediated immunity, T-lymphocytes in the spleen were examined by flow cytometry. Differences in immune responses are affected by the amount and method of *in ovo* DNA inoculation.

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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 objectives of our study 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. Identification of one live MG vaccine strain in broiler breeders was investigated. Neighboring commercial layers were most likely inadvertently infected by using a vaccine bag that had contained MG vaccine the previous day. The organism then spread within the flock and to the neighboring broiler breeders. This and other recent examples emphasize the need for proper use of live mycoplasma vaccines.

Clinical infectious synovitis (caused by MS) was noted in Minnesota turkey flocks that were negative by standard serological tests. An isolate from this flock was used to challenge experimental turkeys. Turkeys infected via the upper respiratory infection were culture positive but remained negative on all serological tests. This emphasizes the need for further investigations in situations in which clinical signs are seen but antibody tests are negative.

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

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A second neuraminidase gene cloned from *Pasteurella multocida*

“Sometimes there breaks out in the poultry-yard a disastrous disease, commonly known as chicken cholera. The animal which is prey to this infection is without strength, trembles and has drooping wings.” These words were spoken by Louis Pasteur in 1880 when he revealed his fowl cholera vaccine to the Paris Academy of Sciences. Pasteur, now known as the father of modern microbiology, developed the first attenuated-live vaccine effective against infection. Live vaccine strains are still used today to prevent fowl cholera; however, the available live vaccine strains are often too pathogenic for use in chickens, and killed vaccines induce protection against only closely related strains of *Pasteurella multocida*, the bacterium responsible for the disease. We propose to create a recombinant vaccine that will induce protection against all *P. multocida* strains. We have cloned and sequenced a neuraminidase gene present in all strains of *P. multocida* and have protected chickens from fowl cholera using recombinant protein from this gene. We are the first to discover that *P. multocida* contains multiple neuraminidase genes. The

objectives of this study are to 1) determine the DNA sequence of *nanB* the second neuraminidase gene cloned from *P. multocida*, 2) determine the substrate specificity of the two neuraminidases, 3) create isogenic mutants deficient in production of the two active enzymes, and 4) complement mutants with cloned copies of specific neuraminidase genes.

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Rapid identification and epidemiological typing of food-borne pathogens by PCR

Many foods including poultry products have a short shelf life in the supermarket. Microbial testing will require sensitive and rapid methods for detection of biological hazards and typing of these food-borne pathogens. Molecular biology has provided the key tools for rapid identification of pathogens. The objectives of this study are to 1) develop a rapid method for identifying food-borne pathogens *Campylobacter*, *Escherichia coli* O157:H7, and *Salmonella* using multiplex polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA); and 2) type *Salmonella* and *Campylobacter* by multiplex, restriction fragment length polymorphism (RFLP) and random amplification polymorphic DNA (RAPD) PCR. Several PCR-based methods were developed to replace current immunological methods to identify important *Salmonella* and *E. coli* serotypes. The primers were designed to target and amplify the O and flagellar antigen genes. PCR tests were developed for rapid detection of *E. coli* O157 serotypes and *Salmonella* serovars, B, D1, and C1. ELISA modified the current *E. coli* O157 PCR test towards a cost-effective detection method for screening a multitude of samples. A RFLP PCR for flagellin genes provided further serological typing of *Salmonella* by matching RFLP patterns against a database of known *Salmonella* flagellin gene RFLP patterns. Once identified down to the O and flagellin serotype, *Salmonella* can be



The laboratory of Dr. Mark Jackwood uses molecular biological methods to develop vaccines and diagnostic tests for important avian diseases.

differentiated further at the genetic level by molecular techniques involving pulsed-field gel electrophoresis or random polymorphic DNA PCR. *Salmonella enteritidis*, however, remains resistant to discrimination by these typing methods. Several *Salmonella* virulence genes have been identified as potential candidates for molecular typing by RFLP PCR or DNA sequencing of *S. enteritidis* and other *Salmonella*.

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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 on the *in vitro* and *in vivo* expression of matrix proteins; fibrillar procollagen I and collagen III; proteoglycans, aggrecan, and decorin; and expression of regulatory proteins TGF β , CTGF, and Hsp47. Specific aims are, first, to determine the roles of TGF β and Hsp47 and their interaction in expression of embryonic and posthatch fibrillar collagens. Second, to define biomechanical parameters of gastrocnemius tendons undergoing stress-related structural changes. Third, to determine structural changes in collagen(s) and proteoglycans in gastrocnemius tendons subjected to altered load. In tendon explant cultures subjected to surgical trauma, there was an acute upregulation of TGF β mRNA followed by sustained upregulation of CTGF mRNA in another 48 hours with concurrent down regulation of TGF β mRNA. Hsp47 mRNA exhibits no difference to either injury or heat in explant cultures. Biomechanically, exercise produces a response related to the parameter of stiffness compared with controls suggesting accelerated maturation of the tendons. Pilot work in environmental chambers indicated that, first, we need to cycle a high temperature for seven to eight hours minimum. And second, we want the density of birds at approximately 1 square foot per bird. The finding of a ruptured tendon in the group fed an occidiostat, 3-nitro, was interesting.

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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) misted into an egg incubator according to commercial guidelines against an *Escherichia coli* bacteria known to be pathogenic to chickens. This product was also misted into another incubator at one-fourth the commercial concentration but was combined with EDTA-Tris in a ratio shown to increase the activity of the disinfectant against this particular bacteria in the laboratory. When the commercially blended disinfectant was compared with distilled water or the disinfectant/EDTA-Tris compound, no significant difference was found in egg shell permeability and egg weight or moisture loss during the incubation period. Aerosol bacterial counts measured inside the incubator during incubation were actually increased with the addition of EDTA-Tris to the disinfectant when compared with bacterial counts measured in the incubators misted with straight disinfectant or even distilled water. Fertility and hatchability of the eggs exposed to the various treatments were not affected. Early embryonic mortality was significantly higher in the eggs misted with distilled water when compared with eggs exposed to either disinfectant mixture. One-day-of-age chick weights were not significantly different, although male chicks were slightly heavier than female chicks.

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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 teaching experiences for students in the Master of Avian Medicine and other graduate programs.

The new lab database was placed on-line January 4, 1999 and has already provided significant improvements in administration and accounting for the diagnostic lab. More improvements are scheduled for this year. Lab reports can now be provided by e-mail and fax directly from within the system without the need for an intermediate hard copy.

The polymerase chain reaction (PCR) technique has become a more integral part of the diagnostic laboratory as seen by the tripling

of the numbers of tests performed during this reporting period. 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 has been adopted and refined, which has improved turnaround time from 1.5 days to 3 hours. Other techniques for performing large volume processing of PCR samples are being pursued and will provide even better turnaround for large submissions. Research will continue and new PCR tests will be applied to diagnostics as applications are developed and other ways are investigated to improve service to poultry industry clients.

Diagnostic Services Laboratory activity is represented by 5,361 accessions, 25,216 bacterial procedures, 315 antimicrobial susceptibilities, 33,823 enzyme-linked immunosorbent assay (ELISA), 24,782 infectious bronchitis virus-hemagglutination inhibition (IBV-HI) tests, 748 agar gel precipitin tests, 30,100 histopathology slides, 2,063 diagnostic PCR tests, and 998 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

Pathogenic viruses, which present a continual hazard to the commercial poultry industry, are the basis of our current research. More than 600 virus isolation cases were submitted last year to the virology laboratory at the Poultry Diagnostic and Research Center. Specifically, diseases caused by infectious bronchitis virus (IBV), avian leukosis virus subgroup J (ALV-J), and infectious bursal disease virus (IBDV) are the focus of this laboratory's ongoing research.

Variant IBV strains are common to the U.S. and world poultry industries. Numerous unique IBV isolates from diseased commercial flocks in Georgia have been identified. The protection provided by currently available commercial vaccines is not as satisfactory as expected. Therefore, challenge studies are conducted and evaluated in order to develop products that provide better protection against these IBV variants.

A new and ubiquitous exogenous strain of avian leukosis virus belonging to the subgroup J has recently been reported. An increase in condemnations at processing plants, mortality

with or without tumors, a decrease in egg production, and depletion of chick uniformity are some of the effects of this virus. Because of the recent identification of ALV-J, minimal genomic information is available. Currently, virus isolation in specific cell culture along with the use of molecular techniques such as RT-PCR and sequence analysis are being used to generate data for progressive research on ALV-J pathogenicity and oncogenicity.

IBDV is an acute and highly transmissible virus, which can cause clinical disease and mortality along with immunosuppression in young chickens. Variant IBDV isolates from problematic commercial broiler flocks are selected for evaluation in challenge studies and clinical analysis. Molecular analysis is used to rapidly identify and classify IBDV strains. This has led to the recent discovery and identification of a very virulent strain of IBDV for the first time in the American continent. Monitoring of IBDV field isolates allows for modification of vaccination procedures in order to provide improved protection against pathogenic viruses.

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The role of a large molecular weight plasmid in the pathogenesis of avian *Escherichia coli*

Financial losses caused by colibacillosis costs the poultry industry hundreds of millions



Dr. Miguel Ruano from Ecuador inspects inoculated embryos at the Virology Section of the Poultry Diagnostic and Research Center.

of dollars annually. Virulence traits of the bacterial organisms that cause this disease are encoded by specific genes. These virulence-specific genes are located either on the bacterial chromosome or large molecular weight plasmids. *Escherichia coli* strain V-1 is a well-characterized, pathogenic avian strain isolated from a broiler chicken with colisepticemia, and it contains several plasmids. Two of these plasmids are high molecular weight plasmids. The high molecular weight plasmid, pWT3, has been characterized via restriction enzyme mapping and sequence analysis. This plasmid contains F-plasmid and transposon sequences, sequences encoding for (B and D) colicin production, virulence plasmid genes similar to those of several species of bacteria, and resistance to several antibiotics and arsenite.

We are looking for a genetic marker (designated epv) that indicates the putative virulence gene located on the virulence plasmid, pWT3 (of *E. coli* strain V-1). The ability of the organisms to kill chicken

embryonated eggs has been used as a measure of virulence for the organisms tested. When electroporated into the lab strain DH5 α , pWT3 did not bestow a stable ability to kill chicken embryos in the chicken embryo lethality assay. Analysis of data involving the weights of embryos postinoculation has not been useful in this study.

Initially, an isolate was created that lacked the plasmid pWT3 but was otherwise identical to isolate V-1. Colicin plasmids can stably integrate into the chromosome and continue to function. This is the case with our V-1 mutant; the plasmid never reoccurred in the strain, but the genes present on the plasmid were present in the V-1 mutant genomic preparations. Clearly, some other knockout mutants will have to be created in order to determine the contribution of this plasmid to the pathogenesis of colibacillosis.

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Fish

Georgia's aquaculture industry continues to expand with its greatest increase in channel catfish. Since 1995, pond acreage for catfish farming has grown from 6,000 to 8,000 acres. Major areas of disease concern continue to be losses caused by channel catfish virus and the bacteria *Flavobacterium columnare* and *Edwardsiella ictaluri*. Furthermore, there have been extensive losses in Georgia this spring because of the protozoan parasite *Ichthyophthirius multifiliis* or Ich. In addition to Georgia's food fish industry, there is an increasing interest in ornamental fish production. As Georgia's aquaculture industries continue to grow, research aimed at improving the health of aquatic animal species will ultimately help growers reduce production costs and improve profits.

Role of STAT proteins in catfish innate immunity

Nonspecific cytotoxic cells (NCC) are the teleost equivalent of mammalian natural killer cells. NCC lyse protozoan parasites and tumor cells. NCC may also participate in antibacterial resistance through the elaboration and release of cytokines and amplification of inflammatory responses. We have recently identified an antigen receptor protein-1 (NCCRP-1) on the membrane of NCC. Crosslinkage of this receptor with monoclonal antibody or tumor/protozoan antigen initiates a downstream signaling process, which eventually activates cytotoxicity. A major consequence of this activation/signaling process is the phosphorylation of many different intermediate proteins necessary for NCC function. We have identified different "species" of phosphoproteins. NCCRP-1 was phosphorylated on both tyrosine and serine residues. In addition, BOX-1 motifs (proline

rich consensus sequences) were found on the N-terminus of NCCRP-1, and these motifs are known docking sites for JAK kinases. When NCC membrane lysates were treated with the chemical crosslinker DSS, it was found that JAK-2 kinase was physically associated with NCCRP-1. The transcriptional activators that link JAK kinases to increased gene expression are known as signal transducers and activators of transcription (STAT) proteins. Although STAT proteins are constitutively expressed in most cells, upon activation they become phosphorylated, dimerize, and are translocated into the nucleus where they bind DNA. In the present study, we have detected the presence of STAT 6 in the cytosol of NCC.

Furthermore, we were able to follow the translocation of STAT 6 into the nuclear fraction following activation of NCC with PMA/Ca ionophore (a proven enhancer of cytotoxic function). These results may provide a link between the signal transducer functions of NCCRP-1 needed for cytotoxicity and transcriptional activation, which leads to cytokine production.

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Immunoregulation of streptococcal infections in tilapia: apoptosis vs necrosis

The immediate innate cytotoxic and cytokine mediated immunological responses of tilapia (*Oreochromis niloticus*) to environmental or microbial stressors may be the model for studies of mechanisms of teleost resistance to infectious diseases. Tilapia injected intravenously with killed *Streptococcus iniae* produce an immediate enhancement of nonspecific cytotoxic cell (NCC) lytic activity. Activation of NCC is mediated by soluble factors released into the peripheral circulation. Passive transfer of increased cytotoxicity is achieved following in



Dr. Liliana Jaso-Friedmann studies the molecular mechanisms of fish immunity.

vitro treatment of control NCC with serum from fish injected intravenously with *S. iniae* (i.e., a stressor response). This increased cytotoxicity was produced at the single cell level of activated NCC and was not generated by increased numbers of NCC mobilized into the peripheral blood. To study the mechanism(s) of serum factor activation of NCC, we predicted that enhanced cytotoxicity was derived from regulation of apoptosis of these cells by soluble protection factors released into the serum. To study this, we developed *in vitro* and *in vivo* apoptosis model systems. *In vitro* incubation of NCC with HL-60 target cells produced apoptotic lesions in both effector and target cells determined by DNA hypoploidy analysis. NCC does not undergo fratricide (i.e., self-killing) in the absence of target cells. Pretreatment of NCC for 4 hours with serum containing the apoptosis protection factors (i.e., serum that also activated NCC killing of HL-60 targets) prior to coculture with HL-60 cells prevented target induced DNA hypoploidy of NCC. *In vivo*, the activation of NCC following IV injection with *S. iniae* exhibited characteristics of cytokine amplification associated with antigen recognition. Levels of activation of NCC cytotoxicity differed depending on the lymphoreticular tissue compartment and on the identity of the bacterial isolate. All *S. iniae* isolates did not produce the same levels of NCC activation. To further investigate possible mechanisms of activation, NCC purified from different tissue (spleen, anterior kidney, and peripheral blood leukocytes) were examined by flow cytometry for expression of FasL. High levels of cytoplasmic (but not membrane) FasL were found. To examine the relationship between FasL and stress serum activation, NCC was treated *in vitro* with stress serum, and whole cell lysates were made. Lysates were probed by Western blot examination with many different antisera to proteins/enzymes involved in programmed cell death (PCD). Stress serum activated NCC had increased signals associated with FasL cellular apoptosis susceptibility (CAS) gene, and Fas associated death domain (FADD) expression. Multiple pathways of apoptosis regulation were also suggested by experiments demonstrating the initiation of PCD by crosslinkage of a membrane antigen receptor (e.g., NCCRP-1) on NCC with monoclonal antibody. Additional pathways of PCD

induction of NCC included treatment with camptothecin. In the camptothecin experiments, and similar to the HL-60 induction of apoptosis, pretreatment of NCC with stress serum inhibited DNA hypoploidy. These studies indicated that both NCC and soluble apoptosis protection factors participate in tilapia innate resistance to stress responses. Activation of innate immunity may precede the generation of active inflammatory responses and augment antigen processing to significantly enhance the development of acquired immunity.

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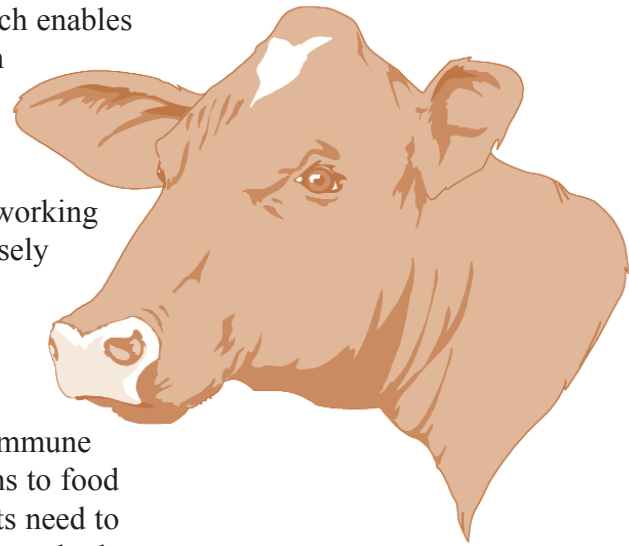
DNA vaccine dose titration of the channel catfish humoral immune response

Americans are eating more fish products. The decline in commercially important wild fish stocks and steady demand for fish food items has enabled aquaculture to become a rapidly emerging component of Georgia agriculture. Channel catfish farming accounts for approximately half the total aquaculture production in the United States. As with most commercially important food animals, there is a pressing need for safe, effective, and inexpensive vaccines in the channel catfish culture industry. DNA-mediated vaccines, or plasmid vaccines, are novel variations on the concept of subunit vaccines. The long-range goal of this research is to develop safe, effective, and affordable plasmid vaccines for infectious diseases important to the channel catfish culture industry. The current objective in pursuit of this goal is to determine the optimal plasmid vaccine dose that will generate the best humoral immune response in the channel catfish. Our laboratory has demonstrated that channel catfish will generate a humoral immune response after injection with a plasmid construct containing a foreign protein gene. Our expectations are that at the end of this study we will have determined the best plasmid vaccine dose for inducing a strong, long-lasting humoral immune response in the channel catfish. These observations will facilitate funding for future research towards the development of vaccines targeted against specific channel catfish disease agents, such as channel catfish virus and the bacterium *Edwardsiella ictaluri*.

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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, high grain market, 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, brucellosis, pasteurellosis, pneumonia, Infectious Bovine Rhinotracheitis (IBR), 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.



***Escherichia coli* O157:H7 in sympatric populations of cattle and wild deer**

Cattle are the primary reservoirs of the human food-borne pathogen *Escherichia coli* O157:H7; however, venison was confirmed as a source of human infections, and the organism was isolated from deer feces in the western United States. Currently, there is little information regarding the status of *E. coli* O157:H7 in sympatric populations of cattle and deer. Two previous studies at The University of Georgia demonstrated that inoculated deer carry and shed *E. coli* O157:H7 in a manner similar to cattle. In addition, *E. coli* O157:H7 was detected in 3 of 77 wild deer at a Georgia site where beef and dairy cattle herds were present. Thorough knowledge of the ecology of *E. coli* O157:H7 is essential in developing practices to reduce this pathogen on Georgia and U.S. farms. Specific objectives of this study are to 1) determine the prevalence of *E. coli* O157:H7 in cattle and wild deer at the Georgia site where the organism previously was detected in deer, 2) determine whether the same genomic subtypes of *E. coli* O157:H7 are present in the cattle and deer, and 3) determine whether deer at this site maintain the same subtypes from year to year. A total of 140 fecal samples were collected directly from hunter-killed wild deer at the study site in November and December 1998, and fecal samples were collected from 333 cattle at the site from December 1998 through February 1999. *Escherichia coli* O157:H7 was isolated from 12 (3.6%) cattle but from none (0%) of the deer. Final genomic

analysis of the 3 deer isolates from 1997 and the 12 cattle isolates of *E. coli* O157:H7 is pending; however, preliminary results indicate that the cattle and deer isolates differ. Results to date suggest that deer are not a significant reservoir of *E. coli* O157:H7 for cattle.

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*Food Science and Technology

Multiple nested PCR and AGID in caprine arthritis-encephalitis virus diagnosis

Caprine arthritis-encephalitis virus (CAEV) causes arthritis, mastitis, encephalitis, and pneumonia and is endemic in goat herds throughout the United States. There are more than 300,000 goats in Georgia, and as many as 75% of herds have infected animals. Affected goats may be subclinical carriers and transmission propagated through a herd. Because of decreased milk production, decreased weight gain in meat goats, and occasional death losses, the cost to producers can be substantial. Virus can be transmitted prior to diagnosis because of the limitations of current serologic assays; thus, CAEV eradication programs have so far been unsuccessful. We developed and evaluated multiple nested polymerase chain reaction (PCR) in diagnosis of CAEV in peripheral blood monocytes of an endemically infected Georgia herd by comparing results to those of an agar gel immunodiffusion (AGID) assay in 196 goats. Because lentiviruses have an extremely high rate of mutation, two separate



Dr. Susan Little interprets serology results on goats assayed for antibodies for *Ehrlichia* spp.

conserved sites in the CAEV genome were targeted for nested PCR amplification using a total of 16 different primers to increase the chances of identifying genetic sequences in local (Georgia) CAEV strains. One of our target sites was the *vif* gene, essential for viral processing and transmission, whereas the other was a *gag* site, a specific conserved sequence found in several West Coast herds. Our studies to date have demonstrated that multiple nested PCR successfully amplified both *vif* and *gag* sequences from CAEV infected goats and compared favorably with AGID. Using both tests, we have found 55% of the 196 goats tested were positive for CAEV. In contrast to reports from other regional CAEV isolates, many different primer sequences were necessary to generate positive PCR results, and cDNA sequencing of amplified viral genes from field isolates has revealed marked genetic variability (mutation) even in

sequences thought to be highly stable/conserved. With comparison of viral genomic isolates and primer refinement, this nested PCR assay should provide a valuable adjunctive diagnostic test to serology in CAEV eradication efforts in Georgia.

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Sequential Western blot analysis of bovine humoral immunity to paratuberculosis

Johne's disease (paratuberculosis) caused by *Mycobacterium avium* subs. paratuberculosis (MPTB) is a chronic wasting disease of ruminants that spreads slowly and may be present in a herd for years prior to diagnosis. In the United States, it is estimated that the prevalence of infection is between 5% and 20%. Infected cows have diarrhea, decreased milk production, and body condition. Losses to the dairy industry alone exceed \$1.5 billion per year. Though controversial, MPTB

may also be a human pathogen. Crohn's disease is a chronic transmural inflammatory disease of the intestinal tract that has clinical and pathologic features similar to paratuberculosis. The sequential humoral immune response to MPTB is not well studied and this lack of information hinders development of serologic tests that target molecules recognized early in the immune response. The objective of this project is to analyze by Western blot, the sequential humoral immune response of calves infected with MPTB. Our long-term goal is to develop serologic diagnostic assays that have greater potential to diagnose this chronic disease than is currently possible. Detection of early infection would allow producers a better tool to identify and eliminate MPTB-infected cattle and limit the spread to other animals and the environment. Gordon A. Hullinger, Murray E. Hines II, John R. Cole, and Charles A. Baldwin
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Endemic *Ehrlichia* in cattle: potential to confound tests for heartwater

Heartwater, caused by *Cowdria ruminantium*, is an exotic tick-transmitted disease with potential for introduction into the Southeast. Diagnostic serologic tests for *C. ruminantium* recently have been shown to result in false positive reactions in areas where ruminants are exposed to endemic, antigenically related members of the genus *Ehrlichia*. To determine the degree of exposure of Georgia ruminants to those endemic *Ehrlichia* most likely to cross-react serologically with *C. ruminantium*, blood samples were collected from cattle and goat herds. Samples were then tested by indirect fluorescent antibody (IFA) and diagnostic polymerase chain reaction (PCR) assays for antibodies to or DNA fragments of *Ehrlichia chaffeensis*. Samples were also tested by PCR assay for an *Ehrlichia* commonly found in white-tailed deer in Georgia. Initial serologic results reveal a high prevalence of antibodies reactive to *E. chaffeensis* in domestic goats, but not in cattle. Likewise, PCR testing revealed direct evidence of *E. chaffeensis* in domestic goats, and this organism was recovered in cell culture from one goat. Evidence of the deer *Ehrlichia* that also is believed to cause false positives on serologic tests for heartwater was not found in any goats or cattle tested. The data gathered from this study provide evidence of exposure of domestic ruminants in Georgia to endemic *Ehrlichia*, enabling more accurate interpretation of *C. ruminantium* serology.

Testing of sheep from endemic regions is currently underway.

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The role of parasite adhesion proteins in the disease process

Parasites exploit molecules on the surface of host cells by producing adhesion proteins capable of binding to them. The parasite's goal is to enhance its survival, which contributes to the development of disease in humans and animals. The adhesion proteins produced by the parasite are therefore of considerable interest, as blocking their interaction with host molecules should interfere with an important step in the disease process. The focus of work in this laboratory is to better understand these interactions between parasite adhesion proteins and the host molecules they bind. The starting point of this research is a family of adhesion molecules in the parasite *Plasmodium falciparum*, an agent of human malaria. During the past year, the complete sequence of the gene encoding a new adhesion molecule has been obtained, and work is underway to determine how it interacts with target molecules on host cells. Using the DNA sequence of this gene, several other putative adhesion molecules have been identified. There are significant similarities between

P. falciparum and several parasite pathogens of veterinary importance, most notably species *Babesia* (parasites of cattle, horses, and dogs). The techniques and reagents developed for the study of adhesion molecules in *Plasmodium* are now being used to detect similar molecules in *Babesia*. The discovery of novel adhesion molecules in *Babesia* will allow these techniques to be adapted to the detection of adhesion molecules in a wider array of pathogenic parasites that impact human and animal health, such as *Cryptosporidium* and *Eimeria*. These adhesion molecules are expected to provide important new targets for the development of vaccines and antiparasite drugs.

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Cytokine and T cell responses to mucosal BRSV vaccination

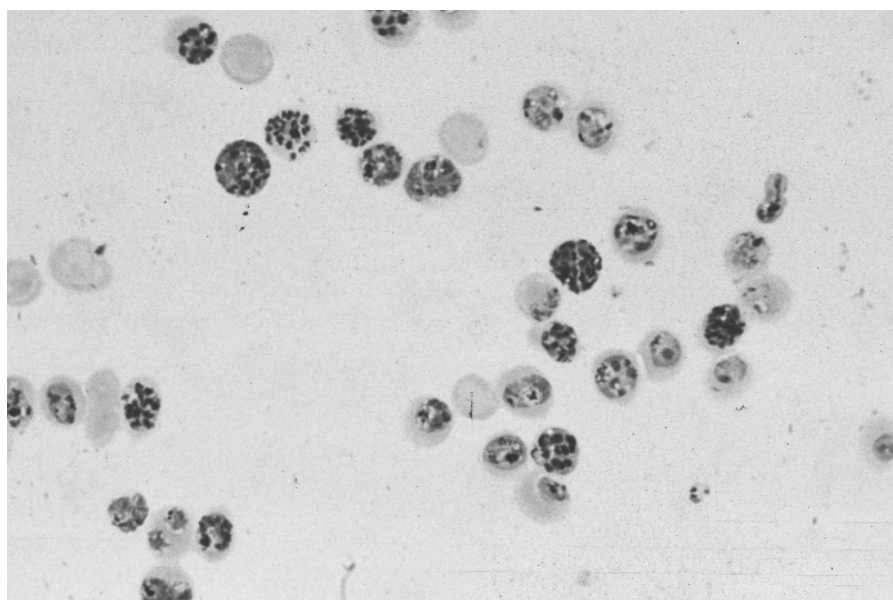
In Georgia and across the United States, respiratory disease is a leading cause of sickness and death in cattle, particularly in calves. Bovine respiratory syncytial virus (BRSV) is a common cause of this problem. Although BRSV vaccines exist, their efficacy is debatable; moreover, vaccination has at

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 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 prior to 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.

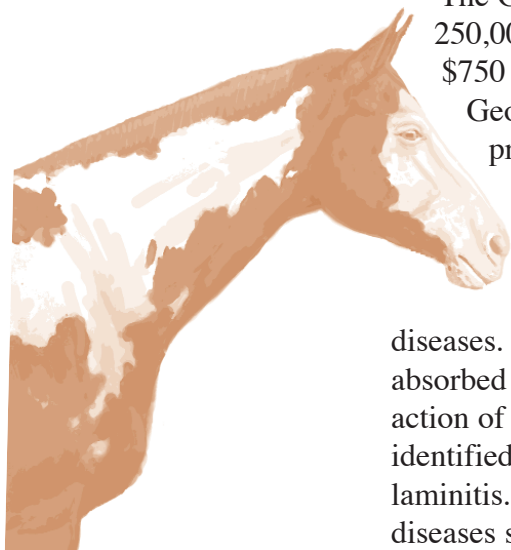
The immune response is controlled in part through the release of proteins called cytokines by T-lymphocytes and other cells. Preliminary research suggests that vaccines can be tailor-made to induce T cell and cytokine responses that are helpful and not harmful. Dr. Woolums and Dr. Brown will measure 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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Red blood cells injected with *Plasmodium falciparum*. These parasites produce specialized binding proteins to infect new red blood cells.

Horses



The Georgia horse industry is experiencing significant growth as more than 250,000 horses reside in this state. Equine-related services contribute about \$750 million to the state's economy. These numbers are expected to rise as Georgia's population continues to grow. The number of new equine training programs in the Southeast continues to expand, and more professional trainers have moved to the Southeast. With this growth comes an increasing concern about equine colic and laminitis. These diseases cause major losses to the equine industry. Thus, VMES researchers have focused on identifying better ways of treating horses with these diseases. To date, researchers have determined the effects of bacterial toxins absorbed during serious episodes of colic, developed treatments that inhibit action of some toxins, developed improved techniques to anesthetize horses, and identified the earliest changes that occur in the digit of horses that develop acute laminitis. It is the researchers' goal to learn more about the development of these diseases so that they can design appropriate measures to prevent them in the future.

Preventing LPS-monocyte interactions with anti-equine CD14 monoclonal antibodies

Endotoxemia is associated with leading causes of death in horses of all ages. Therefore, our long-term goals are to identify the molecular mechanisms responsible for the deleterious effects of endotoxin and then to develop appropriately targeted therapeutic inventions. During the past year, we have made significant progress to meet these goals. This includes cloning of equine CD14, which includes the principal endotoxin receptor. Comparison of the DNA and protein sequences of this receptor to those of CD14 from other species has revealed two regions that are essential for binding endotoxin and transmitting proinflammatory signals to the interior of the cell. In the future, we plan to bioengineer

specific alterations in the latter region of CD14 to generate a recombinant protein that will bind endotoxin strongly but not transmit the deleterious signals into the cell. Alternatively, the cloned gene may also be used to generate recombinant equine CD14 protein and then monoclonal antibodies directed against the receptor. Similar anti-CD14 antibodies have been shown to prevent endotoxin binding to CD14 in other species and thus effectively block endotoxin's ill effects. In additional research, we have also identified a new class of endotoxin antagonists derived from a specific type of plant bacteria. Our data show that these natural antagonists efficiently block endotoxin's action in horse cells. In summary, we now have three exciting new ways to interfere with the effects of endotoxemia in horses and have

developed unique tools for studying the molecular basis of endotoxin's actions.

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Dr. Ray Kaplan conducting field investigations on equine cyathostomes (parasitic worms).

Mechanisms of resistance to macrocyclic lactone drugs in equine cyathostomes

Parasite drug resistance is recognized globally as one of the greatest health threats to grazing horses, with drug-resistant cyathostomes (small strongyles)

being the group of parasites most likely to impair equine performance and cause colic. Drug resistance in the cyathostomes now exists to all classes of available dewormers except the avermectins/milbemycins (AM, macrocyclic lactones, ivermectin/moxidectin). It is not known why AM-resistant cyathostomes have not yet emerged in horses; however, most parasitologists agree that it is a question of when, not if, resistance to this drug class will appear. When AM resistance does appear in cyathostomes, the frequent and widespread movement of horses will cause rapid dispersion of resistant parasites and great harm to the equine industry. The glutamate-gated chloride channel (GluCl) protein is the putative receptor and site of action of the AM drugs and also is very likely involved with drug resistance. Currently, no AM-resistant strains exist to study, and nothing is known about the biology or genetics of the GluCl gene in cyathostomes. Therefore, molecular diagnosis is not possible, leaving traditional tests of drug efficacy that can detect resistance only after treatment failure. Lack of knowledge regarding mechanisms of resistance will therefore leave the veterinary and equine community with absolutely no method of testing for low-level resistance and no effective means to control the spread of resistant worms. The focus of the work in this laboratory is to study the molecular changes in cyathostomes that are responsible for drug-resistance, with the goal of developing polymerase chain reaction (PCR)-based assays to detect drug-resistant cyathostomes. During the past year, three ponies were acquired from a herd infected with a highly characterized population (population S, University of Kentucky) of cyathostomes. These ponies will soon be started on a specialized drug-treatment protocol to select for AM-resistant worms. Genetic changes in these drug-selected worms will be studied over time. Additionally, numerous population S worms were recovered, identified to species, and stored for use in this study. A fragment of the glutamate-gated chloride channel gene from *Cylicocyclus nassatus* was amplified from cDNA by PCR, cloned, and sequenced. Comparison with other parasite GluCl sequences showed a high level of identity between the sequences. The remainder of the *Cyc. nassatus* GluCl gene is currently being cloned. Further characterization of this gene will lead to additional studies designed to evaluate the role of this protein in AM resistance.

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A novel method of jejunal anastomosis in horses using a hyaluronate membrane

Small intestinal strangulations are one of the most serious causes of abdominal pain in horses. Despite surgical intervention, mortality rates are unacceptably high. Treatment often involves resection and anastomosis of the affected segments of intestine. Debate exists as to the preferred method for small intestinal anastomosis in the horse. This study compared three jejunal anastomosis techniques with respect to adhesions, stomal area, healing, and surgery time. We hypothesize that a single-layer, appositional anastomosis covered by a hyaluronate membrane (HA-membrane) will 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 (group 2, n=6), or single-layer appositional pattern covered by an HA-membrane (group 3, n=6). All horses were euthanatized 21 days after surgery. The abdominal cavity was evaluated for adhesions and anastomotic healing. Intestinal luminal diameters at the anastomosis and adjacent jejunum were measured using ultrasonography. Histopathology was performed to evaluate anastomotic healing. Fibrous adhesions were associated with the anastomoses in one of six horses from group 1, five of six horses from group 2, and one of six horses from group 3. The mean percent reduction of jejunal diameter at the anastomoses for group 1, 2, and 3 horses was 44%, 34%, and 28%, respectively. Mean surgery times for performing the anastomoses was 33 minutes, 25 minutes, and 28 minutes, respectively. The results of this study suggest a single-layer, appositional anastomosis covered by an HA-membrane may provide a safe and reliable method of small intestinal anastomosis in horses, reducing the morbidity, mortality, and economic losses associated with small intestinal surgical diseases in horses.

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An HA-membrane is being applied to a jejunal anastomosis in a horse to promote healing and prevent intra-abdominal adhesions.

Swine



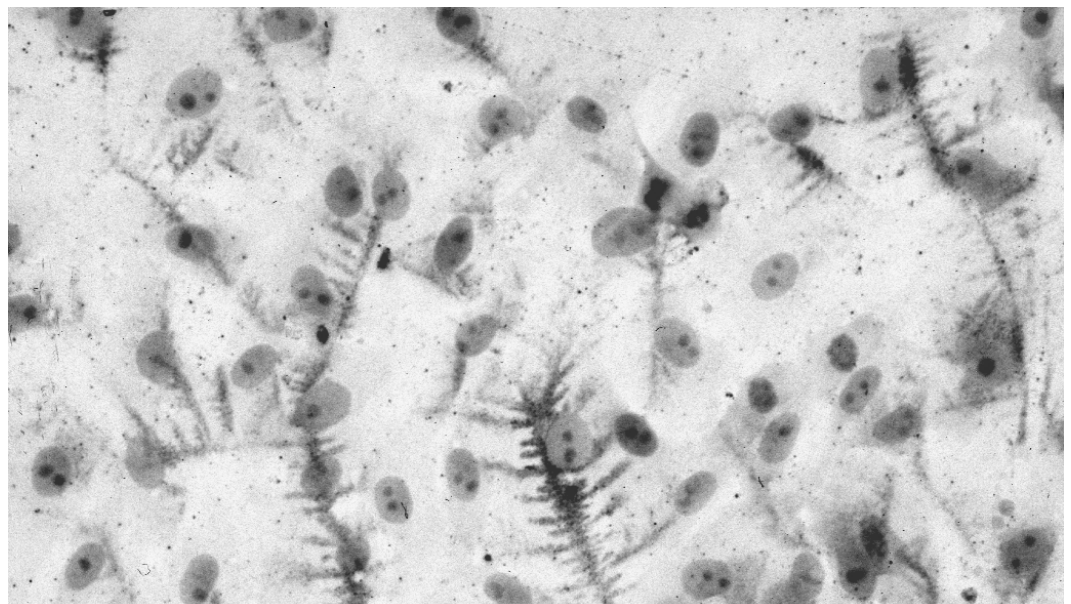
The productivity of swine herds continues to improve nationally. Much of this improvement is because of new technology such as environmentally controlled housing, artificial insemination, and high health strategies such as multiple-site production. This innovative technology along with macroeconomic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

Porcine reproductive and respiratory syndrome virus infection of porcine endothelial cell cultures

Porcine reproductive and respiratory syndrome (PRRS) virus is an important cause of abortion and pneumonia in pigs. Diagnosis of this disease can sometimes be difficult, and the virus cannot always be isolated from suspected field cases using the currently available cell lines. Although the macrophage is the primary target of virus replication for PRRS virus, it is difficult to develop and maintain cultures of macrophages for routine work. There is evidence that PRRS virus naturally infects endothelium, and endothelial

cultures are fairly easily developed and could be used for routine diagnostic work. Our objective was to test porcine endothelial cell cultures as an alternative and possibly more effective method for isolating this virus. We developed aortic endothelial cell cultures from pigs and attempted to infect them with a reference strain and several field strains of the PRRS virus. PRRS virus did not replicate in the endothelial cell cultures we developed. From our work it seems likely that the tropism of PRRS virus for endothelial cells is low. *Elizabeth W. Howerth, David E. Stallknecht, and A. Wayne Roberts*
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Immunohistochemical staining of primary porcine aortic endothelial cell cultures for von Willebrand factor antigen. Note positive immunostaining along cell surfaces confirming that the cells are endothelium.

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, decreased respiratory capacity, and decreased function.

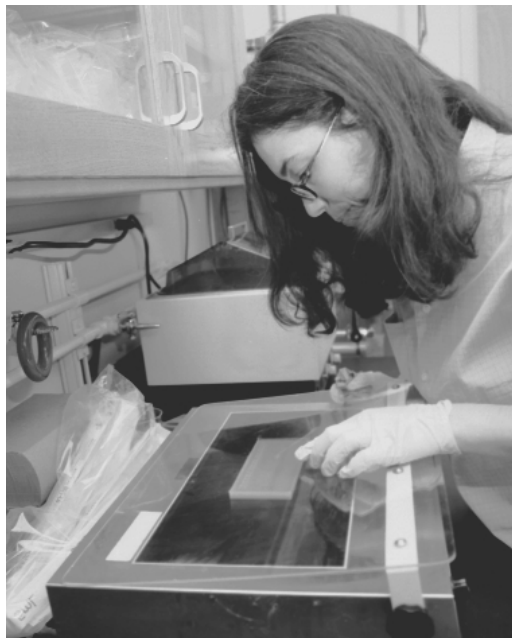
However, unlike other research areas, there are no federal funds and only limited state funds to support projects specifically for companion animals. The VMES has been useful in assisting new clinical faculty in their initial research projects, but the vast majority of funding has come from foundations and private industry. 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.



Feline immunodeficiency virus: *in vitro* and *in vivo* cell tropism

Infection of cats with the feline immunodeficiency virus (FIV) results in an early flulike illness, followed by a long asymptomatic period, and eventual death of the patient caused by immunodeficiency. The disease in cats can be transmitted through blood inoculation (biting), sexual contact, and *in utero* to kittens. Thus, the disease closely resembles the course of HIV infection and presents a valuable model for investigating possible vaccines against an immunodeficiency virus.

In our laboratory, we investigate how FIV causes immunodeficiency. Lymphocytes expressing the CD4 molecule are at the center of a functional immune system. HIV infects mainly CD4 lymphocytes, and the cells die as a result of the viral infection or are killed by the immune system. FIV infects many different lymphocytes; however, only CD4 lymphocytes disappear as the disease progresses. Thus, although the target cell of the viruses differs, the outcome of infection is essentially the same. In our studies, we have determined that the effect of viral infection is different for



The VMES has conducted molecular studies on the pathogenesis of feline immunodeficiency virus.

T-lymphocytes, B-lymphocytes, and monocyte/macrophages. The virus induces rapid cell death in T-lymphocytes, replicates to a great degree without inducing cell death in

monocytes/macrophages, and persists without much replication or cell damage in B-lymphocytes. This indicates that early intervention might have to be directed at those cells that sustain viral production, namely monocyte / macrophages. Further, these cells are the first targets of natural infections by lentiviruses, and vaccine design should either provoke an immune response able to prevent or curtail productive infection of monocyte/macrophages.

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Dietary conjugated linoleic acid: Effect on tumor growth and metastases

Because of the increased quality of pet care in the United States, the pet population is living to older ages. Associated with this increase in longevity is an increase in the prevalence of cancer in the pet population. Cancer is one of the leading causes of death in pets. In the majority of cases, death from cancer is not caused by an inability to control the primary tumor but instead from the inability to prevent or treat the spread of cancer. This inability to control the spread of the disease is also the leading cause of cancer death in people.

Prostate carcinoma of the dog has recently been identified as a model for human prostate cancer. Prostate cancer is the most commonly diagnosed neoplasm of men and the second leading cause of male death in the United States. The dog is the only nonhuman species in which prostate cancer occurs spontaneously and has a similar pattern of spread to distant organs. Because pets share a similar environment and in many cases diet with their owners, factors that influence cancer in dogs

may influence cancer in people. Diet has been shown to play a role in the development, treatment, and management of cancer in both the human and animal patient. Conjugated linoleic acid (CLA), a fatty acid found predominately in meat and dairy products has been shown to have anticancer properties. Conjugated linoleic acid may alter growth of the primary tumor as well as inhibit the spread of tumor cells to distant organs. This study will evaluate the effects of dietary CLA on the ability of canine prostate cancer cells and human breast cancer cells to grow and spread to distant sites when they are inoculated into athymic (immune incompetent) mice. Completion of this study will further elucidate the role of CLA in the inhibition of cancer in animals and in man.

Karen Cornell

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Pharmacokinetics and efficacy of transdermal fentanyl patches in cats and their effect on serum cortisol concentrations

Adequate pain control in cats is frequently neglected in veterinary clinical practice. A method needs to be developed to provide effective analgesia for control of postoperative pain in cats. Transdermal delivery of opiate analgesics offers an attractive means of maintaining analgesia for an extended period. Although several studies have been published describing the use of transdermal fentanyl patches (TFP) in dogs, only one study has been published describing their use in cats. The fentanyl concentrations achieved, the efficacy, and any potentially serious side effects of TFP have not been well characterized in this species.

The objectives of this project were to investigate the pharmacokinetics and clinical use of TFP in cats. The cats were assigned to the following groups. Group I cats received fentanyl patches but did not undergo anesthesia or surgery. Group II cats received fentanyl patches and were anesthetized during a portion of the study period. Group III cats did not receive fentanyl patches and were anesthetized during a portion of the study period. Group IV cats received fentanyl patches and underwent anesthesia and ovariohysterectomy (spay). Group V cats did not have fentanyl patches placed and underwent anesthesia and ovariohysterectomy. Group V cats were provided analgesia in a manner similar to that used in clinical practice.



Dr. Karen Cornell propagates canine prostatic cancer cells in the laboratory.

The effect of TFP on pain, behavior, serum cortisol levels, heart rate, respiratory rate, blood pressure, body temperature, blood glucose, feed intake and body weight was investigated in the five groups of cats in an attempt to evaluate the efficacy of these patches for the control of postoperative pain. *Christine M. Egger and Leigh E. Glerum*
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Bartonella henselae infection in cats, immunity based on route of exposure

We conducted a study to determine the effect of the route of inoculation on immunity against *Bartonella henselae* infection in cats. Cats are the primary reservoirs for this organism that causes cat scratch disease and bacillary angiomatosis in people. Determining the appropriate route will facilitate development of a vaccine against this infection in cats and thus reduce the spread of infection to people. Cats were infected by either IV inoculation of whole blood or intradermal inoculation of organisms. Infection was determined by weekly culture of blood measuring intracellular and extracellular bacterial counts. Specific serum antibody responses were also determined weekly. Cats from both groups became infected after the first challenge. Seroconversion was noted in all cats during the course of infection. Following a three-week observation period, all cats were treated with antibiotics to clear infection. Subsequently, the challenge infection was repeated in the identical manner as before. At that time, only the cats given IV inoculation were infected. The qualitative differences in serum antibody response to outer membrane proteins of the organism are currently being compared in both groups of cats. This will enable us to target specific antigens for vaccine development.

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Pharmacokinetics of the antihyperglycemic agent metformin in cats

The feline form of diabetes mellitus shares many of the same characteristics as those associated with type-2 diabetes mellitus in people. Many of the type-2 human diabetic patients can be treated with oral antidiabetic drugs. Metformin is a new oral drug that has recently been approved in the United States for human type-2 diabetics. It improves diabetes

control by decreasing glucose absorption in the gut, by increasing the uptake of glucose from blood into cells where the glucose can be used for energy, and by decreasing triglycerides in the blood. This drug could be an attractive treatment for the diabetic cat if we can establish a dosage that will be safe and effective. The goal of this study was to establish the pharmacokinetics of metformin in cats. With this information about the drug's disposition in the cat, we established the dose and the interval at which it needs to be given to yield plasma concentrations that have been documented to be effective in humans. We showed that the general disposition of metformin in cats was similar to that reported for humans. We also found that metformin was eliminated principally by the kidneys and should therefore not be used in cats with substantial dysfunction of the kidneys. The results from this study will now allow us to enter into clinical trials with metformin.

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Distribution of NMDA glutamate receptors in the canine brain assessed by autoradiography using [3H] MK-801

The objectives of this proposal are to 1) develop a reliable technique to determine glutamate (NMDA) receptor distribution in the canine brain and 2) establish a normal cerebral regional distribution of glutamate (NMDA) receptors in the dog.

Seizures in humans and animals are related to the excitatory neurotransmitter glutamate. An excess of the transmitter or an increase in the amount of receptors in the brain for the transmitter has been proposed as mechanisms for abnormal brain activity including seizures. This remains to be definitively proven and potentially utilized in future therapies.

Autoradiographical mapping of cerebral receptors for glutamate will be used to demonstrate the alterations that exist in pathological states and the changes that therapy used for these states invokes. This information is vital to the understanding of the pathophysiology of seizures and will help with the investigation of more effective treatments. *Simon R. Platt, John J. McDonnell, Thomas F. Murray, Gaylen L. Edwards, K. Paige Carmichael, and Fred W. Berman*
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Financial Highlights

Research Funding

Funding Source*	Fiscal Year '98	Fiscal Year '99
VMES Budget	\$2,984,133	\$3,174,529
Federal Grants and Contracts	1,327,683	1,619,584
State Grants and Contracts	142,650	179,825
Private Grants and Contracts	2,904,553	2,347,355

*Excluding carryover funds

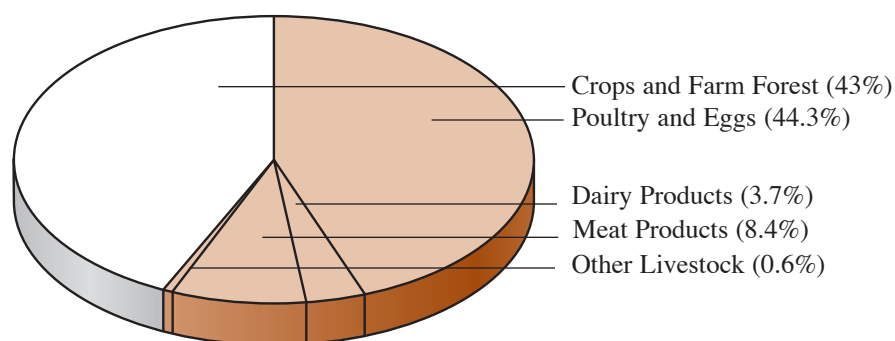
Georgia Livestock and Poultry: Inventories and Values^a

Species		Number on Farms and/or Produced	Production Value
Cattle	Beef	1,354,000	\$620,360,000
	Dairy	96,000	323,530,000 ^b
Hogs		1,640,000	209,200,000
Poultry	Broilers	1,202,500,000	2,386,382,000
	Nonbroilers	21,034,000	18,443,000
	Eggs	5,126,000,000	375,907,000
Horses and Ponies		251,000	125,500,000

^aBased in part on information published by the Georgia Agricultural Statistics Service, Athens, Georgia

^bIncludes value to dairy cattle and milk produced

Georgia Farm Cash Receipts



Research Contracts and Grants

- Barton, M.H.** Evaluation of the ability of polymyxin B sulfate to bind endotoxin in an *in vivo* equine model. Morris Animal Foundation, \$16,266.
- Brackett, B.G.** Ovarian cortex transplantation in the SCID mouse. Reproductive Biology Associates, \$6,812.
- Brown, C.C.** Pathogenicity of pigeon paramyxovirus-1 for domestic poultry. U.S. Poultry and Egg Association, \$26,411.
- Brown, S.A.** Choice of antihypertensive agents in cats. Morris Animal Foundation, \$33,360.
- Comparison of two dietary approaches to managing canine chronic renal failure. The IAMS Company, \$25,431.
- Effects of dietary antioxidants and omega-3 polyunsaturated fatty acids on immune function and progressive uremia in dogs with chronic renal failure. The IAMS Company, \$174,356.
- Effects of dietary fiber on colonic trapping of nitrogen in dogs with induced chronic renal insufficiency. The IAMS Company, \$26,534.
- Brown, T.P.** Mobilizing national resources to combat emerging food animal diseases using poult enteritis mortality syndrome in turkeys as a model. North Carolina State University, \$78,374.
- Prevention of the effects in broilers of avian leukosis-virus subgroup J (ALV-J). U.S. Poultry and Egg Association, \$74,096.
- Study of spiking mortality of turkeys. North Carolina State University, \$23,041.
- Budsberg, S.C.** Evaluation of the application of transdermal diclofenac for the improvement of rear limb function in dogs with chronic stifle osteoarthritis. Blue Ridge Pharmaceuticals, Inc., \$40,698.
- Preemptive analgesic efficacy of Meloxicam in comparison with postoperative butorphanol in dogs undergoing stifle stabilization for the repair of a cranial cruciate rupture. Boehringer Ingelheim Animal Health, \$10,871.
- Coffield, J.A.** Mechanism of bolulinum toxin action. Thomas Jefferson University, \$18,915.
- Crowell-Davis, S.L.** Canine separation anxiety, a disorder of dogs characterized by inappropriate behaviors in the absence of an attachment figure. Novartis Animal Health U.S., Inc., \$3,000.
- Dickerson, H.W.** IBV serotype rollout project. Georgia Research Alliance, \$30,000.
- Molecular approaches toward the control of ichthyophthirius infection, U.S. Department of Agriculture, \$300,000.
- Research training experience for veterinary medical students. Merck Company Foundation, \$30,000.
- Edwards, G.L.** Role of dorsomedial medulla oblongata in Amylin action on gastrointestinal activity and food intake. American Diabetes Association, \$74,315.
- Evans, D.L.** Catfish nonspecific cytotoxic cells: Receptors and signalling. U.S. Department of Agriculture, \$150,000.
- Fayrer-Hosken, R.A.** Development of a natural injectable sterilant vaccine for dogs and cats and elucidation of a synthetic sterilant vaccine for dogs and cats. Georgia Research Alliance, \$50,000.
- Immunosuppression in African elephant using PZP vaccine. Humane Society of the United States, \$26,458.
- Finco, D.R.** Beneficial effects of dietary phosphorus (P) restriction and antioxidant therapy in cats with induced chronic renal failure. The IAMS Company, \$116,800.
- Ultralow phosphorus diet on dogs with induced chronic renal failure. The IAMS Company, \$32,300.
- Validation of Iohexol clearance as a metric of glomerular filtration rate in the cat and dog. Pfizer, Inc., \$34,888.
- Fischer, J.R.** Epidemiologic investigation of coot and eagle brain lesion syndrome. Arkansas Game and Fish Commission, \$60,000.
- Garcia, M.** Development and application of RT-PCR assay for detection of ALV-J from meat-type chickens. U.S. Poultry and Egg Association, \$55,198.
- Development of polymerase chain reaction diagnostic method capable of distinguishing among infectious laryngotracheitis strains. U.S. Department of Agriculture, \$5,004.
- Hoening, M.** Development of a specific and sensitive assay for feline insulin and proinsulin. Morris Animal Foundation, \$25,450.
- Effect of dietary fiber on carbohydrate and lipid metabolism in the healthy cat. Ralston Purina Company, \$5,500.
- Effect of high protein diet on glucose tolerance and lipid profiles. Ralston Purina Company, \$32,248.
- Obesity in the cat: Investigations into the etiology of hepatic lipidosis. The IAMS Company, \$91,000.
- Howerth, E.W.** Infection of domestic pigs with pseudorabies virus isolated from feral pigs. National Pork Producers Council, \$18,500.
- Pathogenesis of vesicular stomatitis in horses. Grayson Foundation, \$28,703.
- Jackwood, M.W.** Development of a recombinant vaccine for infectious bronchitis virus. Hoechst Roussel Vet., \$8,936.
- Recombinant vaccines for infectious bronchitis virus. U.S. Department of Agriculture, \$7,398.
- Jaso-Friedmann, L.** Role of cytokines in amplification of tilapia nonspecific cytotoxic cell activity. U.S. Department of Agriculture, \$20,000.
- Kleven, S.H.** Avian mycoplasmosis. U.S. Department of Agriculture, \$4,874.
- Studies on *Mycoplasma synoviae* strain K4463B: Virulence, antigenicity, and antigenic relationship to type strain. Intervet, Inc., \$6,604.
- Lee, M.D.** Creating a broadly protective fowl cholera vaccine for broiler-breeder chicken. U.S. Poultry and Egg Association, \$26,965.
- Little, S.E.** Evaluation of the use of Preventic (9% Amitraz) collars as adjunctive treatment for canine demodectic mange. Allerderm/Virbac, \$15,382.

- Lukert, P.D.** Bovine enteric diseases challenge model. Merial Limited, \$80,209.
- Development of experimental challenge models for bovine enteric diseases in newborn calves and testing of candidate vaccines by challenge studies in newborn calves. Georgia Research Alliance, \$79,825.
- Maki, J.** *Ictalurus punctatus*: A model to study mucosal immunity. National Institutes of Health, \$79,270.
- Maurer, J.J.** DNA fingerprinting of *Salmonella* sp. from poultry by pulsed-field gel electrophoresis. U.S. Poultry and Egg Association, \$65,661.
- The distribution of virulence specific genes among avian *Escherichia coli* isolates. U.S. Department of Agriculture, \$2,611.
- McCall, J.W.** Antifilarial drug testing in dogs. World Health Organization, \$156,041.
- Experimental chemotherapy of filariasis and screening of filaricides. World Health Organization, \$63,268.
- Filariasis repository-research service. National Institutes of Health, \$235,514.
- Furnish *Brugia malayi* adult worms and/or *B. malayi* infective larvae. National Institutes of Health, \$90,028.
- Medleau, L.** Frontline TopSpot therapy for flea allergy dermatitis in cats. Merial Limited, \$13,734.
- Treatment of flea allergic dermatitis in cats using Frontline TopSpott. Merial Limited, \$1,831.
- Murray, T.E.** Affinity labels for opioid receptors. University of Maryland, Baltimore, \$37,490.
- Dextrorotatory opioids as probes for PCP receptors. National Institutes of Health, \$293,048.
- Dynorphin analogues as K-opioid receptor antagonists. University of Maryland, \$89,248.
- New reactor for drug discovery libraries. Bend Research, Inc., \$32,400.
- Technology for generation of drug candidate libraries. Bend Research, Inc., \$5,000.
- Marine/Freshwater Biomedical Sciences Center. Oregon State University, \$10,000.
- Nettles, V.F.** Assess possible risk factors for exposure to *Ehrlichia*. Centers for Disease Control and Prevention, \$43,277.
- Development and evaluation of alternate baits for delivery of V-RG rabies vaccine to gray and red foxes. Georgia Research Alliance, \$20,000.
- Development of scientific information on the animal traps for selected wild vertebrates species by providing necropsy data in injuries associated with the use of animal restraint devices. U.S. Department of Agriculture, \$75,000.
- Oral vaccine delivery to raccoons. U.S. Department of Agriculture, \$7,500.
- Southeastern cooperative wildlife disease study. Southeastern Association of Fish and Wildlife Agencies, \$184,300.
- Peterson, D.S.** Characterization of DBL-domain proteins in *Plasmodium falciparum*. \$161,943.
- Rawlings, C.A.** Balloon catheter for radiation delivery in the caprine model. Proxima Therapeutics, Inc., \$24,839.
- In vivo* study of biodegradable urethral stents with proximal and distal stent body, proximal and distal loop gold bands in the canine cystoscopy model. Indigo Medical, Inc., \$20,000.
- Laparoscopic technique for induction of interstitial laser coagulation lesions by the Indigo's laser fiber in the canine. Indigo Medical, Inc., \$31,688.
- Reeves, D.E.** A survey of patterns and persistence of antimicrobial resistance on swine farms using three different antimicrobial use strategies. National Pork Producers Council, \$25,000.
- Effect of competitive exclusion on *Salmonella* in pigs. U.S. Department of Agriculture, \$29,700.
- Safety evaluation of pre-licensing serials of swine influenza, killed virus, USDA product code 19A5.10 under field conditions. Bayer Corporation, \$5,860.
- Rowland, G.N.** Evaluation of mechanisms of tendon failure in heavy meat-type birds. U.S. Department of Agriculture, \$1,415.
- Sander, J.E.** Investigation of hatchery disinfectant efficacy and effect on broiler production. U.S. Department of Agriculture, \$3,480.
- Stallknecht, D.E.** Validation of a swine model for vesicular stomatitis. U.S. Department of Agriculture, \$20,216.
- Wildlife reservoirs for the H5 and H7 avian influenza viruses. U.S. Department of Agriculture, \$10,000.
- Thayer, S.G.** Investigation of natural disease outbreaks and field trial studies. U.S. Department of Agriculture, \$26,026.
- Thompson, F.N.** Transruminal and transomasal migration of ergot alkaloids. U.S. Department of Agriculture, \$19,550.
- Villegas, P.** Inoculation study of Marek's disease virus. Pfizer, Inc., \$149,553.
- Isolation, identification, and control of avian viruses. U.S. Department of Agriculture, \$9,230.

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* Publications resulting from research conducted by faculty in the College of Veterinary Medicine and the Veterinary Medical Experiment Station