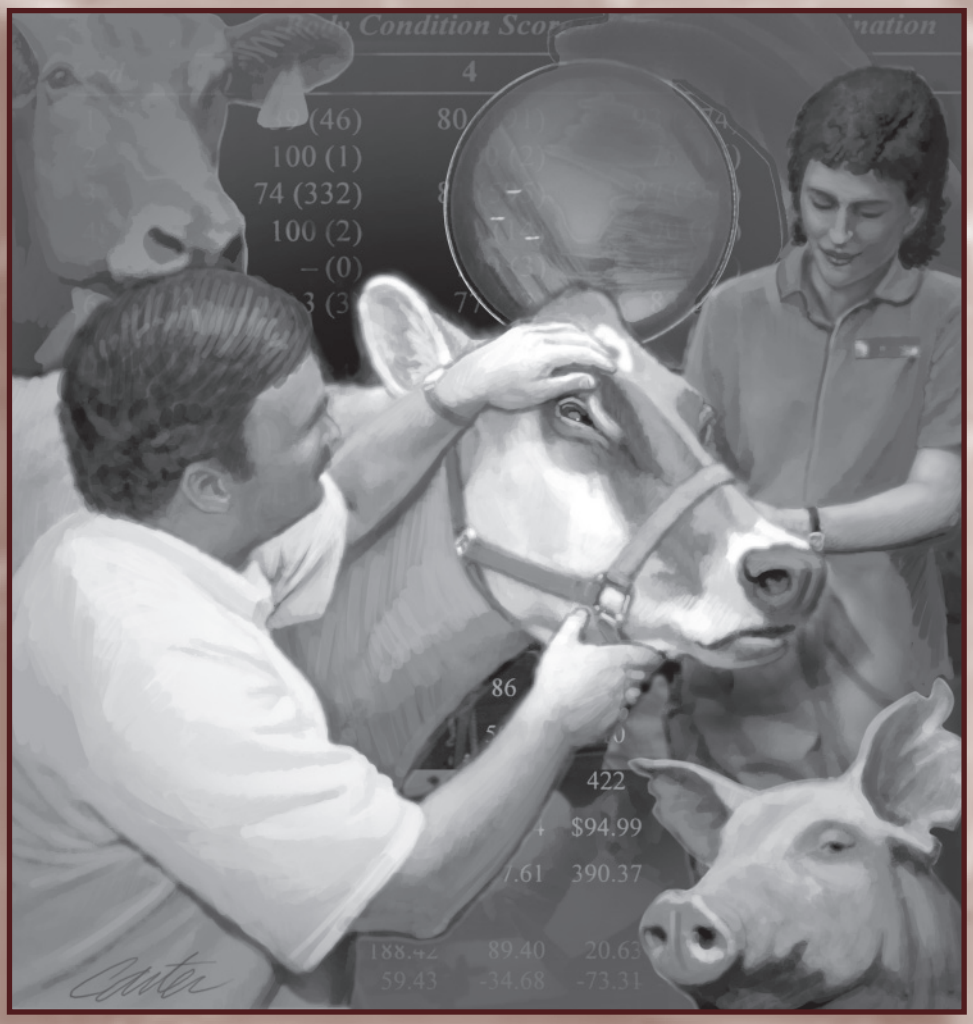
VMES 2002

Science in Service to Animals



FOOD ANIMAL HEALTH & MANAGEMENT PROGRAM

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

VMES 2002

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College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602

July 1, 2001 to June 30, 2002

26th Annual Report

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

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VMES Objectives

The Veterinary Medical Experiment Station (VMES) supports a wide range of research that impacts on many aspects of our lives; the food we eat and the clothes we wear, our physical, emotional, and economic health, and the quality of our environment. VMES research includes efforts to improve the productivity and health of poultry and livestock, to better the quality of life for companion animals, and to improve public health through disease surveillance. This year's research is profiled in our 2001 - 2002 VMES annual report.

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

Our objectives are as follows:

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

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Report of the Director

It is with pleasure that I introduce the 26th Annual Report of the Veterinary Medical Experiment Station (VMES). As I write this, I am completing my fifth year as Director and look forward to the challenges of the coming years. Although we are faced with increasing budgetary constraints in the face of uncertain state and national economies, the VMES continues to play a critical role in animal and human health. For instance, the VMES will serve as an important element of our state's and the nation's defense against agroterrorism. The United States Department of Agriculture is implementing a plan to establish a unified network of public agricultural institutions to identify and respond to high-risk microbial pathogens in the nation's food and agricultural system. The University of Georgia College of Veterinary Medicine has been chosen, based on its relevant expertise in this area, to be part of a core network of five animal diagnostic laboratories with Biocontainment Level 3 capabilities. (Biocontaiment Level 3 is a designation given to research facilities with the ability to work safely with highly contagious microbial pathogens.) As new opportunities for research in the area of emerging infectious diseases and biodefense arise, we will seize them. For example, new funding initiatives from the NIH will provide resources for basic research in the diagnosis, prevention, and treatment of infectious zoonotic diseases. By responding to the immediate biodefense needs of U.S. agriculture, we have the opportunity to enhance veterinary science in basic and applied areas such as microbial pathogenesis, drug discovery and vaccine development.

This year's cover story focuses on the Food Animal Health and Management Program (FAHMP), a new program that was initiated through a significant enhancement to the VMES budget over the last three years. As you will read in the accompanying article, the FAHMP brings a team approach involving field veterinarians and animal and veterinary scientists to address all aspects of disease and food animal production management.

Our mission remains critical to the people of Georgia. The VMES plays a primary role in research on animal health problems of present and future concern to our state's livestock and poultry industries as well as its wildlife resources. Our food animal industries are valued at well over $3 billion. Sales of livestock, poultry and their products account for more than half of Georgia's annual farm income. A continued commitment at the state level to support research on animal health is a smart investment, particularly in view of the fact that there is limited federal and private funding targeted specifically for animal health research.

The 26th Annual Report provides a brief overview of many of the VMES-supported projects during the fiscal year of 2001-2002. Additional information on any of these projects can be obtained by contacting the VMES office by phone, email or website, or directly from the investigators themselves. A list of publications is provided. These peer-reviewed papers represent a selection of VMES supported work and other research originating at the College of Veterinary Medicine.

Harry W. Dickerson

Food Animal Health & Management Program

Georgia's economy and quality of life are built on a long and proud history of agricultural productivity. As a result, Georgia is a national leader in many areas of agricultural production, and its prosperity depends upon maintaining a well-balanced and productive agricultural sector. The Food Animal Health and Management Program (FAHMP) was developed to address the needs of the state's beef, dairy and swine producers. The organization of the FAHMP was based upon the University of Georgia's poultry production programs that enjoy an international reputation for excellence.

The FAHMP brings together a team of veterinarians and scientists to address fundamental aspects of disease and animal production management. Due to its diverse makeup, the team will be able to approach problems and new trends in production from both basic and applied aspects. The principal aims of the FAHMP are to address problems that impact meat and milk production in Georgia; develop a core of leaders in food animal health; meet the public's demand for high quality, safe meat and dairy products; and provide consultation services to private veterinarians serving Georgia's food animal industry.

The members of the program team have developed a new, highly focused program for the development of food animal production system leaders. The program, bestowing a Masters of Food Animal Medicine, MFAM, is designed to provide veterinarians a clear path to becoming leaders as food animal health service providers. The FAHMP will require the demonstration of a "real world" skill set driven by the career track and long term vocational goals of veterinarians enrolled in the program. The veterinarians in the program will also design, execute, and evaluate trials or field programs. These trials will be within the area they plan to work and will be in cooperation with end users (such as production companies, biologic and pharmaceutical companies, or government organizations). Depending on their individual career goals, the graduates will have an intimate working knowledge of issues confronted by the beef, dairy, or swine industries. Graduates will be able to address pre-harvest and post-harvest food safety, production economics, epidemiology and management, and regulatory issues.

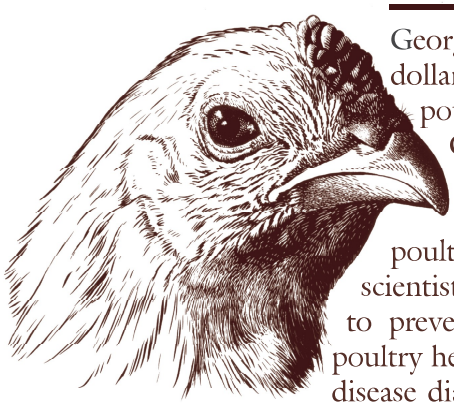
The FAHMP also is a research organization. Members of the program include veterinarians and scientists working on basic problems in food animal disease, neonatal and vaccine immunology, antibiotic resistance, reproductive efficiency, epidemiology and nutritional methods that improve animal health, and their application. The overarching goal of the research arm of the program is to provide biological, pharmaceutical, nutritional and management-based solutions to real world production problems.

The FAHMP has a team of experienced field investigators with expertise in health and management issues of beef, dairy cattle, and swine. One of their functions is to provide immediate "backup" assistance and advice to veterinarians currently working with food animal producers in Georgia. The FAHMP is working with companies to provide the products that will allow producers to meet the ever-changing regulatory environment in food animal production. The program has initiated collaborations in vaccine evaluation and development with the veterinary biological industry. Members of the program are interacting with companies interested in developing new nutritional products, developing ways to reduce the need for antibiotics, and improving implants used in the production of meat. Members of the program are actively working with producer organizations on the development and analysis of management programs to provide the optimal economic return to producers of food animals. An additional goal of the FAHMP members is to develop the basis for a politically neutral clearinghouse for the dissemination of scientific and economic information impacting the development of policy and regulation of the industry. The FAHMP will be of service to all organizations attempting to sustain and improve the production of meat and milk. Therefore, the program is in an excellent position to become a national resource for the unbiased assessment of information on which fair and equitable policies can be built.

We are proud of the outstanding history of Georgia in agriculture. It is our goal, as members of the FAHMP to provide continuing support and innovation to the future of food animal production in Georgia.

PI: David J. Hurley (dhurley@vet.uga.edu)
CoPIs: David Reeves, Mel Pence, Dana Cole, Amelia Woolums, James N. Moore

Poultry



Georgia's poultry industry dominated the state's animal agricultural dollars with nearly $2.42 billion in annual revenue in 2001. The state's poultry industry is continuing to expand as broiler production in Georgia increases. 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. A major recent effort has been initiated to characterize "silent" laryngotracheitis, which was first detected and described in our laboratories. Researchers are also focusing on the reduction of potential human pathogens on poultry products nationwide and on ways to prevent the development of resistance against antibiotics.

During FY 02, avian medicine faculty were investigators or co-investigators in new extramural funding of $3,776,136 from 10 projects. This was primarily from USDA, U.S. Poultry and Egg Association, and private sources. Twelve intramural projects totaling $450,394 were funded for FY 2002. Our faculty and graduate students have been active in presenting their research at national and international meetings. During FY 2002, there were 42 papers published in refereed journals, and 123 scientific and industry presentations.

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

Investigation of Natural Disease Outbreaks

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

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

An example of field investigations includes scenarios such as: serological assessment in a broiler operation with severe condemnations at processing showed significant titers against Connecticut-like infectious bronchitis virus. Addition of a Connecticut strain vaccine to the broiler vaccination program ended the condemnations and the financial losses due to this virus.

Improvements in the lab database continue with functional and additional data search capabilities and simplified maintenance. Lab reports are being sent by email in .pdf format and faxed directly from within the system without the need for an intermediate hard copy. Offering lab data by secure web site is being investigated.

The polymerase chain reaction (PCR) technique is an integral part of the diagnostic laboratory as seen by the consistent demand for these tests. PCR techniques for infectious bronchitis virus, Mycoplasma, Infectious Bursal Disease, Infectious Laryngotracheitis Virus, and Avian Leukosis Virus-J provide mostly same-day results. Techniques for large volume processing and faster turnaround are continually being evaluated.

Diagnostic Services Laboratory activity is represented by 6,294 accessions, 36,200 bacterial procedures, 180 antimicrobial susceptibilities, 103,437 ELISA tests, 44,770 IBV-HI tests, 42,598 *Mycoplasma* plate agglutination tests, 3,716 agar gel precipitin tests, 3,033 diagnostic PCR tests, and 1,651 necropsies.

P.I.: Stephan G. Thayer (sthayer@arches.uga.edu)
Co-investigators: Kleven, S.H., Brown, T.P., Garcia, M., Glisson, J.R., Hofacre, C.H., Jackwood, M.W., Maurer, J.J., Rowland, G.N., Sander, J.E., Sellers, H.S., Vezey, S.A., Villegas, P.

Clinical Investigation of Poultry Diseases

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

studied 4 problem broiler farms to determine the cause of poor performance. Also, studies were completed evaluating the heating of an oil emulsion *Pasteurella multocida* bacterin on tissue reaction/immunity in broiler breeders; the effect of feed restriction on hypoglycemia spiking mortality syndrome in broilers; the incidence of *Salmonella* in litter of North Georgia broiler farms; the prevalence of IBV during the downtime in broilers; and the effect of formaldehyde usage on *in ovo* injected eggs.

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

PI: Dr. Charles Hofacre (chofacre@arches.uga.edu)
Co-PI: Dr. John Glisson and Dr. Jean Sander

Detection of Foodborne Pathogens Using *r*RNA Signature Sequences and Acroarrays

Many foods including poultry products have a short shelf life in the supermarket. Microbial testing requires sensitive and rapid methods for detection of biological hazards. We have been successful in development and validation of several polymerized chain reaction (PCR)-based tests for detecting important foodborne pathogens like *Salmonella* and *Campylobacter.* We have been able to incorporate *invA*-specific, nested PCR into screens of poultry samples for *Salmonella*. Compared to the gold standard, culture, the PCR had excellent test specificity (96%) and sensitivity (95%). To improve test throughput, we have developed a PCR-ELISA with similar specificity and sensitivity to our nested PCR. Similarly, we have developed and started to validate a PCR-ELISA test for the detection and enumeration of *Campylobacter jejuni/coli* on chicken carcasses. We have moved toward developing a single test for the detection of multiple foodborne pathogens using signature sequences within *rrn* as targets of an oligonucleotide capture probe in macroarrays. Unfortunately, we have been unable to detect hybridization of labeled PCR product to membrane despite good amplification and excellent incorporation of dig-labeled nucleotides into the PCR amplicon. However, these oligonucleotides do work as part of a PCR-ELISA. We propose in the next year to redesign the oligonucleotide capture probes, creating 50-mer capture probe, length generally observed for oligonucleotides used in microarrays and empirically optimize specificity and sensitivity of the macroarray. If we should fail to obtain adequate results, then we will move toward developing ribosomal rna gene (*rrn)*-signature oligonucleotides into a single PCR-ELISA for detecting multiple foodborne pathogens.

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

Avian Mycoplasmosis

The objectives of this proposal are to study improved measures for the control of avian mycoplasma infection, to improve detection methods, to study the pathogenesis of avian mycoplasmas, and to study the incidence of avian mycoplasmas.

RAPD is being routinely used to fingerprint *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) isolates. Isolates which appear to be similar may in fact be different if a second or even a third primer set is used. PCR using primers for genes with strain polymorphisms are being used. Primers for mgc2 show promise as a diagnostic PCR from which sequencing could be used to identify strains. A study is underway to compare various PCR procedures for diagnostic and epidemiological purposes. We have begun to use a PCR for MS strains using primers from the vlhA gene. This gene shows polymorphisms which are now being used to further fingerprint MS isolates.

A house finch-like isolate of MG isolated from turkeys in the Midwest shows excellent promise as a vaccine. The strain is avirulent for both chickens and turkeys and in laboratory challenge studies appears to be very efficacious.

MG 6/85-like vaccine isolates have been made from vaccinated and unvaccinated commercial layers in Georgia which showed production drops. Chickens challenged with these isolates did not develop signs or respiratory lesions, but these isolates colonized chickens quite effectively.

Studies on the incidence of avian mycoplasma species and production of control antisera against MG and MS will continue.

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

Investigation Into Factors Affecting Hatchability and Chick Quality

Chick quality, characterized by size, lack of abnormalities, maternal antibody levels, and absence of infections, is essential to productive poultry production. Many factors, involving management of the breeder flocks and the hatchery, can have an influence on these parameters. The sanitation of commercial poultry hatcheries is an area that can have a serious effect on the incidence of infectious disease in young poultry and requires continual

2002 Regents Professor and head of the Poultry Disease and Research Center, Dr. Stanley Kleven has been researching avian mycoplasmas and the diseases they produce for over 30 years.

evaluation. This includes evaluation of various disinfectants used in the hatchery, the monitoring of the bacterial population in these environments, and the effect of these disinfectants on both hatching eggs and bacteria. These parameters are measured through in vitro testing in the laboratory by simulating field situations. This involves the application of bacteria to various surfaces and evaluating the efficacy of various disinfectants to kill these bacteria when used at different dilutions, varied contact periods, and their application in the presence of organic material. In addition, it is important to evaluate the application of these disinfectants to hatching eggs during the incubation period. This is accomplished through a misting system within the incubators spraying a measured volume of disinfectant into the machines at measured time intervals. The disinfectants used in this manner are evaluated for their effect on egg moisture loss, hatchability of fertile eggs, chick yolk sac contamination, and chick viability.

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

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

The mission of the diagnostic virology laboratory is to provide accurate and timely diagnostic virology services for the U.S. poultry industry, conduct applied research on current avian disease isolates from the field, and improve detection and isolation methods for monitoring avian viruses. During 2001-2002, the diagnostic virology lab processed 578 cases and isolated 687 viruses. This past year virus isolation for avian leukosis was added as a full-time service. Six hundred forty-six whole blood samples were submitted for leukosis isolation and subsequent antigen capture ELISA.

Several new molecular-based assays have been incorporated. PCR is now available in a multiplex format for enteric coronaviruses and astroviruses. We are currently evaluating the sensitivity and specificity of this assay directly from field samples. In addition, an RT-PCR is available for reoviruses. Our goal is to enhance our diagnostic offerings for enteric viruses of poultry.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of Infectious Laryngotracheitis Virus (ILTV) has been used in epidemiological studies to distinguish ILTV vaccine strains (Vac) and broiler isolates (Br) from backyard flock isolates (By).

A live avian adenovirus vaccine (serotype 8) is currently being evaluated both pathologically and serologically in its ability to protect against inclusion body hepatitis caused by different serotypes of adenoviruses that were isolated over the past two years. Recent outbreaks of inclusion body hepatitis have been classified as serotype 11 adenovirus.

During the past year, we have gathered evidence that a mild infectious laryngotracheitis virus (ILTV) is circulating in broiler flocks in north Georgia and surrounding states. The condition is characterized by mild tracheitis, swollen sinuses and conjunctivitis with no mortality and minimal serological response. We are investigating the spread of the disease in broilers, evaluation of laboratory isolation and diagnostic procedures, and farm clean-out.

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

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

Despite widespread vaccination, infectious bursal disease virus (IBDV) continues to cause economic losses to the poultry industry. Within serotype 1 there are classic, variant, and very virulent viruses. The U.S. poultry industry is most affected by the presence of antigenic variants that can be responsible for vaccination failures. The VP2 gene of IBDV has been the target for molecular classification of the virus since it is the major host protective antigen responsible for inducing serotype-neutralizing antibodies. Advancements in nucleic acid technology have led to the identification and classification of antigenic variants by RT-PCR/RFLP analysis. In addition, potential antigenic regions have been identified within the hypervariable region of the IBDV VP2 gene that may be important for pathogenesis. Molecular analyses in this region of VP2 can be used to group these field variants based on their unique genetic properties. Recent studies have indicated a potential for segment reassortment between serotype 1 and serotype 2 in very virulent European isolates of IBDV. The objectives of this proposal are to conduct an epidemiological study of IBDV field isolates from the southeastern U.S. Field isolates will be chosen for the study based on unique RFLP patterns obtained using the current IBDV typing system at PDRC. We will amplify both segments (A and B) of the IBDV genome from the field viruses using RT-PCR and clone the resulting products for sequencing. Finally, phylogenetic analyses of the complete genomic sequences of the IBD variant viruses will be compared to previously published IBDV gene sequences and examined for evidence of segment reassortment.

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

Development and Characterization of Infectious Laryngotracheitis (ILT) Recombinant Virus

Restriction fragment length polymorphism (RFLP) of the ILTV genome was one of the first molecular assays developed for differentiation of ILTV strains. This technique has proven to be technically cumbersome. In addition, in some cases the similarity among RFLP patterns of ILTV strains makes interpretation of results difficult. In our laboratory we have developed a PCR-RFLP assay where the glycoprotein E gene is amplified by PCR, and the amplification product is digested with two restriction enzymes allowing discrimination of CEO vaccines and CEO vaccine subpopulations. The RFLP polymorphism observed for the gE gene relies on two nucleotide differences observed among vaccine strains. Although it is easy to perform and useful for tracking vaccine strains in the field, the discrimination potential of the gE PCR-RFLP assay is limited, particularly when it comes to differentiate field isolates from vaccine strains. In order to exploit other genes for differentiation of ILTV strains, we have analyzed the sequence of four glycoprotein genes (gI, gE, gC and gM) and one regulatory gene (ICP27) for five unrelated field isolates, two vaccine strains, and the USDA challenge strain. Nucleotide and predicted amino acid sequence comparisons suggested that backyard flock isolates (2-36-91, 6-48-88) and broiler isolate (8954) were different from vaccine strains in at least three genes. Broiler breeder isolate 1-219-01 and broiler isolate 9030 were closely related to CEO vaccine strain. In both of these flocks evidence of direct and indirect exposure to CEO vaccine was provided. Although sequencing of five genes allowed discrimination, clearer, faster molecular assays for differentiation of ILTV strains are needed to improve the epidemiological tools and for better understanding of the different levels of virulence among strains. The long-term goal for FY 2003 is to establish a reproducible set of assays to further characterize molecularly and in vivo ILTV strains 2-36-91,6-48-88, 8954, 1-219-01 and 9030.

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

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

Specific research involving infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), and avian adenovirus, along with concluded research on avian leucosis virus subgroup J (ALV-J), are the focus of this continuous proposal.

IBV recombination potential was characterized in vitro by RT-PCR and sequence analysis between the Massachusetts and the Delaware 072-like strain. Separately, adaptation of field strains of IBV to replicate in the intestinal tract of chickens has been performed via serial passages in SPF chickens. Procedures have been established to create and maintain intestinal ring tissue culture and are being utilized to further propagate these strains. Challenge studies will be performed, and selected vaccine candidates will be evaluated.

Dr. Holly Sellers prepares eggs for inoculation of live avian viruses, increasing their numbers to provide sufficient material for accurate testing.

Variants of IBDV have been characterized in our laboratory via RT-PCR/RFLP, heteroduplex mobility assays (HMA), sequence analysis and in vivo pathological studies. In addition, selected chicken anemia virus (CAV) field isolates have also been characterized via sequence and clinical analysis. Molecular and clinical analysis of these isolates have precluded future characterization of CAV and IBDV co-infection via in situ hybridization techniques.

Selected pathogenic avian adenovirus field isolates have been characterized via molecular and clinical methods. Serological responses induced by these isolates will be assayed by an avian adenovirus ELISA that is utilized by a U.S. primary breeder and compared with the virus neutralization assay.

Classical and molecular studies involving ALV-J were completed. These studies included comparative analysis of different cell cultures for viral propagation, identification of RT-PCR ALV-J positive samples undetected by virus isolation, and the design of potential inhibitors of ALV-J replication.

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

Control of Infectious Bronchitis Virus (IBV)

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

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

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

3. To create an infectious clone for IBV.

Objective 1 is ongoing. We are currently evaluating an IBV isolate designated 95-7728. That virus is highly pathogenic, but appears to be similar to Arkansas.

For VPL production, the spike and envelope genes were subcloned into the pIRES vector which allows expression of the two genes simultaneously in cell culture. Expression of spike has been verified, but rabbit antisera against the Beaudette envelope protein is thus far unreactive.
To create an IBV infectious clone, we ligated 9 separate Mass41 clones into 5 overlapping segments, which represent the entire IBV genome. The CMV and T7 promoters were ligated to the 5' end clone, and the BHG poly A signal and a poly A tract onto the 3' end clone. The clones are now ready to be joined together and inserted into the BAC vector.

PI: Mark W. Jackwood, (mjackwoo@arches.uga.edu)

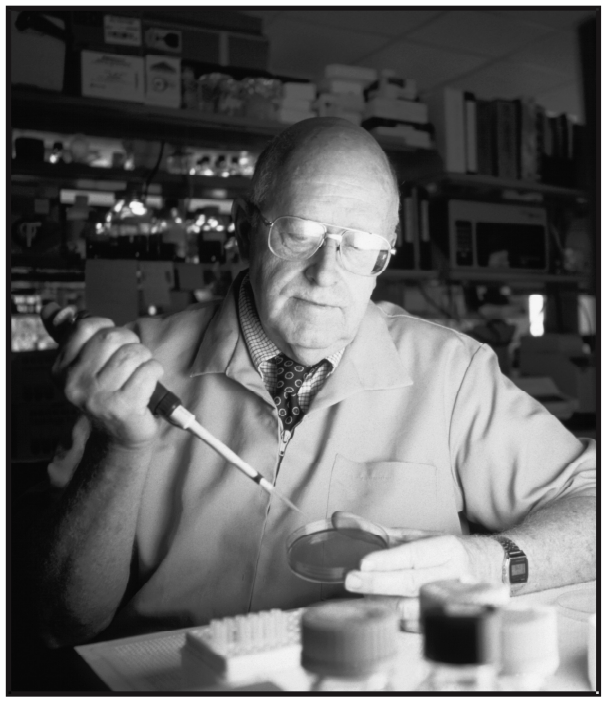
Is Infectious Bursal Disease Virus the Cause of Broiler Proventriculitis?

Proventriculitis is a common, naturally occurring disease of commercial broiler chickens that causes proventricular rupture and carcass contamination during routine processing. Infectious Bursal Disease Virus (IBDV) is implicated as a cause of proventriculitis, and vaccination for IBDV is marketed as a preventative. However, since no direct cause and effect relationship has been established between IBDV and proventriculitis, we propose that the immunosuppression caused by IBDV allowed a second pathogen to directly produce proventriculitis.

In this study, we first examined the direct effects of IBDV on the proventriculus. We experimentally reproduced proventriculitis in chickens using tissues from naturally occurring cases. These damaged proventriculi were examined using a newly developed real time RT-PCR for IBDV and none was detected in either the acute or chronic cases. Furthermore, we infected chickens with each of eight different strains of IBDV. No viral tropism or localization in the proventriculus was seen with any of these IBDV strains.
Secondly, to determine if IBDV is required to produce proventriculitis, we induced chemical immunosuppression in chickens without IBDV, and in chickens with IBDV neutralizing antibody. Both groups developed severe proventriculitis nearly identical to naturally occurring cases. These results show that severe immunosuppression, rather than IBDV, is a prerequisite for subsequent development of proventriculitis.

We will continue these studies to identify the specific agent(s) responsible for direct production of proventricular necrosis in immunosuppressed chickens. These results will be the first steps specifically targeted toward either exclusion of the true causative agent(s), or protection against their effects by vaccination.

PI: Tom P. Brown (tbrown@arches.uga.edu)
CoPI: Mary Pantin-Jackwood



Dr Richard Wooley inoculates a MacConkey agar plate to reisolate E. coli *from challenged adult poultry.*

Comparison of Three Adult Challenge Methods for Avian Colibacillosis with the Embryo Lethality Assay

In an earlier publication (*Avian Dis.* 44:318-324, 2000), 10 isolates of *Escherichia coli* from clinical cases of colibacillosis and 10 *E. coli* from the intestinal tracts of normal broilers at slaughter were assayed by the embryo lethality test to determine their virulence status. The assay was repeated five times in order to establish reproducibility and determine the statistical parameters of the test. The assay has been shown to be capable of discriminating between highly virulent, moderately virulent, and avirulent isolates of *E. coli*. In the present study, the same 20 isolates were used in adult challenge methods. These tests included the intravenous, the cellulitis (subcutaneous), and the aerosol (intratracheal) challenge methods. Comparison of the percent mortalities and selected phenotypes was also done. Test results were then statistically compared to the data from the embryo lethality assay. Correlations were observed between the embryo lethality assay and the intravenous and cellulitis methods. Correlations were observed for percent deaths, lesions, and body weights. No correlation was observed between the three correlative tests (embryo lethality assay, intravenous test, and cellulitis test) and the intratracheal challenge method. The use of the embryo lethality assay, intravenous test, or subcutaneous test will give comparable results in determining the virulence of an avian *E. coli*.

PI: Penelope S. Gibbs and Richard E. Wooley (wooleyr@vet.uga.edu)

Georgia's aquaculture industry is steadily expanding, with its greatest increase occurring in channel catfish production. Pond acreage for catfish farming has continued to grow every year. Other species being developed for aquaculture include striped and largemouth bass, yellow perch, and tilapia. In addition to Georgia's developing food-fish industry, there is an increasing interest in ornamental fish production, particularly koi, and cultured shellfish. It is estimated that aquaculture production in all countries will have to expand at least twofold to meet world demand for fisheries products over the next 25 years.

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

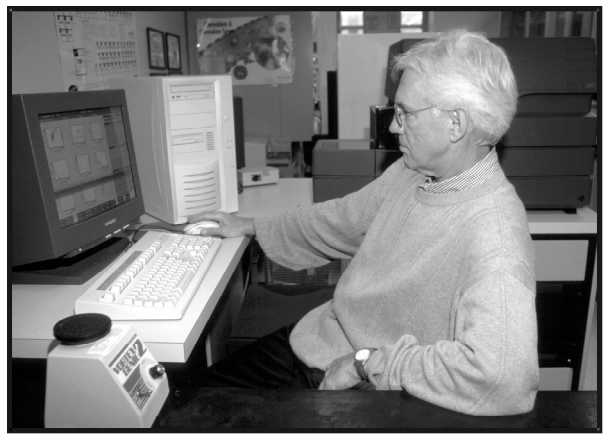
Regulation of Expression of the Fas Ligand Gene in Tilapia NCC

Morbidity and mortality due to infectious diseases in fish farming cause great monetary losses to the industry. While adaptive immunity is present in teleost fish, secondary responses are not very effective. The lack of understanding about immune responses in fish has made vaccine development both difficult and costly as a solution to the problem. The teleost innate immune system is known to play a fundamental role in the prevention of many important opportunistic infections, and it has thus become crucial to learn more about the many pathways of natural defense. Nonspecific cytotoxic cells (NCC), the phylogenetic precursors to mammalian natural killer (NK) cells, are the best-characterized cytotoxic effectors of natural immunity in teleost fish. NCC are known to kill protozoan parasites, virus-infected cells and tumor cells. In addition NCC, through the production of cytokines, are active participants of antibacterial immune responses. The pluripotent activities of NCC place these cells as very important participants in fish health. We have previously shown that NCC from tilapia kills target cells through the production and secretion of Fas ligand. Parasites (*Tetrahymena*) undergo apoptosis following encounter with soluble FasL (sFasL) released by NCC. Soluble FasL perfuses into the fish skin/integument, amplifies inflammatory responses, and, most importantly for our concept of parasite resistance, sFasL kills parasites by binding to a Fas receptor-like molecule on the parasite. In the present application, I propose to study the mechanisms that regulate the expression of Fas ligand in NCC. The mammalian FasL protein and gene have been sequenced, but there is a complete void of similar information of the teleost FasL ortholog. Unlike in mammals, where FasL is expressed on the membrane of lymphocytes, teleost FasL performs its function as a soluble cytokine and thus it is a secretory protein in NCC. Although we have not purified or sequenced the teleost FasL protein, all evidence indicates that FasL is essential for the function of teleost NCC and that it is utilized as a major pathway for killing target cells. Before this important cytokine can be manipulated for improvement of fish health, it is critical that its structure and regulation of expression be fully elucidated.

PI: Lilliana Jaso-Friedman (ljaso@vet.uga.edu)

Development of Poly- and Monoclonal Antibodies Against *Piscirickettsia salmonis*

Piscirickettsia salmonis is a fastidious, obligate intracellular bacterial pathogen of salmonids and is the etiologic agent causing an emerging disease in salmon farms throughout the world. During the next few years we plan to develop rapid immunological and molecular-based methods for the detection of *P. salmonis* in fish and perhaps even in waters surrounding fish growing facilities. The development of an immunological detection method would: 1) enable microbiologists to assess the potential for disease caused by these emerging pathogenic microorganisms; 2) improve the ability of fish growers to protect their investment from damage by taking the appropriate measures to control the spread of this disease; 3) determine the species/subspecies diversity of this group of pathogens.



Dr. Donald Evans, professor in the Department of Medical Microbiology and Parasitology is an acknowledged expert in fish immunology. He is sitting at the console of a flow cytometer, an instrument which is used to measure varied characteristics of cells such as DNA/RNA content.

We hypothesize that the *P. salmonis* isolates from various geographical locations will present different protein repertoires. This variation in protein expression may represent differences in virulence. To begin the process of assessing the diversity and exploring the pathogenesis the overall goal of this proposal is to develop immunologic tools which can be utilized in the identification, characterization and cloning of immunoreactive *P. salmonis* proteins. Once these proteins have been identified, their use as diagnostic and vaccine candidates will be determined. This will be accomplished by the following: Produce rabbit polyclonal antibodies against *P. salmonis* to be utilized as a diagnostic tool and in the future to screen a genomic library for immunoreactive proteins; develop mouse monoclonal antibodies against *P. salmonis* to be utilized for diagnostics and in differentiating isolates. These monoclonals will, in the future, be utilized for in situ experiments to begin exploring the cellular pathogenesis of this bacterium and for screening genomic libraries for immunoreactive proteins.

Mice have been immunotolerized to chinook salmon embryo (CHSE214) host cell antigens by use of the cyclophosphamide protocol. *Piscirickettsia salmonis* cells have been IP injected in the mice. Blood serum from the mice has been tested by ELISA, and an optical density four times greater was observed on *P. salmonis* infected CHSE 214 cells when compared to the titer on uninfected CHSE 214 cells. The spleens have been harvested and fused. Selection of hybridoma cells is proceeding.

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

Striped bass (*Morone saxatilis*) represent an important commercial and recreational fish with significant economic benefits to the boating and tourism industries. In recent years there has been heightened concern regarding the health of striped bass populations in eastern coastal waters of the United States. An epizootic of mycobacteriosis was reported in the Chesapeake Bay, which was characterized by lesion prevalence as high as 30-50%. Histological examination of skin lesions and internal organs revealed granulomatous inflammatory responses accompanied by the presence of acid-fast bacilli. A higher prevalence of granulomatous lesions in visceral samples than in skin indicated that many stripers are asymptomatic. Subsequent bacteriological studies revealed that infections were associated >70% of the time with a new species, *Mycobacterium shottsii*, sp. nov. The significance of heavy mycobacterial infections in native striped bass from coastal waters of the eastern U.S. is currently unknown. The high prevalence of *M. shottsii* infections in striped bass could potentially cause human infection in people that handle these infected fish. This proposed research will define the virulence mechanisms associated with this new and emerging pathogen. In association with ongoing epidemiological studies that will define the extent of the spread of this agent along the eastern U.S. coast, the work described here may indicate a direction toward appropriate treatment and prevention strategies for afflicted fish and potentially human populations.

PI: Fred Quinn (fquinn@vet.uga.edu)

DNA Receptors and Innate Immunity in Catfish

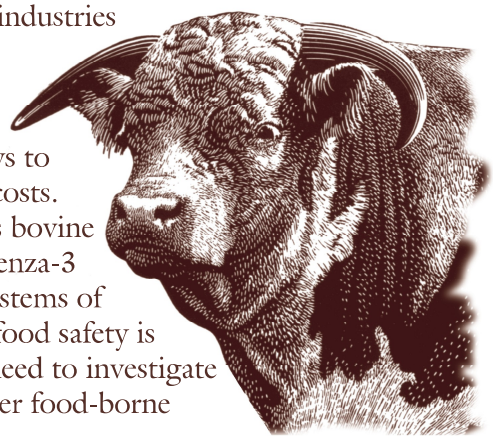
Oligodeoxynucleotide (ODN) and bacterial DNA (bDNA) binding proteins on teleost nonspecific cytotoxic cells (NCC) were identified. An 18 kDa cytosolic and membrane protein was purified from NCC. Results of primary peptide sequencing and database searches tentatively identified p18 as a histone-like protein (HLP). Based on these results, degenerate primers were synthesized and PCR analysis was done using a cDNA expression library from activated catfish NCC as template DNA. Sequencing the PCR products and translation revealed the presence of a (small) conserved amino acid homology region with human H1. Additional experiments were done by flow cytometry to examine the presence of DNA binding proteins on NCC membranes. A commercial anti-H1 antibody bound 54% of ODN activated NCC. Western blot analysis of membrane preparations from purified NCC (using this same anti-H1 antibody) revealed the presence of additional ODN binding proteins with molecular weights of 29 kDa and 12-14 kDa.

Preliminary conclusions from these data indicated that NCC express a molecular weight-heterogenous population of DNA binding proteins. Finally, in an effort to determine the possibility that NCC may secrete antibacterial proteins, studies were done to examine the anti-bacterial activity of supernatants generated from NCC stimulated in vitro with bDNA or ODN. These data indicated that NCC secrete anti-bacterial proteins that lyse *Streptococcus iniae*. Future studies are planned to obtain the full length amino acid sequences of the antimicrobial proteins and to study their molecular and immunological properties.

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

Cattle, Sheep, and Goats

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. Today's cattle producers are working with narrow profit margins and must watch their expenses more closely than ever. Consequently, biomedical researchers are providing these industries with ways to maintain healthy animals, which will help reduce production costs. Mastitis, Johne's disease, pasteurellosis, pneumonia, infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), parainfluenza-3 (PI-3), and leptospirosis continue to challenge the immune systems of Georgia's cattle herds. Ruminant herd health as it pertains to food safety is also a major concern to consumers and producers. Scientists need to investigate pathogenic *Escherichia coli*, *Salmonella*, *Campylobacter*, and other food-borne organisms as to their origin, transmission, and prevalence.



Soluble CD14 as an initiator of neonatal inflammatory and immune responses.

The transition from the womb to the world is one of the most stressful times in the life of any animal. This is especially true for farm animals, such as cattle, sheep, and swine, as there is no transfer of immune protection from the dam to the developing fetus during gestation. These animals are born immunologically naïve and require a specific event, or series of events, to initiate development of their immune systems.

Passive transfer of pre-formed antibodies from the dam to the neonate via colostrum has long been recognized as an important factor in the survival and development of newborn farm animals. It also has become apparent that animals receiving "colostrum substitutes" (e.g., antibodies extracted from milk whey during the production of cheese or even as frozen pools of donor colostrums) do not do as well as animals receiving plenty of colostrum from their mothers. Therefore, colostrum contains elements other than antibodies that are important in the protection and development of the newborn.

In other species, CD14 is an important protein in the response to bacterial invaders and in the development of lymphocytes involved in adaptive immunity. We hypothesize that CD14 is likely to play a significant role in triggering the capacity of newborns to mount inflammatory and adaptive immune responses. Our group has based this hypothesis on several recent findings.

First, we have demonstrated that calves receiving colostrums lacking cells from the mother were developmentally delayed (Reber, 2000 – PhD dissertation, and Reber et al., 2000 CRWAD). When compared with calves receiving whole maternal colostrum, calves receiving cell-free colostrum were less capable of presenting antigens or responding to antigens in adaptive immune responses. Second, we have demonstrated that cells in colostrum express CD14 on their surface and release CD14 into culture media coincident with the development of adaptive immune changes that occur in newborns. Third, we have measured an increase in the expression of the "quality control" proteins in the adaptive immune response in calves receiving maternal cells, corresponding with changes occurring in adaptive immune function.

During the past few months, we have cloned the genes for the CD14 protein from RNA taken from white blood cells of normal dairy cattle. This RNA was converted to DNA and the DNA for CD14 was selected using the polymerase chain reaction (PCR) method involving a series of nucleotides present in all known sequences of CD14. The DNA for CD14 was placed into a plasmid that allows us to produce it in larger quantities in bacterial cells. We then sequenced the plasmid DNA from bacterial colonies having evidence of the gene insert on selective and differential medium (using antibiotic resistance genes). The resulting sequences documented that we had isolated the complete CD14 gene for cattle, and that we had also accomplished our goal of making smaller gene "fragments" that can be used to evaluate their biological activity.

Most recently, we have transferred the bovine CD14 genes into yeast cells by electroporation. This process will allow us to produce the CD14 protein in large quantities, which can be readily purified and used to assess its function as a trigger of the maturation of monocyte cells (blood precursors) into macrophages (inflammatory cells and inflammation managers) and dendritic cells (cells that initiate adaptive immune response and regulate its function). These evaluations are currently underway.

The ultimate goal of this project is to use the natural biological trigger of inflammatory and immune development to improve the health and growth of food animals. Achieving this goal will allow us to make vaccines that provide immune protection at an earlier age in food animals and make them less expensive to produce. We also believe that this will be an important method to reduce the use of antibiotics in animal production by producing stronger, healthier animals, and thereby reducing environmental exposure to disease and producing fewer animals with acute illness.

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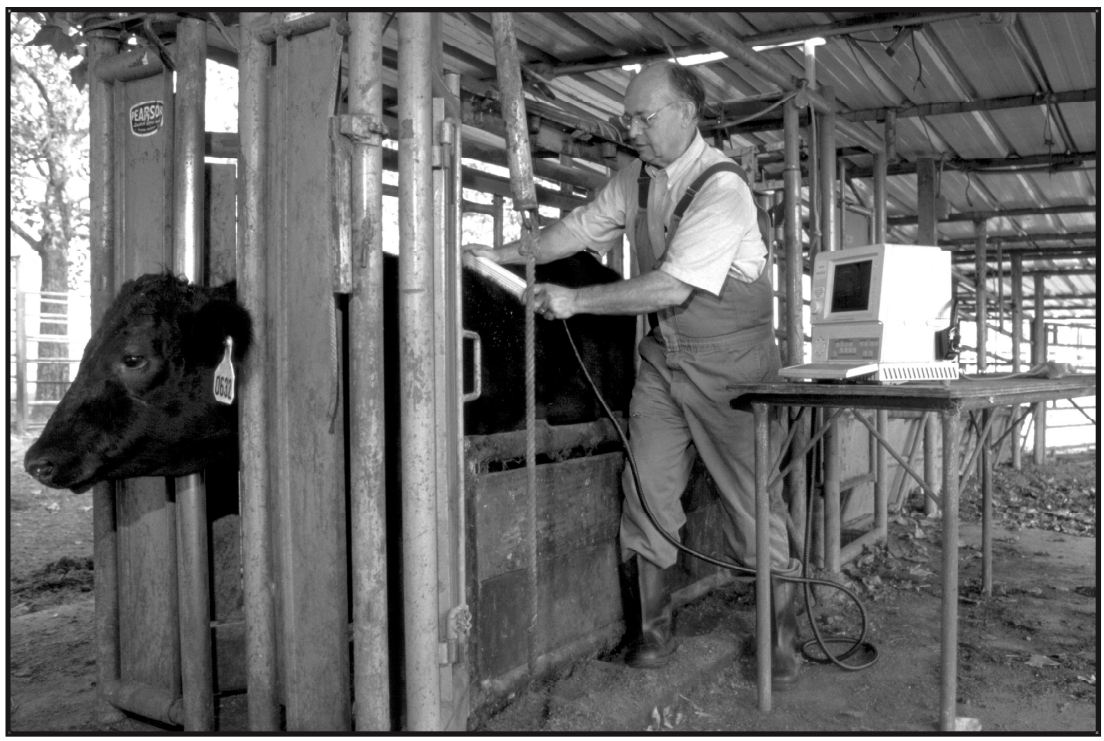
CoPIs: Amelia Woolums, Tatsuyuki Okinaga

Non-invasive bovine embryo sexing via sperm-mediated transgenesis

The need to enhance bovine reproductive efficiency in order to expand world production of beef and milk, coupled with the need for better tools for achieving this goal, provide motivation for this research. Our recent efforts have targeted development of technology to enable sperm mediation of exogenous gene transfer. The immediate goal is embryo sexing. The successful accomplishment of the introduction of novel DNA, using sperm cells as vectors with incorporation into the embryonic genome, promises a way for making cows with disease resistance genes and other desirable traits as the DNA determinants become available. In vitro production of bovine embryos of known sex, cryopreservation and direct embryo transfer (i.e. carried out as artificial insemination is currently done) promise increased profitability in both dairy and beef production by cutting costs of unwanted offspring. An easy and non-invasive way of embryo sexing is now necessary for complementing our current capabilities to carry out the other aspects of these relevant new breeding technologies. Our strategy, involving candidate promoters Sry and Xist to direct sex-dependent expression of the green fluorescent protein reporter-gene (designed to allow distinction of differences in fluorescence upon brief microscopic examination), requires additional research. Although sperm uptake of novel DNA could be demonstrated, the appropriate delivery for genomic incorporation at fertilization remains a technological barrier.

PI: B. G. Brackett (bracket@vet.uga.edu)
CoPIs: R. A. McGraw, S. Sirisathien, and H. Hernandez



Dr. Mel Pence of the Veterinary Diagnostic and Investigational Laboratory in Tifton and the Food Animal Health Program examines a cow for pregnancy using a portable sonograph.

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

Response of the Equine Peritoneum to Abdominal Surgery and Surgical Trauma

Equine gastrointestinal diseases causing abdominal pain (colic) are devastating problems to the equine industry and represent a major cause of morbidity and mortality in horses. Intestinal strangulations are one of the most serious causes of abdominal pain in horses and, despite surgical intervention, long term survival rates only approach 50-60%. In many cases this poor prognosis for survival is due to the development of postoperative intra-abdominal adhesions. Adhesions cause clinical problems when they compress or anatomically distort the intestine. This may lead to intestinal constriction, incarceration, or volvulus, predisposing the patient to intestinal obstruction and signs of abdominal pain. The additional number of abdominal surgeries performed in horses as a result of postoperative adhesions represent a significant financial burden to horse owners.

Despite technological advances in surgery and perioperative care, adhesions continue to be a common and costly complication of abdominal surgery in horses. The development of adhesions has been attributed to insufficient peritoneal fibrinolytic activity. In this study, the fibrinolytic response of the equine parietal and visceral peritoneum to abdominal surgery and surgical trauma were evaluated using a proven model of serosal trauma that consistently results in adhesion formation. Tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) activities were measured in equine parietal and visceral peritoneum and in peritoneal fluid during abdominal surgery and surgical trauma. We also evaluated the effect of precoating the small intestine with a 0.4% solution (sodium hyaluronate) on the surgically induced alterations in peritoneal fibrinolytic activity. We hypothesized that tPA and PAI-1 activity are present in normal equine parietal and visceral peritoneum, and that abdominal surgery and serosal trauma result in a hypofibrinolytic state evidenced by significant decreases in tPA activity with corresponding increases in PAI-1 activity. Secondly, we hypothesized that precoating of the small intestine with a 0.4% HA solution would significantly attenuate this surgically induced hypofibrinolytic state.

A ventral midline celiotomy was performed in 8 horses under general anesthesia. In all horses, peritoneal fluid and peritoneal and jejunal seromuscular biopsy specimens were taken immediately after opening of the abdominal cavity (time 0). In control (group 1, n=4) horses, two liters of sterile saline was used to lubricate the intestine during manipulation. In HA (group 2, n=4) horses, two liters of HA solution was used to lubricate the intestine during manipulation. The entire small intestine was placed in a sterile, transparent plastic bag equipped with a purse string closure that retains the intestine without compromising mesenteric circulation. Serosal abrasion was performed at 10 sites on the small intestine, at one-meter intervals starting one meter oral to the ileocecal orifice. Small intestinal seromuscular biopsies from abraded and non-abraded sites, and peritoneum, and peritoneal fluid were obtained at 30 minute intervals for a total of 120 minutes of surgery time. At the completion of the study, all horses were euthanized with an overdose of sodium pentobarbital solution.

Samples were immediately rinsed in saline, placed in an airtight tube, snap frozen in liquid nitrogen and stored at -70°C. Samples will be assayed for that tPA and PAI-1 activity. We expect to quantitate tPA and PAI-1 activity in normal equine parietal and visceral peritoneum, and to demonstrate that exploratory celiotomy and surgical trauma result in decreased peritoneal fibrinolysis characterized by significant decreases in peritoneal tPA activity with corresponding increases in PAI-1 activity. Secondly, we expect that precoating of the small intestine with a 0.4% HA solution will significantly attenuate this surgically induced hypofibrinolytic state. Characterization of the fibrinolytic response of the equine parietal and

visceral peritoneum to abdominal surgery and surgical trauma will provide insight into the development of new pharmacologic strategies for adhesion prevention.

PI: Eric Mueller (emueller@vet.uga.edu)
CoPIs: Fred J. Caldwell, Randall Eggleston

Generation of Equine-specific Genetic Markers: Application to Endotoxemia

Endotoxemia, caused by the translocation of endotoxin (lipopolysaccharide, LPS), released from gram negative bacterial cell walls, into the bloodstream, is associated with the leading causes of death in horses of all ages. It is known that single nucleotide polymorphisms (SNPs) in certain genes (e.g. Toll-like receptor 4 and TNFa) increase the susceptibility of the host animal to endotoxemia. Thus, knowledge of such mutations would allow for studies that demonstrate a correlation between these mutations and susceptibility to disease. However, equine genome research has lagged significantly behind that of other domestic animal species with relatively few gene sequences available. Currently, fewer than 3800 equine sequences have been deposited into public databases while more than 170,000 bovine sequences are available. To address this paucity of information regarding the equine genome in a fast and cost effective manner, we have begun to produce equine expressed sequence tags (ESTs) using a largely automated, high throughput process developed for a much larger sorghum EST program, presently comprising about 120,000 ESTs. In order to rapidly determine equine gene sequences, we have used plasmids randomly isolated from 3 equine cDNA libraries (monocytes, liver, and mesenteric lymph nodes) as templates for sequencing of either 5' and/or 3' ends of equine cDNAs. All sequencing data has moreover been fully integrated with a bioinformatics system that provides full data storage and analysis. Bioinformatics analysis includes: 1) removal of vector and *E. coli* sequences, 2) assignment of PHRED quality scores to all sequences, 3) clustering of 3' sequences to identify unique genes (unigenes), 4) assignment of provisional annotation using HT BLAST, 5) determination of the electronic expression profile of each gene in a given library, and 6) identification of polymorphic markers such as SNPs and microsatellite repeat sequences. We have obtained 3575 equine ESTs from monocyte, liver, and mesenteric lymph node libraries. The average length of these ESTs, after removal of vector sequence, is 601 bp at a PHRED score of Q16 (97.5% accuracy). ESTs shorter than 100 bp were rejected.

Clustering of the 3' sequences demonstrates that these ESTs represent 1,666 unique genes of which a preliminary analysis indicates only 149 have previously been deposited in the public domain (e.g. GenBank). All EST sequences have been uploaded to GenBank. The generated ESTs will serve as essential tools for examining associations between genetic mutations, altered gene expression profiles, and susceptibility to disease or other quantitative trait loci such as enhanced performance. In this regard these ESTs can immediately serve as target in microarrays to examine changes in gene expression profiles during specific diseases. Alternatively, our bioinformatics analysis of these ESTs can identify single nucleotide polymorphisms (SNPs) and microsatellite repeat sequences that might prove useful in mapping of the equine genome. As a result of this work we have obtained additional funding from the Grayson Jockey Club to continue this research and have applications pending with the Morris Animal Foundation and the USDA.

PI: Michel Vandenplas (mvdplas@vet.uga.edu)

Comparative biomedicine investigates how a particular disease affects one species versus another; that is, how a disease manifests itself for example in a mouse versus a human or cow. Researchers can compare diseases between species because different species often share substantial genetic information. Scientists study data such as symptoms, disease progression, treatments, mortality, and so on. Thus, one species serves as a disease model for another. And interestingly, both species may benefit. For example, researchers study cardiomyopathy in dogs and humans and both have benefited in the short- and long-term. In the abstracts that follow, VMES scientists discuss their research using comparative biomedicine involving hypertension, malaria, toxicology, and respiratory disease.

Antioxidants and Adhesion Molecule Expression in the Lung

Oxidative stress plays a role in the pathogenesis of many respiratory diseases in food-producing animals in Georgia. A large number of inflammatory mediators are found in the respiratory tract in pulmonary diseases. Within the inflamed airway, inflammatory cells produce cytokines, like tumor necrosis factor alpha (TNF) and reactive oxygen species (ROS), which have deleterious effects to the airway. Intercellular adhesion molecule-1 is an adhesion molecule that increases adherence of leukocytes to the airway endothelium and epithelium resulting in an influx of inflammatory cells into the lung. In previous studies we have demonstrated that TNF induces ICAM-1 gene and surface expression on airway epithelial cells. Recently, our data suggest that this increased expression can be inhibited by at least two antioxidants that differentially alter the binding of oxidant-sensitive transcription factors to the TNF-responsive region within the ICAM-1 promoter. Understanding how antioxidants modulate transcription factors that are directly involved in adhesion molecule regulation is essential to understanding their role in lung diseases. Further defining cytokine-enhanced expression of adhesion molecules such as ICAM-1 in the lung may provide targets for therapies to benefit animal health in airway diseases.

PI: Thomas Krunkosky (tmkrunko@vet.uga.edu)
CoPI: Carla Jarrett

Mechanisms of JSRV Oncogenesis In Sheep In Vivo

Ovine pulmonary adenocarcinoma (OPA; also known as jaagsiekte or sheep pulmonary adenomatosis) is a naturally occurring neoplasm of sheep with striking similarities to some forms of human lung adenocarcinoma. We have demonstrated the causal association between a retrovirus known as jaagsiekte sheep retrovirus (JSRV) and OPA by the isolation of an infectious and pathogenic molecular clone ($JSRV_{21}$). Presently, the main objective of our laboratory is to understand what are the molecular mechanisms governing cell transformation by JSRV.

We have recently reported that expression of the JSRV envelope (ENV) induces transformation of immortalized rodent fibroblasts in classical transformation assays. We showed that the antiapoptotic cell signaling pathway initiated by phosphoinositide-3 kinase (PI-3K) is induced in JSRV-transformed NIH3T3 but not in the parental cell line. A single point mutation in a tyrosine residue (Y590) in the cytoplasmic tail of the transmembrane domain of the JSRV ENV abolishes its capacity to induce cell transformation, but not the capacity to mediate viral entry. Y590 is part of a YXXM motif that is a putative binding site for PI3-K. Thus, the activation of the PI3-K/Akt pathway appeared to be a major event in transformation of rodent fibroblasts by JSRV.

JSRV can induce transformation also of immortalized chicken fibroblasts (DF-1). Interestingly, in chicken fibroblasts Y590 is not critical for transformation. We have developed cell lines derived from DF-1 cells transformed by an avian retroviral vector expressing the JSRV ENV (RCASBPA+JSenv) or the mutated JSRV Env (RCASBPA+JSY590F or RCASBP+JSY590D). Akt phosphorylation was detected in DF-1 transformed by the wild type JSRV Env, but not in DF-1 cells transformed by the mutants RCASBPA+JSY590F or RCASBP+JSY590D. Thus, activation of the PI3-K/Akt pathway is not necessary for transformation of immortalized chicken fibroblasts. Because immortalized cell lines have a deregulated cell cycle that favors the process of immortalization, we sought to repeat the transformation assays in primary cells, as the latter better mimic the properties of cells in vivo. DF-1 cells might have particular signal transduction pathways activated that favor the transformation potential of the JSRV Env mutants. However, we have been able to transform also primary chicken embryo fibroblasts (CEF) with both RCASBPA+JSEnv and the mutants RCASBPA+JSY590F or RCASBP+JSY590D. Vectors expressing a chimeric envelope protein containing the cytoplasmic tail of the JSRV-related endogenous betaretroviruses were unable to transform either DF-1 or CEF cells. These results suggest that (i) the JSRV Env is able to also induce transformation of primary cells; (ii) the cytoplasmic tail of the TM domain of the JSRV Env is a major determinant of transformation and (iii) other mechanisms, rather than PI3-K/Akt

activation, play a major role in JSRV-induced cell transformation in vitro.

Experiments are in progress to understand whether the expression of the JSRV envelope alone is sufficient to induce transformation of cells in vivo. Studies on JSRV and OPA can serve as an intellectual background to further understand the mechanisms of pulmonary oncogenesis.

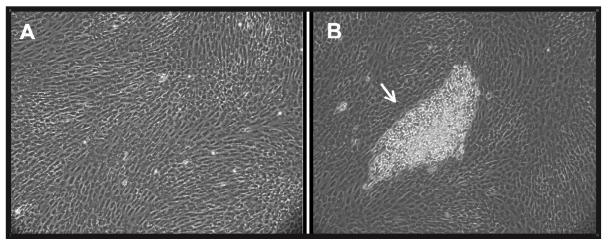
PI: Massimo Palmarini (mpalmari@vet.uga.edu)

Evaluation of Chalcone Derivatives in a Murine Model of Canine Neoplasia

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

One goal of this study was to standardize our production of reliable, predictably behaving models of both canine prostatic carcinoma and osteosarcoma. Using BALB/c-*nu/nu* (athymic) mice, we have been able to reliably produce transplanted canine prostatic carcinomas with aggressive, metastatic behavior. The canine osteosarcoma cell line was not reliably tumorigenic in the athymic mice so this cell line was not included in the study.

In preliminary studies, we encountered difficulty in solubilizing the test compounds into a form that would be safe and reliable for administration. Although we were able to administer the compounds orally, poor water solubility and the potential for ineffective drug delivery were concerns. The University of Georgia Department of Chemistry has since developed several water-soluble chalcone derivatives which are currently undergoing in vitro testing of anti-angiogenic activity at Emory University.



JSRV-Env transformation of 208F cells. 208F show normally a strong contact inhibition and very uniform morphology (panel A). The transfection of 208F with an expression plasmid for the JSRV Env (panel B) induces the appearance of transformed foci (arrow) 6- 10 days post-transfection.

Studies to evaluate the water-soluble chalcone derivatives in our murine models are scheduled to begin within the next 3 months. Compounds will be chosen for evaluation based on anti-angiogenic activity in in vitro assays.

A successful collaboration between the Department of Chemistry and the College of Veterinary Medicine has been formed through this study. The ultimate goal of the collaboration is the development and evaluation of new anti-angiogenic agents for treatment of both veterinary and human cancer patients. Agents demonstrating efficacy and safety in the mouse model will progress to clinical trials in veterinary oncology patients at the University of Georgia.

PI: Nicole Northrup (northrup@vet.uga.edu)
CoPIs: Karen Cornell, Nancy Stedman

A Peptide-Based ELISA for Flavivirus Serology

A peptide, termed FLP-1, was synthesized corresponding to a region of high potential antigenicity in the envelope (E) protein of West Nile virus (WNV). As the amino acid sequence selected was virtually identical to the comparative region in the St. Louis encephalitis virus (SLEV) E protein, this peptide (if reactive) would provide a flavivirus-specific diagnostic reagent for detecting antibodies directed against both viruses. When used as antigen in an ELISA format, FLP-1 was shown to be reactive with avian WNV-specific antibodies. Preliminary evidence suggests that this peptide approach may be as sensitive or, perhaps, more sensitive than other ELISA formats currently being used in the diagnosis of flavivirus infection. A pair of rabbits were subsequently immunized with FLP-1 in an attempt to elicit an antibody response against the peptide. Crude rabbit anti-peptide antisera was then used as a primary antibody against both WNV and SLEV-infected cell lysates in a Western blot. The anti-peptide antisera recognized the E protein of both viruses. The antisera was non-reactive against uninfected cell lysates. The incorporation of FLP-1 and anti-FLP-1 antibody (upon final purification) into an ELISA format will likely provide a highly sensitive immunoassay for the detection of flavivirus-specific antibodies from wild bird sera. Based upon the initial success of this peptide-based approach, development of a WNV-specific peptide ELISA is currently underway.

PI: David Stallknecht (dstall@vet.uga.edu)

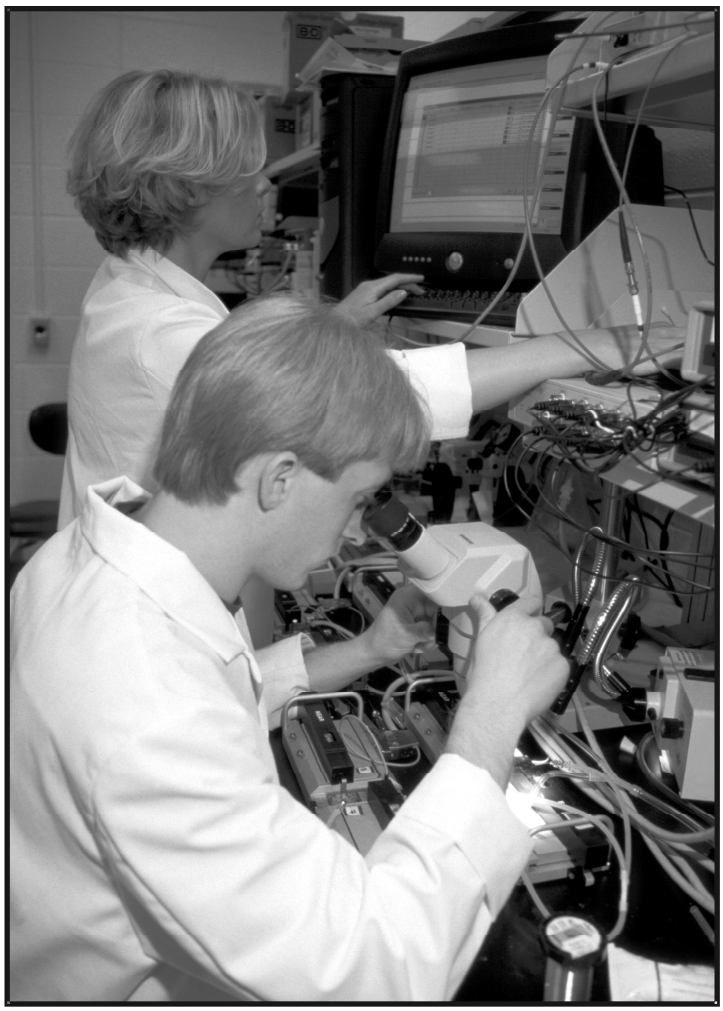
S-nitrosocysteine Recognition Sites in Hypertension

Endothelium-dependent vasodilation is impaired in Spontaneously Hypertensive (SH) rats. The mechanisms responsible for this impairment of vascular function have not been established. Endothelium-derived S-nitrosothiols and especially L-S-nitrosocysteine play important roles in controlling the tone of resistance arteries regulating arterial blood pressure. We have provided evidence that L-S-nitrosocysteine relaxes vascular smooth muscle by activation of redox-sensitive stereoselective recognition sites (SS-Rs).

The objectives of this investigation were to examine whether the vasodilator potency of endothelium-derived L-S-nitrosocysteine is diminished in resistance arteries of SH rats as compared to age-matched normotensive Wistar Kyoto (WKY) rats, and whether the diminished potency of L-S-nitrosocysteine is due to oxidation of cysteine residues in stereoselective S-nitrosothiol recognition sites in vascular smooth muscle.

We have determined first that the vasodilator potencies of L-S-nitrosocysteine and endothelium-dependent vasodilators were substantially impaired in SH as compared to WKY rats; whereas, the vasodilator potency of the nitric oxide-donor, MAHMA NONOate, was not. Secondly, we have determined that treatment of SH rats with the angiotensin converting enzyme inhibitor, captopril, markedly improved L-S-nitrosocysteine and endothelium-dependent vasodilation in SH rats. Thirdly, administration of the disulfide-bond reducing agent, dithiothreitol, markedly improved the vasodilator potencies of L-SNC and endothelium-dependent vasodilators in SH rats, whereas, it did not affect nitric oxide-mediated vasodilation.

PI: Stephen J. Lewis, Ph.D. (slewis@vet.uga.edu)



Researchers in the laboratory of Dr. Steven Lewis are using a stereomicroscope to examine a small artery encased within a wire myograph. This allows them to measure active changes in the diameter of the artery.

Research Funding

Funding Source*	Fiscal Year 2001	Fiscal Year 2002
VMES Budget	$3,569,225	$3,893,561
Federal Grants and Contracts	2,830,617	8,131,854
State Grants and Contracts	107,468	772,808
Private Grants and Contracts	2,779,141	2,075,962

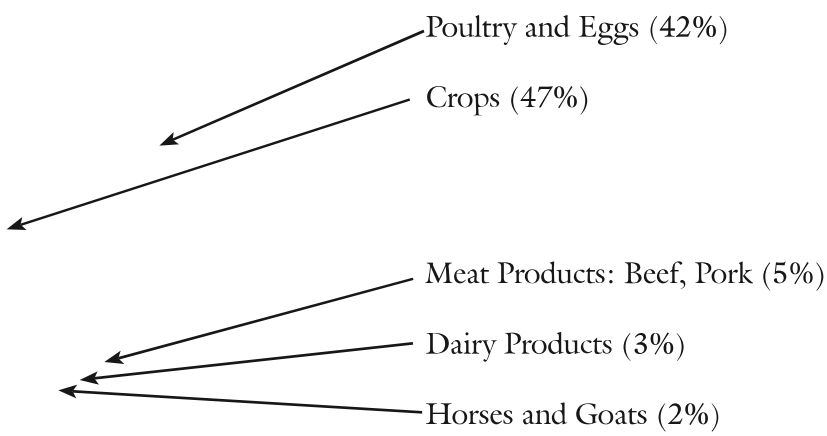
*Excluding carryover funds

Georgia Livestock and Poultry: Inventories and Values[a]

Species		Number on Farms and/or Produced	Production Value
Cattle	Beef	1,145,373	$364,087,741
	Dairy	93,935	285,322,028[b]
Hogs		147,462	105,000,459
Poultry[b]	Broilers	1,247,300,000	2,432,235,000
	Layers	20,994,000	261,754,000
	Eggs	5,086,000,000	368,000,000

[a]UGA College of Agriculture and Environmental Science, "2001 Georgia Farm Gate Value Report" (AR-02-02)
[b]Georgia Agricultural Statistics Service, Georgia Farm Report, May 15, 2002, Volume 2: Number 09

Georgia Farm Cash Receipts[a]



[a]UGA College of Agricultural and Environmental Sciences, "2001 Georgia Farm Gate Value Report" (AR-02-02)

Brackett, Benjamin. *Platelet-activating factor and bull sperm fertilizing ability.* Select Sires; $10,000.

Brown, Corrie. *Cuban agricultural scientific exchange*. The Rockdale Foundation, Inc.; $10,000.

Brown, Corrie. *Further investigations into virulence characteristics of Newcastle disease virus strains using infectious clones.* U.S. Poultry & Egg Association; $56,235.

Brown, Corrie. *Education globalization in animal and poultry agriculture.* Univ. of Arkansas; $68,509.

Brown, Corrie. *Preparing veterinarians to deal with global issues in animal health, trade and food security.* FIPSE - U.S. Dept. Education; $171,257.

Budsberg, Steven. *Evaluation of clinical efficacy and safety of flupirtine for treatment of chronic pain associated with osteoarthritis secondary to hip dysplasia in dogs.* Bayer Corporation; $98,739.

Edwards, Gaylen. *Ingestive peptide controls of alcohol intake.* NIH; $144,800.

Edwards, Gaylen. *Metabolic regulation of growth and development.* Pennington Biomedical Research Center; $678,851.

Ferguson, Duncan. *Molecular genetic approach to development of a feline Thyrotropin*. Morris Animal Foundation; $123,438.

Fischer, John. *Development of scientific information on the animal traps for selected wild vertebrates species by providing necropsy data on injuries associated with the use of animal restraint devices.* USDA-APHIS; $13,430.

Fischer, John. *Persistence of pseudorabies virus in feral swine populations*. USDA-APHIS; $50,000.

Fischer, John. *Cooperative Agreeement for developing & evaluation of data relative to disease relationships that may involve wildlife, domestic livestock & poultry.* USDA-APHIS; $300,000.

Fischer, John. *Cooperative agreement for developing and evaluation of data relative to disease relationships that may involve wildlife, domestic livestock & poultry.* USDA-APHIS; $350,000.

Frazier, Kendall S. *Effects of FF-2216 on renal fibrosis and anemia in the 5/6 rat remnant kidney model*. Fibrogen, Inc.; $14,967.

Fu, Zhen. *Noninvasive delivery of skin-targeted rabies vaccines*. Vaxin Inc.; $33,000.

Fu, Zhen. *Regulation of rabies virus transcription and replication.* NIH; $1,255,800.

Halper, Jaroslava. *Advanced training in molelcular biology.* Ministry of Science and Technology, Pakistan; $9,000.

Halper, Jaroslava. *The effect of enrofloxacin on proteo-glycan composition in equine superficial digital flexor tendons.* American Quarter Horse Association; $42,737

Hartmann, Katrin. *To further Dr. Hartmann's research in feline retroviral infection.* Univ. of Munich, Germany; $50,817.

Hoenig, Margarethe. *Diabetes and impaired glucose intolerance*. Pfizer Limited; $10,489.

Hoenig, Margarethe. *Effect of diet on glucose tolerance and lipid metabolism in the cat.* Ralston Purina; $14,551.

Hoenig, Margarethe. *Insulin resistance in the obese cat.* Ralston Purina; $83,823.

Hoenig, Margarethe. *Effect of talibegron hydrochloride (SCH 417849, ZD2079) oral liquid on glucose metabolism in obese cats.* UGARF - University of GA, Research Foundation; $114,944.

Hofacre, Charles. *Poultry: A food animal model for following antimicrobial resistant enterococci.* USDA-CSREES; $786,350.

Howerth, Elizabeth. *Pilot study: Prevention of uronemiasis in marine fish.* Morris Animal Foundation; $7,422.

Jackwood, Mark. *Infectious bronchitis virus in turkeys?* U.S. Poultry and Egg Association; $50,000.

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

Kaplan, Ray. *Novel methods for sustainable control of gastrointestinal nematodes in small ruminants.* Fort Valley State University; $38,981.

Kleven, Stanley. *Evaluation and comparison of various procedures and methods for PCR analysis of* Mycoplasma gallisepticum. U.S. Poultry & Egg Association; $43,516.

Lewis, Stephen. *Three dimensional animations of signal transduction processes.* UGA – CAIT; $29,272.

Lewis, Stephen. *Role of rho kinase in ischemia-reperfusion-induced research vasoconstriction in insulin-dependent diabetes.* Junior Diabetes Foundation; $55,000.

Li, Wan-I (Oliver). *Identification of angiogenic activities in lactobacillus secretion.* Ye Cherng Industrial Products, Co.; $38,090.

Lukert, Phil. *Development of experimental challenge models for Georgia State University bovine enteric diseases in newborn calves: testing of candidate vaccines by challenge studies in newborn calves.* Merial Limited; $50,000.

Mahaffey, Edward. *Surveillance for West Nile Virus Encephalitis (WNVE) and other arboviral pathogens.* GA Dept. of Human Resources; $13,400.

Mead, Daniel. *West Nile Virus surveillance in West Virginia.* West Virginia Dept. Health and Human Services; $46,336.

Moore, James. *LPS-binding protein and the major LPS receptor in horses with colic*. Morris Animal Foundation; $25,882.

Moore, James. *New synthetic endotoxin antagonist incorporating structural features of the lipid-A of rhizobium sin-1.* American Heart Association; $60,000.

Moore, James. *Three dimensional animations of signal transduction Mechanism.* USDA-CSREES; $99,999.

Moore, Julie. *T-cell memory and protection against placental malaria.* NIH; $1,707,928.

Mueller, Eric. *Response of the equine peritoneum to abdominal surgery and surgical trauma.* American College of Veterinary Surgeons; $9,994.

Murray, Thomas. *Brevetoxin-induced neuronal death*. Oregon State University; $15,000.

Murray, Thomas. *Neuroprotective actions of cannabinoid agonists.* Cheryl C. Miller Fellowship, *NIH;* $71,580.

Murray, Thomas. *Hypoxia and fetal regulation of breathing movements*. University of California at Los Angeles; $74,269

Murray, Thomas. *Dynorphin analogs as kappa opioid receptor antagonists*. University of Maryland; $194,762.

Murray, Thomas. *Neurotoxins from marine algae and cyanobacteria*. Oregon State University; $339,129.

Northrup, Nicole. *A multi-site safety and efficacy evaluation of LDI-100 in canine patients with malignant mast cell tumors.* Milkhaus Veterinary Products, Inc.; $4,975.

Palmarini, Massimo. *Oncogenesis in retrovirus-induced lung cancer*. NIH-National Institutes of Health; $1,033,252.

Peroni, John. *Functional analysis of equine laminar arteries.* Grayson Foundation; $44,089.

Prasse, Keith. *Core Animal Diagnostic Laboratory.* USDA-CSREES; $2,000,000.

Sanderson, Sherry. *Comparison of two premium diets - veterinarian vs owner.* Ralston Purina; $82,973.

Stallknecht, David. *West Nile Virus Surveillance.* Ga Dept. Human Resources; $145,449.

Stallknecht, David. *Peridomestic avian species as amplifying hosts and sentinels of WN and SLE viruses in Georgia.* Centers for Disease Control; $557,000.

Vandenplas, Michel. *Equine genes, microarrays and responses to gram-positive toxins.* Grayson Jockey Club; $76,000

Villegas, Pedro. *Molecular and pathogenic analysis of co-infection with infectious bursal disease and chicken anemia.* U.S. Poultry & Egg Association; $20,000.

Villegas, Pedro. *Pathological and serological assessment of a live avian adenovirus vaccine candidate*. Aviagen NA; $39,628.

Wooley, Richard. *Comparison of several adult challenge methods for avian colibacillosis with results from the embryo.* U.S. Poultry & Egg Association; $13,800.

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Selected Publications*

Alberti, A., Murgia, C., Liu, S-L., Mura, M., Cousens, C., Sharp, J.M., Miller, A.D., Palmarini, M.: Envelope-induced cell transformation by ovine beteretroviruses. J. Virol., 76:5387-5394, 2002.

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Bengis, R.G., Kock, R.A., Fischer, J.R.: Infectious animal diseases: the wildlife/livestock interface. OIE Sci. Tech. Rev., 21(1):53-65, 2002.

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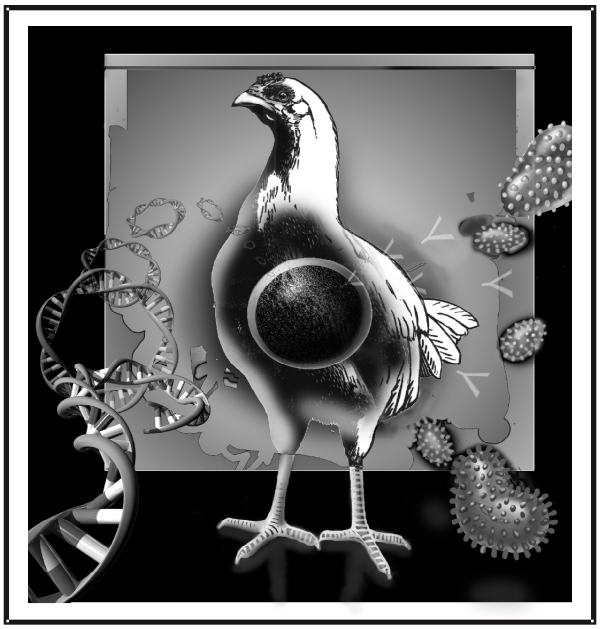

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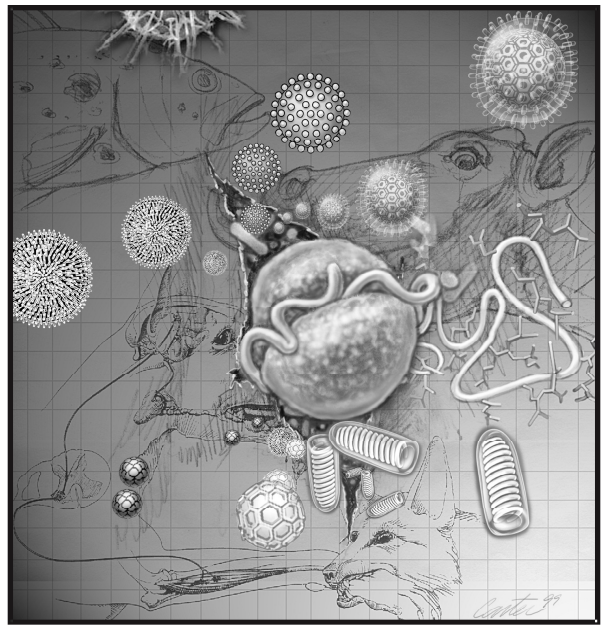

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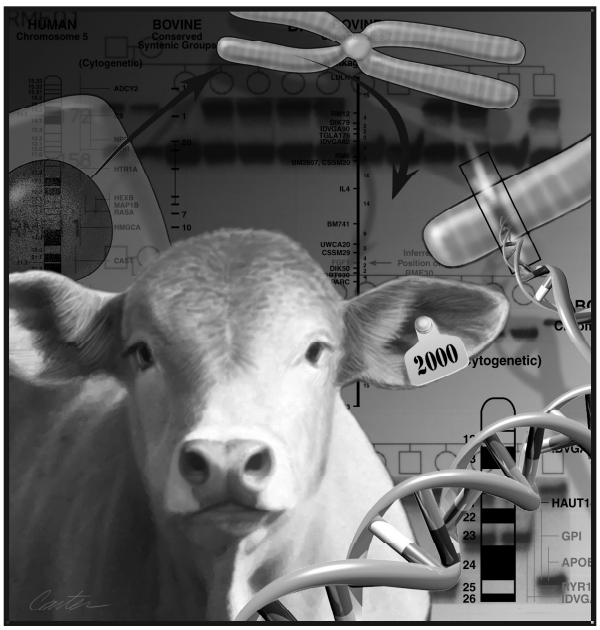

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