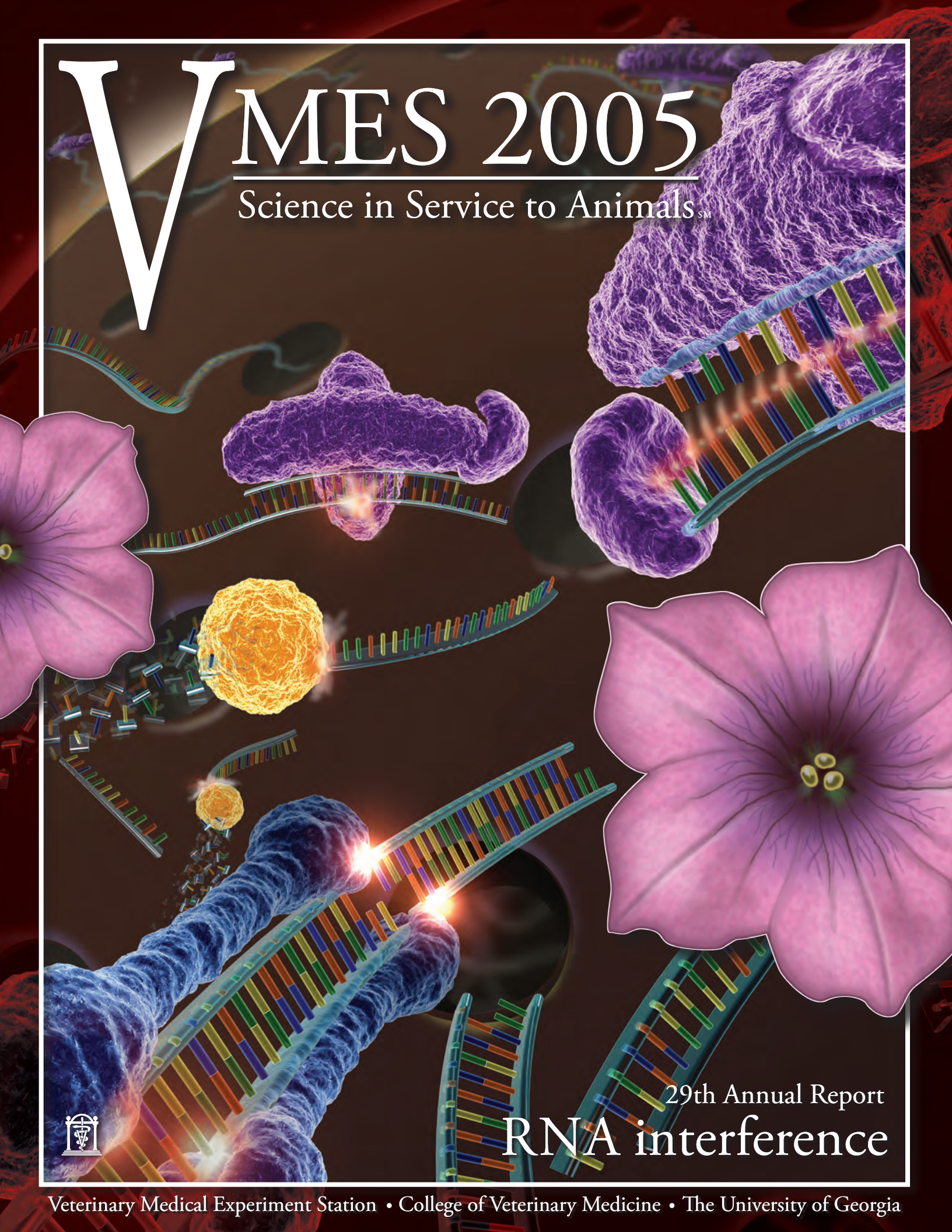


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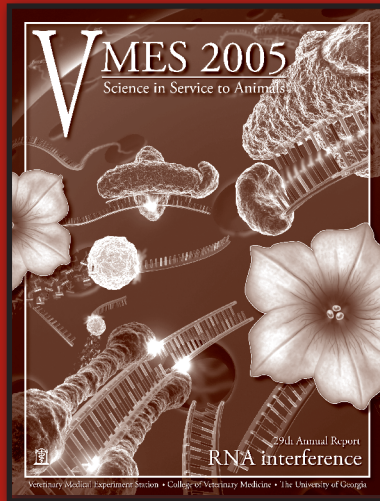
Science in Service to AnimalsSM



29th Annual Report

RNA interference





Cover Description

Only recently elucidated, RNA interference (RNAi) promises to provide revolutionary therapeutic tools against a wide range of diseases. First observed in petunias during genetic experiments involving petal color, the mechanism has been seen to be preserved in plants and animals. Science magazine has declared RNAi as the 2002 “Breakthrough of the Year.” Studies by Dr. Ralph Tripp and others at the College of Veterinary Medicine, The University of Georgia, have shown that RNAi has the potential to rapidly produce therapeutic modalities against a range of disease-producing organisms.



The University of Georgia

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V MES 2005

Science in Service to Animals

29th Annual Report

Veterinary Medical Experiment Station
College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602
July 1, 2004 to June 30, 2005

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 2004-2005 VMES annual report.

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

Our objectives are as follows:

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

Report of the Director

I am pleased to present the 29th Annual Report of the Veterinary Medical Experiment Station (VMES). The project summaries presented in this report represent a spectrum of the research activities of the College, including the development of new therapies against infectious pathogens that affect both humans and domestic animal species. Our cover story by Dr. Ralph Tripp, Georgia Research Alliance Eminent Scholar in Animal Health Vaccine Development, discusses the use of the technology referred to as RNA interference (or RNAi) for the prevention of viral infections, including avian influenza. As you will find from his article, Dr. Tripp and other researchers in the College are at the forefront of research on animal and human viral diseases.

In addition to funding basic and applied research projects, the VMES budget continues to contribute to the support of a myriad of ancillary activities related to animal and human health including: the Athens and Tifton Diagnostic laboratories, a summer research training program for veterinary students, a statistical consulting service, the College's electron microscopy laboratory, and technician and graduate student salary support. Although veterinary research has the potential for great impact in many biomedical fields, support for animal-related research is limited. Thus, the continued commitment at the State level to support research on animal health is a critically important investment. The food animal industries of the State of Georgia are valued at well over \$3 billion and sales of livestock, poultry and their products account for more than half of Georgia's annual farm income. Protection of these resources is paramount to our State's economy.

Veterinary medicine is an indispensable component of our State's public health system. Veterinarians in public practice are focused on zoonotic diseases, food safety, water quality, environmental protection, biomedical research, health education, emergency medicine, wildlife epidemiology, and laboratory animal medicine. In addition, veterinarians are now called upon to strengthen the country's defense capabilities in response to bioterrorism. This is largely due to the fact that over 80% of bio-threat agents are transmissible from animals to humans. At the current time, however, there is a critical shortage of veterinarians, particularly those serving in public health practice with the requisite specialized training. To address this need the College now provides veterinary students the opportunity to obtain training in a public health degree program leading to an MPH in the new UGA College of Public Health

while pursuing the DVM degree. This new program was initiated by faculty with support from the College administration. Dr. David Dreesen, an Emeritus Professor in the Department of Infectious Diseases, currently serves as the Acting Director of the DVM/MPH program and was instrumental in its development.

The 29th VMES Annual Report provides an overview of peer-reviewed, competitive VMES-funded projects conducted during fiscal year 2005 (July 1, 2004 - June 30, 2005). Additional information on any of these projects can be requested by contacting the VMES office by phone, email or website, or directly from the investigators themselves. A list of publications is provided as well. These peer-reviewed papers represent a selection of VMES supported work and other scholarly research originating at the College of Veterinary Medicine.

A summary of the College's research funding is provided below. Over the past year approximately four research dollars were leveraged for each VMES dollar invested.

Finally, I would like to extend a sincere thank you on behalf of the College to Dr. Keith Prasse, our former dean, who retired in February 2005. During his tenure as dean and through his leadership and guidance, the Veterinary Medicine Experiment Station thrived, even during a time of financial constraints and shrinking state budgets. I also extend a hearty welcome to our new dean, Dr. Sheila Allen, who already has exercised her leadership skills in preparation for the opening of our new Animal Health Research Center, which represents a major research investment by the College, University and State. Stay tuned.

Funding Source	FY 2001	FY 2002	FY 2003	FY 2004	FY 2005	FY2006
VMES/VMAR Expenditures	\$3,569,225	\$3,927,297	\$3,672,210	\$3,380,261	\$3,094,649	\$3,148,784
Federal Grants and Contracts	3,452,426	6,962,300	4,768,808	5,624,962	5,746,363	
State Grants and Contracts	4,054,420	4,563,272	4,434,171	3,872,763	4,688,817	
Private Grants and Contracts	2,283,536	1,446,110	715,974	1,677,282	2,221,052	

Harry W. Dickerson

RNA Interference

RNA interference (RNAi) is a naturally occurring process for controlling gene expression in diverse branches of the evolutionary tree ranging from plants, to fungus, to round worms, to man. The power that RNAi wields was first observed in an experiment designed to increase the purple pigment in petunias. In these experiments, researchers were trying to deepen the purple color of the flowers by injecting the gene responsible, but instead of a darker purple flower resulting, the petunias were either variegated or completely white. This surprising phenomenon was termed co-suppression, since both the expression of the existing gene coding for the purple color and the introduced gene designed to deepen the purple color were suppressed. Co-suppression has since been found to occur in most plants through a process mediated by double stranded RNA (dsRNA) gene suppression.

An important question arises from these findings - why would dsRNA mediate gene suppression? Accumulating evidence suggests that this may be an evolutionary conserved defense mechanism against virus infection. Most viruses that infect plant or animal cells code for dsRNA. Since only single stranded RNA is normally found in a cell, the presence of dsRNA is a danger signal that is typically caused by virus infection. To defend itself, the cell has an enzyme called 'Dicer' that recognizes dsRNA and cleaves it into small interfering RNAs (siRNA) between 19-25 base pairs in length. These siRNAs bind to a RNA-induced silencing complex (RISC) that targets cognate messenger RNA (mRNA), and cleaves it to mediate gene suppression, as once cleaved, it can no longer be translated into a functional protein (See figure).

The basic tenets of RNAi were first demonstrated in 1998 after injection of dsRNA into *C. elegans*, a soil nematode, resulted in highly effective interference of endogenous gene activity. In this study, it was shown that use of dsDNA as opposed to use of the sense or anti-sense strand alone was at least two times more effective in RNAi. Since this discovery, RNAi using dsRNA molecules has become widely used to regulate gene expression in many biological systems and to reveal many cellular processes that were previously not understood. The significance of RNAi to the biological and medical sciences was recognized and heralded as the 2002 "Breakthrough of the Year" by Science magazine.

The therapeutic potential and application of RNAi to silence undesirable host genes, or genes of infectious agents associated with disease, are just being realized. RNAi therapeutics has been used to inhibit oncogenic genes that cause resistance to chemotherapeutic agents, and to treat important infectious viral diseases mediated by respiratory syncytial virus, influenza, HIV, and herpes virus among others. The earliest RNAi applications began using an 'antisense approach' in which long oligonucleotides were designed and used to target cognate RNA to block translation. Antisense RNAi therapeutics was effective, but often associated with off-target effects and unintended toxicity, as these large RNA molecules mediated an 'interferon effect' that exacerbated the proinflammatory response. The recent demonstration of RNAi in mammalian cells using siRNA which does not mediate an 'interferon effect' has positioned siRNAs as the therapeutic of choice by most investigators seeking disease intervention strategies. RNAi therapeutics using siRNA differs fundamentally from antisense and chemical antiviral drugs. siRNAs are natural and formed by an endogenous process that has evolved with cells, the cells carry the machinery needed to mediate gene silencing, and the mechanism of action is gene-specific so there are no off-target effects or cell toxicity. In contrast, cells are not designed to receive organic chemicals or to have their genes silenced by 'synthetic' antisense compounds, and because these therapies are less or not specific, they are notoriously associated with off-target effects and toxicity. Given these advantages, RNAi therapeutics using siRNA molecules is quickly becoming the method of choice to defend against viral and host diseases.

Virus infection is a severe public health problem with substantial, personal, social and economic consequences. There are very few safe and effective antiviral drugs available, and for emerging viral diseases without a vaccine alternative, the lack of antiviral drugs poses a severe threat to public health. The best example of this is pandemic influenza. Vaccines are the mainstay of prophylaxis against influenza, but there are technical and safety issues that must be overcome, and it is impossible to predict what strain of influenza may be associated with the next pandemic. Since a vaccine would not be available in the early stages of a pandemic, it is critical that anti-viral drugs are available to contain and treat infection. Currently, the H5N1 strain of avian influenza has emerged as a leading pandemic influenza candidate. Unfortunately, the current H5N1 strain circulating in Southeast Asia shows evidence of drug resistance to all classes of existing anti-influenza drugs strongly suggesting that mul-

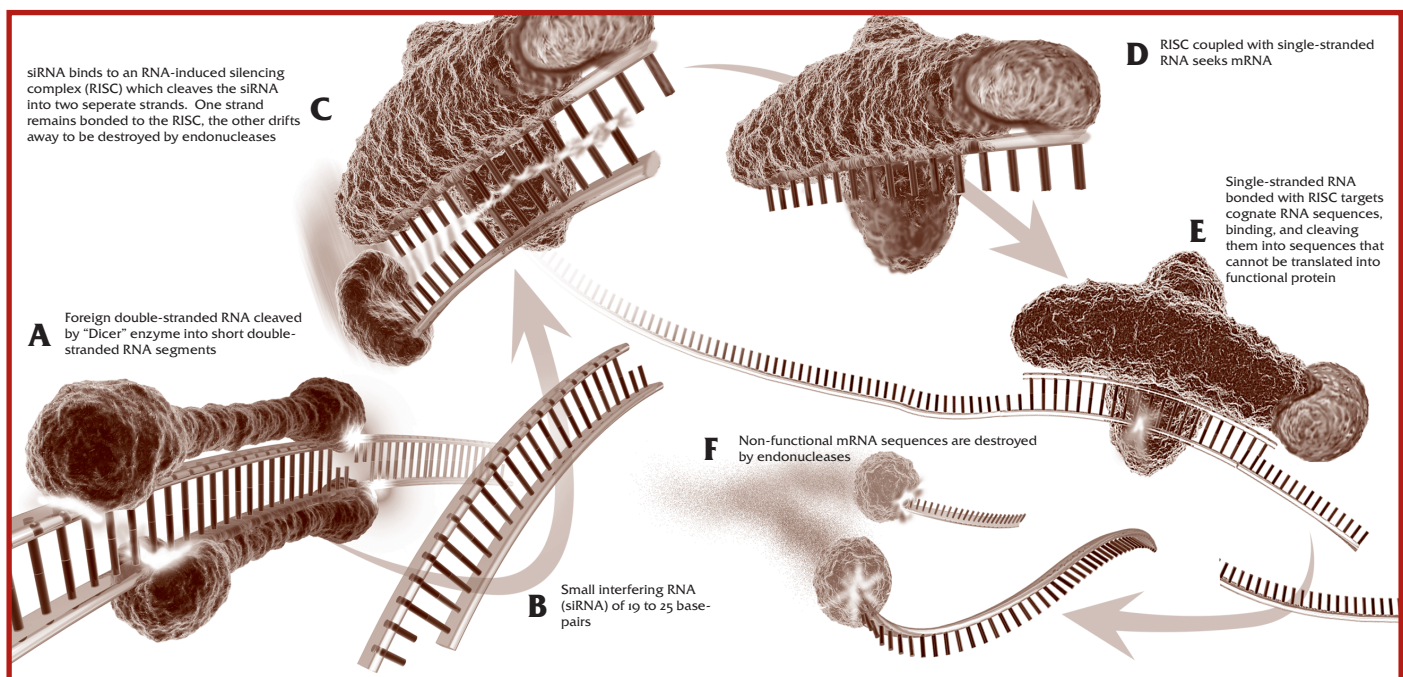
and The Wayward Petunia

multiple anti-viral drug approaches will be required to prevent resistance to existing anti-viral drugs, and that new anti-viral drug approaches are needed to protect the human population from newly emerging influenza virus strains.

Recent studies at the University of Georgia in the laboratories of Drs. Ralph Tripp and Mark Tompkins in collaboration with Alnylam Pharmaceuticals, Cambridge, MA have shown that the power of RNAi can be harnessed to rapidly develop anti-viral drugs reactive for respiratory viruses such as respiratory syncytial virus and influenza virus. These studies have shown that RNAi has the power to create powerful therapeutics that meet the demand for new anti-viral drugs with higher potency, lower toxicity, and having a high degree of specificity. Importantly, this technology allows for development of RNAi-based drugs within months as opposed to several years which is common for development of most anti-viral drugs, e.g. oseltamivir (Tamiflu). Thus, RNAi therapeutics is a new breakthrough solution to address emerging viral diseases, and will provide an unprecedented means to control pandemic flu and other important respiratory virus infections that carry a high disease burden on mankind.

From its earliest discovery in petunias, to our new understanding of its power in disease intervention strategies, all facets of biology and biomedicine are being touched by RNAi gene silencing. It is likely that RNAi therapeutics will impact everyone's life in the near future, and may eventually become an alternative to vaccination as a strategy to control or intervene in disease or disease pathogenesis.

Ralph Tripp, Ph.D.





Dr. Charles Hofacre

The bridge between the full time researchers and the poultry industry is Dr. Charles Hofacre's research laboratory when it comes to food safety. His work is primarily directed at how the poultry industry can reduce the amount of salmonella in their chickens on the farm before it gets to the processing plant. The most recent research was evaluating the effectiveness of vaccination of breeder hens with both a USDA approved live attenuated salmonella vaccine and autogenous killed vaccine to prevent salmonella infection in their offspring. This work has led to the current strategy employed today by the broiler industry of vaccination of breeders to reduce salmonella to the processing plant and hopefully less foodborne illness in people.

The second area of Dr. Hofacre's research in food safety involves finding ways to prevent disease in poultry without having to use antibiotics; therefore, helping to alleviate the concern of many in the human medical field that antibiotic use in food animals can result in antibiotic resistant bacteria in humans. Dr. Hofacre and colleagues in The University of Georgia College of Pharmacy looked at a nutritional, a byproduct of the muscadine grape industry in Georgia, as a means of preventing an intestinal disease in broiler chickens called necrotic enteritis. They found that this byproduct of the muscadine grape may be effective in reducing the mortality and lesions caused by the toxin from the bacterium *Clostridium perfringens* that causes necrotic enteritis. In the past, this disease has primarily been controlled by use of antibiotics. Perhaps in the near future, we will be able to use a natural product like muscadine grape pulp to avoid having to use an antibiotic to prevent this disease.

Bacterial & Parasitic Diseases

From Egg to Carcass: Tracking the Entry of Poultry Foodborne Pathogens into the Food Chain

Virulence genes are frequently found on large "pathogenicity islands" or they are part of mobile genetic elements, such as bacteriophages or plasmids. A species can be pathogenic or non-pathogenic depending on the virulence gene(s) it harbors. There is considerable genetic variability among *Salmonella enterica* as evident with its multitude of serovars, and yet only a handful cause disease in man. Are these differences attributed to variability in virulence gene distribution among specific *Salmonella* serovars? In this study, we examined the distribution of several virulence genes among 470 *Salmonella enterica* isolates, representing 15 serovars, from poultry isolated in the southeastern US. *Salmonella* isolates were screened by DNA:DNA hybridization using DNA probes to several virulence genes associated with prophages. (*sopE*, *ssel*, and *grvA*), *Salmonella* pathogenicity island 1 (SPI1) (*avrA*), virulence plasmid (*spvC*) and fimbrial operons (*lpf*, *sefC*, *stfA*, and *saf*). One of the major genetic differences among poultry *Salmonella* serovars was in the distribution of the virulence plasmid and GIFS2-prophage associated virulence genes, most notably *ssel*. *Salmonella* serovars heidelberg and kentucky were negative for *spvC* while 77% to 67% of enteritidis and typhimurium isolates, respectively, were positive for this virulence plasmid gene. Although GIFS2 prophage virulence gene *ssel* was present several of the *Salmonella* serovars examined, there was considerable difference in its prevalence among serotypes enteritidis (75%), heidelberg (35%), kentucky (1%), and typhimurium (54%). Fimbrial genes were either confined in its distribution to specific *Salmonella* serotypes; ex. *saf* and typhimurium, *sefC* and enteritidis; or varied in their prevalence (50-90%) among the *Salmonella* serovars. The SPI1-associated gene *avrA* was uniform in its distribution among poultry *Salmonella* isolates. While it is important for the poultry industry to reduce *Salmonella* contamination, aggressive eradication/control programs might be best directed first towards elimination of pathogenic serovars from broiler chicken flocks.

PI: Dr. Charles Hofacre (chofacre@uga.edu)

CO-PIs: Dr. Dana Cole, Dr. John J. Maurer, and Dr. Michael P. Doyle

Elucidating the effects of the Microbial Community Structure on the Host Intestinal Mucosa

The long-term goal of our research is to identify the mechanisms involved in the interaction between bacterial communities and intestinal health. The hypothesis of this work is that the composition of the intestinal microbiota can modulate the levels of mucosal inflammation in the small intestine. In Specific Aim 1 we proposed to characterize chemokines produced in the ileal mucosa when certain populations of bacteria are present. We intended to extract mRNA from archived formalin-fixed intestinal samples in order to detect chemokine expression. We discovered that RT-PCR targeting the avian interleukin genes would be difficult to quantitate because the formalin-fixation fragments the mRNA. We need to generate fresh samples for these experiments; therefore, we will pursue this Aim in an external application in which we propose to use the Affymetrix chicken microarray chip to detect chemokine expression. We are also seeking a collaborator that is experienced in microarray expression analysis in order to enhance our ability to interpret the data.

In Specific Aim 2 we proposed to characterize the putative cytotoxins produced by clostridial populations in the ileum. Our preliminary microbial ecology studies had shown that growth-promoting antibiotics elicited ileal communities rich with a unique family of uncharacterized clostridia that had not been cultivated. In order to better understand the biology of these clostridia, we focused on a metagenomic approach to characterize their metabolic potential. Metagenomic analysis integrates molecular ecology, genomic sequencing, and bioinformatics in an effort to formulate biological predictions regarding uncultivated organisms. Since we had detected most of the unique ileal clostridia ribotypes among the cecal bacterial community, we used cecal bacterial pellets to initiate our study. 16S rDNA clone libraries of the cecal community revealed that 70% of the 16S clones represented this family of unique clostridia. We then screened the bacterial pellets for enzyme activities that would indicate toxin expression or specific metabolic pathways. While we detected high levels of glycosidase and phosphatase activity, we did not detect phospholipase C (*Clostridium* toxin A) or protease activity that would indicate cytotoxin expression. Therefore, we produced an expression library of community DNA from the cecal bacterial pellet and screened for hemolysin, phospholipase, and sialidase (mucinase) clones. Two screenings, of over 70,000 clones, were performed to identify putative cytotoxin genes. Sialidase expression was used as a control because the genomes of pathogenic species of *Clostridium* contain 2 different sialidase genes; detecting sialidase clones was important in assessing whether the library was representative. We did not detect cytotoxin clones but we did isolate several sialidase-expressing clones. One clone was sequenced and while its DNA sequence did not exhibit homology to known genes, its derived amino-acid sequence contained conserved domains indicative of sialidases and it exhibits similarity to the family of large molecular weight sialidases. These findings indicate that our metagenome library represented a family of novel clostridia that are intestinal commensals. In order to reveal aspects of their metabolism we randomly selected 96 clones for DNA sequence and metabolomics analysis. We detected genes involved in cell structure and DNA replication as well as those important for carbohydrate and amino acid metabolism. However, we also detected a significant number of genes involved in butyrate and vitamin B biosynthesis. Both of these compounds play a large role in intestinal development and health. It is not surprising that intestinal bacteria are capable of producing these compounds because it has been shown that intestinal microbiota enhance intestinal maturation. What was surprising was the percentage of clones, representing these metabolic pathways, which were detected among the relatively low number of clones that were studied. This finding, while preliminary, suggest that the unique family of intestinal clostridia may be functioning less as ubiquitous commensal bacteria and more as intestinal symbionts.

A better understanding of the complex microbial community in the intestine will encourage new approaches to improved animal health and performance. We submitted a proposal to the USDA (Jan. 2005 - 44.0 Animal Protection) in order to study the potential symbiotic nature of specific cultivable members of the ileal microbial community. In addition, a metagenomic approach can be used to study host/flora interactions for animals. Therefore, future funding will also be sought from the NIH (NIAID) to perform a comprehensive metagenomic analysis of the novel clostridia.

PI: Dr. Margie Lee (leem@vet.uga.edu)
CO-PI: Drs. John J. Maurer, Charles L. Hofacre,
and Barry Harmon

Clinical Investigation of Poultry Diseases

Two studies were completed under this project. The first was an evaluation for *Salmonella* reduction in broilers from breeders vaccinated with live and killed *Salmonella* vaccines. *Salmonella* is one of the major causes of foodborne illness in humans in the U.S. and poultry is one of the sources frequently identified.

It has been found that much of the *Salmonella* in poultry products actually originates from the broilers' parent hen. We found that by vaccinating these hens with a combination of live *Salmonella typhimurium* vaccine when growing and then a killed *Salmonella* vaccine, there was a significant reduction in *Salmonella* in their offspring. It was found with 3 different *Salmonella* serotypes (*Salmonella* kentucky, *Salmonella* hadar, and *Salmonella* heidelberg) that there was a decrease in overall positive broilers and also those that had salmonella had less in the ceca. This leads us to believe that vaccination of broiler breeders may be an ideal strategy to use to reduce *Salmonella* in poultry processing plants.

The second project involved looking at a nutraceutical to prevent the poultry disease necrotic enteritis. The poultry industry uses the growth promotant antibiotics to prevent the clinical and subclinical form of necrotic enteritis caused by the bacteria *Clostridium perfringens*. There is concern that the routine use of antibiotics in food animals may result in resistant bacteria that could then impact the humans' bacteria flora after eating that poultry product. The poultry industry is working very hard to find alternative means to prevent this disease without using antibiotics. It was found that a nutraceutical product that is a byproduct of the muscadine grape may be effective in reducing the mortality and lesions of *C. perfringens*. Also, it appeared to improve the birds' body weight and feed efficiency.

PI: Dr. Charles Hofacre (chofacre@uga.edu)
CO-PI: Dr. Guillermo Zavala and Dr. Stephen Collett

Unique Bacterial Species of the Animal Gastrointestinal Tract as Biomarkers for Point Source Contamination

Water quality and availability has become an important issue worldwide. There have been several waterborne outbreaks of disease linked to contamination of drinking water by human and animal wastes. However, there were several instances where the source of contamination was never identified.

What is the fecal point source for these waterborne outbreaks? Identifying a point source of contamination, human vs. animal waste, is critical in developing strategies to minimize or prevent future outbreaks. Of the bacterial species that inhabit the gastrointestinal tract of animals, several species have been identified that are unique to its animal host. We have compiled from several 16S rDNA libraries of fecal microflora, obtained from human and food animal species, a phylogenetic tree in order to identify animal host specific ribotypes. Several uncultured, bacterial clones were identified that clustered together within our phylogenetic tree, according to animal host. Several bacterial species belonging to the Clostridiales family were identified from cattle, chickens, dog, horse, and pig that appeared to be unique to the animal species of origin. Uncultured bacterial clones representing the *Capnocytophyga-Flavobacterium-Bacteriodes* phylum were also identified from cattle and sheep that appeared to be unique to its animal host. From DNA sequence comparisons of our uncultured bacterial clones against an extensive 16S rDNA database, we were able to identify sequences unique to the clone and its host animal of origin for cattle, chicken, dog, and horse 16S rDNA microflora libraries. PCR will be eventually developed to recognize these unique 16S rDNA sequences for determining the origin of point source fecal contamination.

PI: Dr. John J. Maurer (jmaurer@vet.uga.edu)

Co-PI: Dr. Margie D. Lee, Dr. Dana Cole, Dr. Susan Sanchez, and Dr. Charles Hofacre

Crohn's disease: Potential acquisition through pasteurized milk.

Mycobacterium paratuberculosis causes chronic granulomatous enteritis in cattle and other ruminants known as Johne's disease. The organism is primarily shed in feces, but has also been isolated in milk from cows with clinical disease, subclinical infection and occasionally from bulk milk. Pasteurization of milk is a critical control point in reducing the risk of human consumption of viable *M. paratuberculosis* bacilli. It was recently announced that *M. paratuberculosis* bacilli were isolated from commercially pasteurized milk. Methods for improving the pasteurization process to completely eliminate viable *M. paratuberculosis* bacilli have been proposed and are under consideration. However, before the expense and effort are put forth to potentially change industry processes and standards, we suggest ruling out another possibility: heat-killed *M. paratuberculosis* bacilli serving as an adjuvant to enhance immune response to antigens in the intestinal lumen. Homogenized milk is an oil-in-water emulsion, and the addition of heat-killed *M. paratuberculosis* finishes the recipe for Complete Freund's Adjuvant, a powerful stimulant of immune response. We exposed a strain of mouse routinely used in inflammatory bowel disease studies to live and pasteurized *M. paratuberculosis* bacilli combined with homogenized and pasteurized milk fat to determine if this exposure is associated with the time of onset and nature of observed Crohn's-like illness. We identified pathology and immune responses resembling Crohn's disease in both groups of mice. Because pasteurization, one of the most successful public health measures ever devised and the basis of our milk safety program is an essential component of this pathogenic mechanism, if accurate, this may create a dilemma for the dairy industry, USDA and FDA. We are continuing our studies in the goat model, which more closely resembles human disease than the mouse model.

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

Diagnostic Science

Analysis of recent Infectious Bursal Disease virus field strains

Two different molecular techniques were used to identify field strains of IBDV. The traditional method of reverse transcription-polymerase chain reaction / restriction fragment length polymorphism (RT-PCR/RFLP) was compared to RT-PCR and nucleotide sequence analysis. Strains were detected from samples previously inactivated with phenol from Latin American countries or from samples obtained here in Georgia.

Standard strains were predominantly detected in Mexico. IBDV strains similar to variant E were detected in Colombia and Ecuador. Peru and Venezuela exhibited a higher heterogeneity of IBDV strains because of the detection of standard, Delaware type as well as GLS variant strains. IBDV strains detected from Brazil and Dominican Republic exhibited RFLP patterns identical to very virulent IBDV strains (vvIBDV) prevalent in several countries in Europe, Asia, and Africa.

From the samples analyzed from the United States, 80% exhibited RFLP identical to the variant Delaware E strain. Other classic strains detected included Sal-1, D-78, Lukert, PBG-98, and the standard challenge strain. The variant Delaware A, and GLS were also detected. The analysis of the deduced amino acid sequence of the VP2 hypervariable region from six strains classified as Delaware variant E, revealed some amino acid substitutions from the original variant E strain isolated in the mid 1980s.

Both techniques were able to detect and identify different stains of IBDV; however we found that it is more practical and faster in most cases to go directly to sequencing from RT-PCR. The results shown are a mixture of both sequencing and RFLP findings.

PI: Dr. Pedro Villegas (pedrov@uga.edu)

CO-PI: Alejandro Banda

Diagnostic Avian Mycoplasmosis

This project consists of diagnostic activity for avian mycoplasmas, including mycoplasma isolation and identification, HI tests, and fingerprinting of isolates or specimens by Random Amplified Polymorphic DNA (RAPD) analysis or by sequencing DNA PCR products from the *mgc2* PCR for *Mycoplasma gallisepticum* (MG) or the *vlhA* PCR for *Mycoplasma synoviae* (MS). We also conduct PCR tests for *M. iowae*. In addition, we provided MG and MS sera for test kits distributed among various laboratories in the U.S. Our lab is the de facto reference laboratory for avian mycoplasma diagnosis in the U.S.A. We also consult by person and by telephone regarding field problems with mycoplasma diagnosis or control.

In calendar year we conducted 2414 diagnostic cultures for mycoplasmas, 3103 HI tests for MG, and 3043 HI tests for MS, in addition to RAPD analysis from 32 cases and *mgc2* and *vlhA*.

PI: Dr. S. H. Kleven (skleven@uga.edu)

Co-PI: V. Leiting



Dr. Stanley Kleven

Mycoplasma gallisepticum (MG) and *M. synoviae* (MS) are two important diseases of commercial poultry. MG is a major cause of respiratory disease, resulting in poor performance and processing plant condemnations in growing flocks, and egg production losses in laying flocks. MS is associated with respiratory disease and leg problems. Control of these important infections is especially important because infection spreads from the parent flock to the progeny through the egg. Monitoring of breeding flocks through the National Poultry Improvement Plan helps assure us that breeding flocks are negative. Breeding flocks found to be infected with MG or MS are generally destroyed, resulting in losses of up to hundreds of thousands of dollars. Very few laboratories are equipped to confirm a diagnosis of MG or MS or to do research on these important pathogens. Dr. Kleven's laboratory has become a de facto reference laboratory for avian mycoplasmal infections in the U.S., as well as internationally.

Dr. Kleven's research centers around the practical aspects of improving diagnostic procedures and control methods. For example, their work was instrumental in the development of the first licensed live vaccine against MG, and we showed that it could be used to control field infections and could be a valuable tool for eradicating the infection. They were among the first to show that MS could be an important cause of processing plant condemnations due to respiratory disease. They also were among the first to recognize that MG and MS strains vary in their ability to cause disease, and they have developed methods for using molecular techniques to identify specific field or vaccine strains in diagnostic specimens.

His current projects involve development of improved vaccine strains for MG, determining the role of MS in peritonitis mortality in commercial layer flocks, improving methods for molecular identification of specific strains, and determining the role of *M. iowae* in causing problems with leg weakness and stunted growth in commercial turkey flocks.

Differentiating infected from vaccinated animals (DIVA) using a specific ELISA for the avian influenza N2 protein

Control of avian influenza (AI) is extremely important to prevent emergence of highly pathogenic viruses, and has recently involved vaccinating birds in flocks surrounding the outbreak then monitoring the transmission of the field virus using serology. A key element of this strategy involves differentiating infected from vaccinated animals (DIVA) using a serologic test. The ELISA test has become the gold standard for serologic testing and is used by most diagnostic laboratories. Thus, the goal of this application is to develop an ELISA based DIVA for AI. We obtained a recombinant baculovirus containing the N2 (rBV-N2) gene from Dr. David Suarez (Southeastern Poultry Research Laboratory, USDA/ARS Athens, GA). The rBVN2 virus has the N2 gene of AI that is expressed as a histidine-tag protein. Initial efforts allowed the propagation and titration of the rBVN2 in Sf9 cells. The virus was grown to a titer of approximately 4.0×10^6 pfu/ml and expression of the expected 51kDa histidine-tag labeled protein was detected by western blot analysis using a 6x-histidine monoclonal antibody. Small scale expression experiments using Sf9 cells monolayers estimated that 1 μ g of N2 was produced by 1.0×10^6 Sf9 cells infected at a multiplicity of infection (MOI) of 0.4, and approximately 0.3 μ g of N2 protein was secreted into the media. These results indicated that a substantial level of N2 expression was attained on Sf9 cells. To further optimize N2 expression, we have plaque-purified the rBV-N2 virus and increased protein production of suspension cultures infected at a MOI of 0.1. Once the N2 expression in suspension cultures has been optimized and validated we will proceed to purify the recombinant N2 protein to be used as an ELISA antigen and for N2-monoclonal antibody production.

PI: Mark W. Jackwood, (mjackwoo@uga.edu)
Co-PI's: Dr. M. Garcia, Dr. S. Williams, Dr. H. Sellers, and Dr. G. Zavala

Development of a multiplex RT-PCR to differentiate lentogenic Newcastle disease viruses (NDV) from exotic Newcastle disease (END) viruses

Identification of all NDV isolates is increasingly more important in light of the 2002 outbreak of END in California and adjacent states. Real time RT-PCR has proven to be a valuable tool as observed in the CA 2002 outbreak. However, avian diagnostic labs routinely isolate NDVs that are presumed to be of vaccine/lentogenic origin and lack a rapid and inexpensive test to type the viruses. RT-PCR has become a standard diagnostic tool and most laboratories are equipped with thermal cyclers. Thus, the goal of this project is to develop and validate a multiplex RT-PCR as a confirma-

tory test to rapidly detect and differentiate vaccine and other lentogenic NDV field isolates from END virus. We have constructed primer sets using previously published full-length genome sequences obtained from GenBank for B1, LaSota and the CA02 END isolate.

Our preliminary results indicate that we can successfully amplify B1 and LaSota RNA using the vaccine/lentogen primer set and amplify CA02 END RNA using the END primer sets in both a uniplex and multiplex RT-PCR format. Currently, we are evaluating other mesogenic and velogenic isolates with all primer sets to determine NDV primer specificity. Optimal multiplex RT-PCR conditions will be determined based on our findings.

PI: Dr. Holly Sellers (hsellers@uga.edu)
Co-PI: Dr. Darrell Kapczynski

Diagnostic Detection, Isolation, and Characterization of Avian Viruses

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. The diagnostic virology laboratory provides a vital function in the overall services offered by the diagnostic facility at the PDRC. During 2004-2005, the diagnostic virology isolated 193 viruses. No virus was isolated from 113 samples. Seven hundred and one whole blood samples were submitted for leukosis virus isolation with subsequent LL antigen capture ELISA. Sixty one serum samples were submitted for EDS-76 hemagglutination inhibition testing.

PI: Dr. Holly Sellers (hsellers@uga.edu)
Co-PI: E. Linneman

Investigation of Natural Disease Outbreaks - AV-THA

The major activity of this project is to provide clinical diagnostic support for the commercial poultry industry of Georgia. This is accomplished through the application of field investigation acquisition of flock and farm histories, application of analytical, microbiological, histopathological testing using classical and molecular methods.

Activity is represented by farm visits by Faculty and students. Clinical investigations may include large broiler operations having severe late respiratory disease problems with associated condemnations at processing. Extensive serology testing, virus isolation and PCR testing often reveals a particular

strain of infectious bronchitis virus as the causative agent. Modification of the vaccination program usually brings the problem under control.

The professional staff and students often investigate more long term problems on farms within the region. These are typically multi-faceted problems that take a more long term approach. Many times these are assigned as a student project under direct supervision of an experienced clinician. These investigations bring recommendations and changes in vaccination programs and management practices which frequently allow that grower to become competitive again.

The polymerase chain reaction (PCR) technique has permitted generation of more useful and timely information often in hours rather than days or weeks using classical diagnostic techniques for infectious bronchitis and Mycoplasmas. Also avian leukosis virus-J, infectious laryngotracheitis virus, infectious bursal disease virus, avian adenovirus, Newcastle disease virus, avian pneumovirus, avian influenza virus, avian astrovirus, chicken anemia virus and turkey coronavirus PCRs are available and provide useful and very timely diagnostic information. PCR testing capability now includes Salmonella serotyping for the more common serotypes of Salmonella. Research continues and new PCR tests will be applied to diagnostics as applications are developed.

The Diagnostic Services/Teaching Laboratory is in the process of complete conversion of the lab and accounting system to MS SQL compliance. The integrated accounting system (AccPac) has been upgraded to a SQL version and a web site for delivery of lab reports and for data analysis of client serological data is underway. We are will be including an e-business capability on the web site for accessioning cases, scheduling delivery service pickup and accepting credit card payment for services. In addition clients will be able to check case and account via the secure web site.

Diagnostic Services/Teaching Laboratory activity is represented by 6,431 accessions, 30,215 bacterial procedures, 118 antimicrobial susceptibilities, 118,408 ELISA tests , 56,738 IBV-HI tests, 205,645 total serological tests, 2,853 diagnostic PCR tests, 7,857 histopathology slides, and 1793 necropsies.

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Dr. Michel Vandenplas

In an era when the genomes (genetic makeup) of several organisms have been fully sequenced, comparatively few equine genes have been sequenced and characterized. Consequently, Dr. Vandenplas, as part of a larger equine research team at UGA, has been generating expressed sequence tags (ESTs) to identify and make equine gene sequences available to equine researchers worldwide through public gene sequence databases such as GenBank. These ESTs are partial and highly specific gene sequences derived from equine cDNAs cloned into bacterial plasmids. The team has generated over 14,000 ESTs representing more than 3,000 different equine genes. Clustering and comparison of the ESTs have allowed for the identification of polymorphisms in gene sequences between horses of different breed. These polymorphisms, such as nucleotide insertions and deletions, are useful in determining correlations between gene mutations and disease susceptibility. In addition the team is currently using the 3,000 equine genes they have identified to develop microarrays to examine gene expression profiles during diseases such as colic and laminitis. These microarrays will allow identification of gene networks that are induced or suppressed as a result of the specific disease. All of the gene sequences generated by this team are also available from their local website at <http://fungen.org>.

Immunology

Comparison of fresh and frozen in transfer of mycobacterial immunity

Johne's disease is a major economic problem in raising cattle. It is a chronic infection problem of ruminants, which initially affects the gastrointestinal tract and gradually spreads to regional lymph nodes and other body organs. Johne's disease is widely distributed throughout the world. Herd prevalence estimates indicate that 25% to 35% of dairy herds are infected in dairy production area of the US. *Mycobacterium avium* subsp *paratuberculosis* (Map) is the etiological agent of Johne's disease. This infection is normally acquired by neonates. Ingestion of colostrum and milk contaminated with fecal material represents the major route of infection for newborns. Pasteurization of colostrum is promoted as a way to reduce the transmission of Map. Pasteurization may cause a destruction of immune components in the colostrum, which could reduced the efficacy of passive transfer of immunity. Living maternal cells and/or the products of their lysis in colostrum appear to play an important role in the transfer of immunity from cow to calf, and may be important in transfer of specific mycobacterial immunity to the neonatal calf. Maternal cells also appear to be important in the development of adaptive immune response by neonatal calves.

In the dairy industry, it is common practice to pool colostrum from cows with high levels of antibody and store it frozen for feeding to calves from cows that produce a small quantity or poor quality colostrum. This practice may alter the quality of colostrum by destroying immune cells. However, there is some evidence that the products of the broken down cells may transfer immunity. The goal of this project is to determine the effect of ingestion of live maternal cells from colostrum, as compared to the cell fragments found in frozen colostrum (transfer factor) on the capacity of neonatal immune cells to respond to mycobacterial antigens after ingestion of colostrum. As a negative control, some calves will be fed colostrum that has had the cells removed by centrifugation (acellular colostrum). The objectives of our study are: 1) to determine the phenotype of immune cells circulating in the neonatal calf prior to and after ingestion of fresh, frozen or acellular colostrums, and 2) to determine the function of immune cells in the circulation of the neonatal calf prior to and after ingestion of fresh, frozen or acellular colostrum. The response of immune cells from calves to purified protein derivatives (PPD) from *M. bovis*, *M. avium* and Map will be assessed prior to feeding colostrum, and on days 1, 2, 7, 14, 21 and 28 after ingestion of fresh, frozen or acellular colostrum. The development of the capacity to present and respond to PPD is dependent on the function of major histocompatibility complex (MHC) class II proteins. We will be assessing the correlations between the expression of surface proteins known to regulate the immune response and that serve as indicators of mycobacterial response with the function of the immune cells in each group over a period of 4 week after they receive colostrum.

Fifteen neonatal calves from the University of Georgia Dairy will be used to investigate the expression of MHC class II protein, the expression of cellular activation antigens and cellular response to PPD. To date we have collected data from two calves in each group. Collection of the remaining data will begin in September 2005, when calving resumes.

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Co-PI: Adrian J Reber, Douglas C Donovan

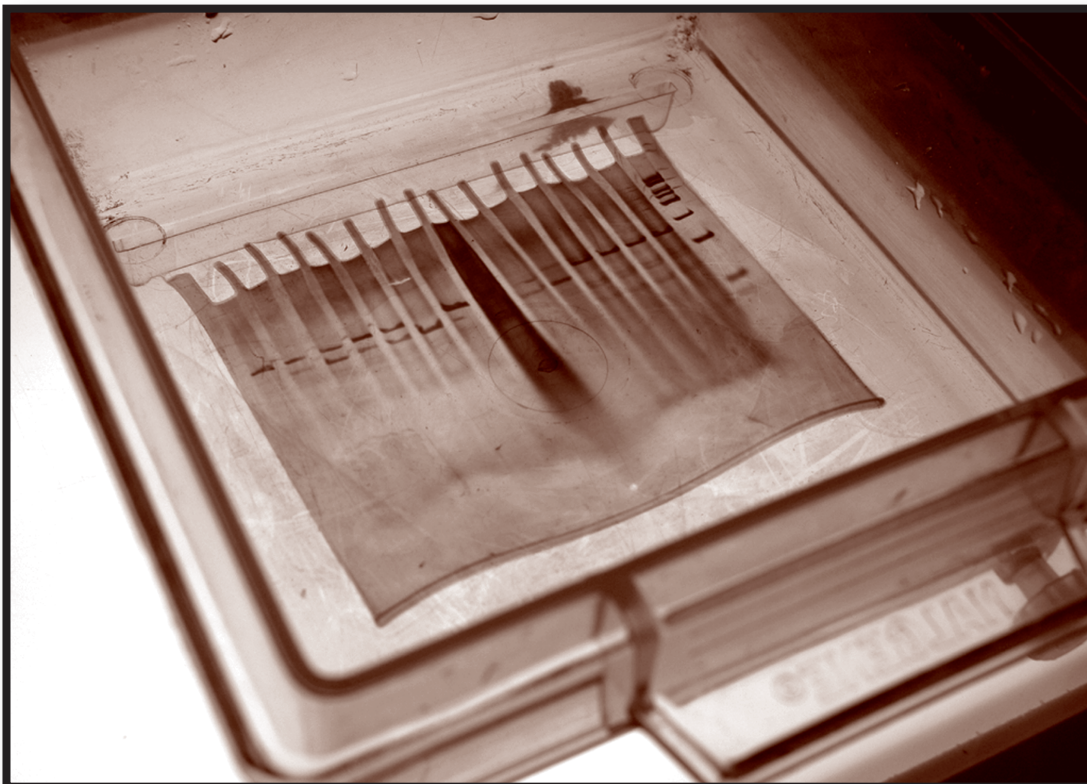
Expression of TNF alpha in Tilapia (Oreochromis niloticus)

Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells are the main killer cell populations of the immune system. The mechanisms by which these cells recognize target cells vary considerably, while the effector molecules used to facilitate target cell death are highly conserved. The main pathways utilized by killer cells consist of granule exocytosis and those mediated by members of the TNF superfamily. Nonspecific cytotoxic cells (NCC) are the first identified cytotoxic cell population in teleosts. We have previously demonstrated the expression of granzymes and Fas ligand in these cells. This is the first report of the expression of tumor necrosis factor-alpha in these killer cells. The significance of tumor necrosis factor (TNF) in animal health has been well documented. In addition to its function in cytotoxicity, TNF is the most important pro-inflammatory cytokine in all vertebrates studied to date. However, little is known about its role in fish health. A cDNA coding for TNF was cloned and sequenced from NCC purified from Nile tilapia (*Oreochromis niloticus*). Factors regulating the transcriptional modulation of TNF in these cells were identified by RT-PCR analysis. The mature form of tilapia TNF was expressed as a recombinant protein and biological activities were analyzed. Using a cross-reacting anti-TNF polyclonal antibody, analysis of TNF expression suggested that tilapia NCC constitutively express the membrane-bound as well as secreted

forms of TNF. Recombinant tilapia TNF effectively induced cytotoxicity in the mammalian cell line WEHI, although to a lesser extent compared to the murine TNF. Treatment with recombinant TNF protected NCC from activation-induced cell death. Recombinant tilapia TNF was also effective in up-regulation of granzyme transcription in tilapia NCC. These data suggest that teleost TNF may play a role in diverse effector functions of cytotoxic cells from ectotherms, similar to the biological functions described for mammalian TNF.

How will our studies of tilapia TNF help improve animal health? While many sequencing projects have provided molecular information about some of the effectors of immunity in fish, there is still a lack of information about the biological roles of some of these molecules. Our studies provide new information about potential roles for TNF in the immune responses of fish. As a direct result of this work, we have been able to determine the biological activities of recombinant tilapia TNF as well as follow its expression in fish following infection.

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Dr. Maricarmen Garcia

Infectious laryngotracheitis (ILT) is a highly contagious and acute disease of poultry responsible for considerable economic losses and trade embargos. The disease is largely controlled by vaccination with live attenuated strains of the virus. Because the apparent genome homogeneity that exists among ILT field and vaccine strains in the United States, most workers believe that vaccine strains have become established in the poultry population, replacing many wild type strains. These circumstances have obstructed our understanding on the origin of ILT outbreaks for many years in the US. In Dr. Garcia's laboratory, they have developed different genotyping methods that allow the rapid identification of ILT strains causing outbreaks in the field. This effort has contributed to understand the origin of outbreaks, and to implement more effective biosecurity measures to interrupt the persistence cycle of the virus in densely populated poultry production areas.

Commercially available ILTV live attenuated vaccines are capable to persist in the field, gain virulence and cause outbreaks of the disease. Therefore development of safer, more effective ILT vaccines is fundamental in the eradication of ILT from the US poultry. Dr. Garcia and her group are currently studying the function of ILT viral proteins, homologous to other animal alpha-herpesvirus, implicated in the virus spread from cell-to-cell. Our objective is to delete viral proteins that function to facilitate the spread of the virus and produce attenuated vaccines that can be easily differentiated from the strains circulating in the field.

Virology

Development of tools to study infectious laryngotracheitis virus (ILTV) glycoproteins involved in viral cell-to-cell spread process (AV-130)

All alpha-herpesvirus express a heterodimer complex, composed of glycoproteins, E and I, the gE/gI complex has been identified as a major facilitator of cell-to-cell spread for some of the alpha-herpesvirus family members. ILTV is an alpha-herpesvirus that spreads between epithelial cells of the upper respiratory track to the trigeminal ganglia, and probably to other nervous system tissues of chickens. Initial propagation of the virus in cell culture is difficult and in many instances not successful. The prerequisite of gE/gI expression for propagation of ILTV in cell culture has not been properly studied because the lack of gE/gI ILTV mutants. Attempts to create gE and/or gI mutants has not been successful so far, and, unlike other alpha-herpesvirus, naturally occurring ILTV gE and gI mutants have not been isolated by conventional viral propagation systems. Taken as a whole these observations suggest that gE/gI expression maybe essential for propagation of ILTV in vitro.

In order to understand the mechanisms utilized by ILTV to spread and the role of gE and gI, viral mutants lacking expression of either one or both these genes are essential. To initiate this task it is fundamental to create specific reagents not available on hand anywhere else. Polyclonal antibodies against the cytoplasmic domain of gE have been raised in mice. The mouse anti-gE polyclonal serum localized the glycoprotein E in the surface of ILTV infected chicken embryo kidney (CEK) cells, and in the surface of the chicken hepatocellular carcinoma cell line (LMH ATCC CRL2117) expressing the gE protein. A protein of approximately 85 to 90 kDa was recognized by Western-blot on ILTV infected CEK cells. One of the objectives of this project was to develop an LMH cell line expression viral glycoprotein E to study its role in viral spread and persistence. However, we found that after consecutive passages of ILTV in LMH cells no viral cytopathic effect or viral nucleic acid was detected. Therefore, contrary to published reports in our hands the LMH cell line does not support ILTV replication. In order to determine if LMH cells do not support ILTV replication because they are not permissive to viral entry the ability of LMH and LMH-gE to support ILTV replication after transfection with viral DNA will be studied. In addition, instead of using LMH-gE cells to study the role of the glycoprotein E on viral spread, CELi cells transiently expressing gE will be utilized to evaluate viral plaque formation as a measure of viral spread.

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Co-PI: Dr. Holly Sellers

Interactions Between ALV Subgroup J and IBDV in White Leghorn Chickens (AV-120)

We have proposed the following objectives for this research:

- Examine the serological response to ALV-J in White Leghorns after infection with IBDV. Virus isolation and virus neutralization assays will determine serological response at various time points. The poultry industry uses these techniques at specific times to screen flocks and eliminate ALV infected birds.
- Examine the incidence of ALV-J viral shedding in White Leghorns after infection with IBDV. Viral shedding will be assessed by cloacal swabs and virus isolation. These samples will be collected at same time as serology samples. The poultry industry screens valuable genetic stocks for ALV shedding and eliminates positives.

- Determine tumor incidence and type and compare to previous reported work with ALV-A and IBDV, and ALV-J in white leghorns. Birds will be kept for 30 weeks to allow time for tumor development. Birds will be examined grossly for tumors and examined histologically for diagnosis and determine if tumor incidence is affected and if manifestations are different.

Results: See Table 1 for virology and serology data. Part of the 18 week data is being retested due to technical problems. Statistical analysis is still pending. Neoplasia did occur in the ALV-J infected groups. Hemangiomas were the most common neoplasia. Unusual tumors were bile duct carcinoma of the liver, histiocytic sarcoma of the spleen and an undifferentiated sarcoma invading the skeletal muscle. There were no myelocytomatosis or lymphoid leukosis manifestations.

PI: Dr. Susan Williams
CO-PI: Dr. Holly Sellers

Table 1. ALV-J virology and serology assay results.

Group	ALV-J virus neutral 4wk	ALV-J virus neutral 10wk	ALV-J virus neutral 18wk	ALV-J virus neutral 30wk
Control	0/24	0/24		0/24
Alvj	0/33	28/33		11/27
IBD	0/36	0/36		0/24
Alvj +IBD	0/37	33/37		7/26

Group	Isolation Blood 4wks	Isolation Blood 10wks	Isolation Blood 18wks	Isolation Blood 30wks
Control	0/24	0/24	0/24	0/24
Alvj	33/33	13/33	27/29	28/29
IBD	0/36	0/36	0/36	0/24
Alvj +IBD	37/37	19/37	28/32	26/26

Group	Isolation Cloacal 4wks	Isolation Cloacal 10wks	Isolation Cloacal 18wks	Isolation Cloacal 30wks
Control	0/24	0/24	0/24	0/24
Alvj	10/33	2/33	18/31	29/29
IBD	0/36	0/36		0/25
Alvj +IBD	5/37	9/37		22/30

Pathogenesis, Transmission and Detection of Reticuloendotheliosis Virus in Japanese Quail.

Reticuloendotheliosis virus (REV) is a retrovirus causing tumors, immunosuppression and mortality in chickens, turkeys and wild birds. REV has been found occasionally in contaminated poultry vaccines. Freedom of REV is required for SPF eggs, poultry vaccines and breeding stock. REV is transmitted horizontally and vertically, and by blood-sucking insects, albeit little is known about natural reservoirs or the dynamics of infection. The objectives of this research include: a) isolation of REV from wild birds (Attwater Prairie Chickens = APC); b) examination of the susceptibility of Japanese quail

to REV isolated from APC; c) studies on the horizontal and vertical transmissibility of REV using a Japanese quail model; d) collection of a variety of clinical samples for detection of REV using an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA), to be developed in our laboratory as part of a separate but complementary research project.

REV was isolated from several clinical samples obtained from APC specimens. PCR primers were designed to amplify, sequence and clone the entire gag gene from a field isolate designated APC-566. The gag gene of APC-566 was successfully amplified and has been sequenced and compared phylogenetically to the gag gene of REV isolates of chicken origin previously reported in GenBank. Efforts to clone the PCR product into a suitable eukaryotic expression vector (pCI-neo) are under progress. Six groups of 60 Japanese quail each are currently being held in isolation to study transmission of REV APC-566. With the exception of a negative control group, each group of quail contains birds that were infected as embryos with the APC-566 isolate. At hatch, each group contained 0%, 5%, 10%, 25%, 50%, and 100% infected quail, respectively. Such experimental quail are currently 5 weeks of age and will be allowed to reproduce to study vertical transmission. REV was detected by PCR in nine (100%) 3-week-old quail that had been infected as embryos, demonstrating that APC-566 successfully infected Japanese quail. REV was not detected in negative control quail of the same age. Samples for REV detection will be collected from all experimental birds in this study, starting at 6 weeks of age. A variety of samples will be collected in the progeny of the experimental quail and the horizontal and vertical transmissibility will be examined. Additional field isolates are currently being propagated in cell culture and their gag gene sequence will be resolved as well in order to determine if gag gene proteins (p30) of REV APC566 are suitable for developing cross-reacting monoclonal antibodies to be used in an AC-ELISA for REV detection.

PI: Dr. Guillermo Zavala (gzavala@uga.edu)
CO-PIs: Dr. Maricarmen García, Dr. Susan Williams, Sunny Cheng and Taylor Barbosa



Dr. Margarethe Hoenig

Obesity is a major problem not only in humans but also in pet animals. It is a risk factor for diabetes mellitus. Dr. Hoenig's group has primarily focused on studying obesity and diabetes mellitus in cats. Cats develop similar defects in insulin secretion and peripheral insulin action as obese people and people with type 2 diabetes mellitus. The laboratory is especially interested in factors that are involved in the progression from obesity to diabetes with a focus on lipid and glucose metabolism. In addition, they are examining nutritional and pharmacological agents that might prevent the defects in insulin secretion and/or action seen in obese cats. Dr. Hoenig's laboratory is also developing feline specific assays which could be used to identify cats at risk for developing diabetes.

Biomedical Science

Documenting Thyroid Hormone Resistance in Obese Cats

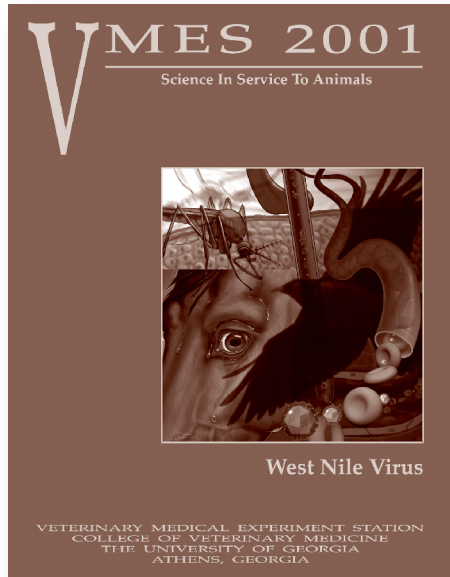
Obesity is a major health problem in pets, especially cats, and is a risk factor for many other diseases, including diabetes mellitus. Obesity is the result of a positive imbalance between energy intake and energy expenditure, but little is still known about the mechanisms involved in its pathogenesis and progression to diabetes. It has been thought that uncoupling proteins are involved in energy expenditure because they produce heat by stimulating the expression of proteins without a coupling to other energy-consuming processes. Thyroid hormone increases basal metabolic rate and it is thought that thyroid hormone elicits this effect by stimulating uncoupling proteins, thereby causing heat production. The goal of this study was to examine if obesity in cats might be caused by resistance to the effect of thyroid hormone on metabolic parameters, including heat production, and expression of uncoupling proteins. Our data indicate that thyroid hormone regulates heat production in both lean and obese cats but this regulation is independent of uncoupling proteins.

PI: Dr. Margarethe Hoenig (mhoenig@vet.uga.edu)

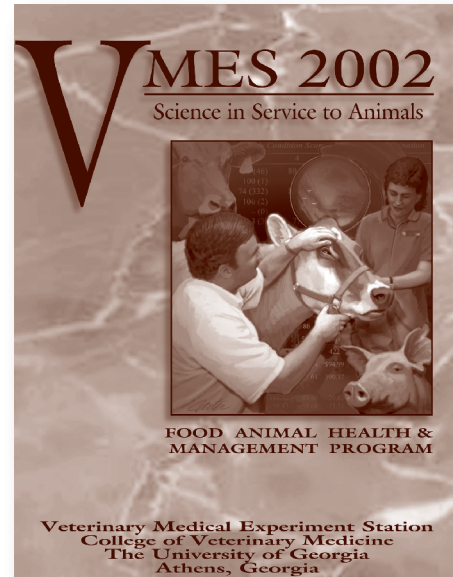
CoPI: Duncan C. Ferguson

VMES - Working For Georgia

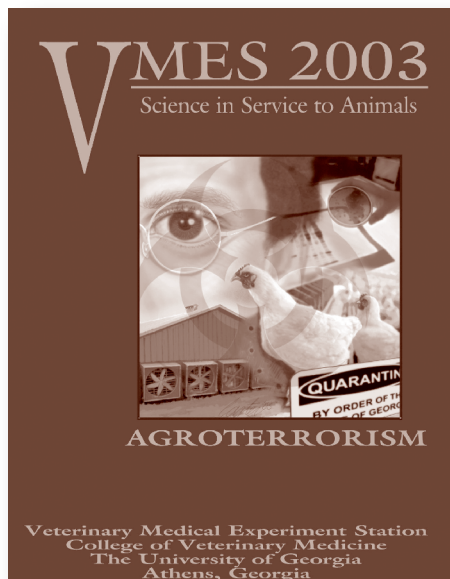
Our Last Four Covers



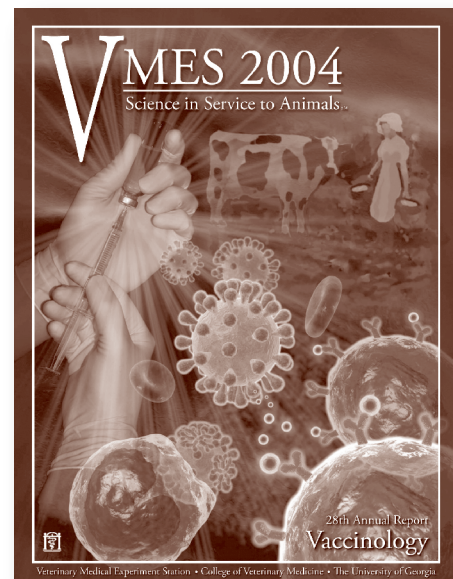
2001
West Nile Virus



2002
Food Animal Health & Management



2003
Agroterrorism



2004
Vaccinology

Research Contracts & Grants

- Allen, Sheila.** Section 1433 Animal Health and Disease Research Funds. \$102,891; USDA-CSREES
- Baldwin, Charles.** Diagnostic services relative to the control, diagnosis, treatment prevention, and eradication of livestock diseases 2004. \$2,013,055; Ga. Dept. of Agriculture
- Brown, Corrie.** Veterinary pathology training - Serbia: Dr. Tomislav Jelesijevic. \$20,530; Iowa State Univ.
- Brown, Corrie.** Pathogenesis of Nipah virus in guinea pigs. \$94,897; NIH-National Institutes of Health
- Brown, Corrie.** USDA Research Support. \$31,399; USDA
- Brown, Corrie.** USDA Research Support. \$5,400; USDA
- Brown, Corrie.** Veterinary curriculum and the future: Public health, food security and agroterror. \$67,559; FIPSE - U.S. Dept. Education
- Brown, Corrie.** Education globalization in animal and poultry agriculture. \$19,467; Univ. of Arkansas
- Brown, Corrie.** Preparing veterinarians to deal with global issues in animal health, trade and food security. \$39,624; FIPSE - U.S. Dept. Education
- Brown, Corrie.** Development of agroterror preparedness curriculum. \$198,000; GA Dept of Agriculture
- Brown, Corrie.** Development of a central repository of information chronicling incubation periods and clinical signs for biothreat agents. \$18,414; SECEBT - Southeastern Center for Emerging Biologic Threats
- Brown, Scott.** A study to evaluate a nutraceutical product in cats. Ipakitine efficacy in feline renal insufficiency. \$56,265; Vetoquinol USA
- Budsberg, Steven.** Evaluation of the COX-2 inhibitory effects of a novel COX inhibitor using an exploratory novel blood assay procedure. \$75,889; Novartis Animal Health
- Budsberg, Steven.** Pilot evaluation of analgesic effects of two proprietary cmpnds in combination with NSAID therapy in alleviation of chronic canine osteoarthritis pain. \$52,523; Boehringer Ingleheim
- Budsberg, Steven.** Evaluation of effectiveness and field safety of oral PHA-739521 at 2 and 4 mg/kg bw compared to negative control of pain and inflammation assoc w/ canine osteoarthritis. \$113,945; Pfizer Inc.
- Coffield, Julie.** Neuromuscular targets of botulinum toxin. \$349,600; National Institutes of Health
- Coffield, Julie.** Identification of botulinum toxin membrane targets. \$294,400; NIH/NIAID
- Cole, Dana.** Estimating the risk of human exposure and resultant spread of highly pathogenic avian influenza. \$24,952; SECEBT - Southeastern Center for Emerging Biologic Threats
- Corn, Joseph.** Distribution of Pseudorabies virus and *Brucella suis* in feral swine. \$50,000; USDA-APHIS
- Corn, Joseph.** Exotic tick surveillance in the southeastern United States and Puerto Rico. \$200,000; USDA-APHIS
- Corn, Joseph.** Survey for the tropical bont tick on wildlife associated with infested pastures on St. Croix, USVI. \$71,521; USDA-ARS
- Crowell-Davis, Sharon.** Evaluation of the efficacy of a synthetic pheromone analogue in the treatment of stormphobia in dogs. A pilot study. \$4,284; CEVA Sante Animale
- Dickerson, Harry.** Veterinary Scholar Symposium 2005. \$28,896; NIH-National Institutes of Health
- Dickerson, Harry.** The Univ of Georgia 2005 Veterinary Scholars Program: A research training experience for veterinary students. \$20,000; Merck/Merial Animal Health Grants Program
- Ferguson, Duncan.** Recombinant feline thyrotropin (fTSH): Immunoassay validation and bioactivity. \$37,706; Morris Animal Foundation
- Ferguson, Duncan.** Recombinant thyrotropin (TSH): Standard for the next generation of canine TSH immunoassays with improved sensitivity. \$50,093; AKC-American Kennel Club Foundation
- Findly, R. Craig.** Differentiation of peripheral B cells, IgZ, and mucosal immunity in channel catfish. \$5,000; USDA-CSREES
- Fischer, John.** Southeastern cooperative wildlife disease study. \$261,700; Various Other States
- Fischer, John.** Cooperative Agreement for develop and evaluation of data relative to disease relationships that may involve wildlife, domestic livestock, and poultry. \$350,000; USDA-APHIS
- Fischer, John.** Investigation of and assistance with wildlife disease problems in the SE region of the U.S. \$231,500; U.S. Dept. of Interior
- Fischer, John.** Southeastern cooperative wildlife disease study: West Virginia. \$15,840; Various Other States
- Fischer, John.** Southeastern Cooperative Wildlife Disease Study. \$168,500, Fish and Wildlife Agencies
- Fischer, John.** Wildlife Services Disease Training. \$150,000; USDA-APHIS
- Fischer, John.** Southeastern cooperative wildlife disease study: North Carolina. \$25,000; Various Other States
- Garcia, Maricarmen.** Genotyping of infectious laryngotracheitis virus (ILTV): New strategies. \$47,840; U.S. Poultry and Egg Assoc.
- Garcia, Maricarmen.** \$3,000; USDA-CSREES
- Hensel, Patrick.** Determination of threshold concentrations of allergens used for intradermal testing in dogs. \$8,873; American Academy of Veterinary Dermatology
- Hines, Murray E.** Efficacy of a spheroplastic whole cell vaccine for the prevention of John's Disease. \$50,085; USDA-APHIS
- Hodge, Thomas.** Identification of host genes required for viral pathogenesis, II. \$0; Hudson-Alpha Institute for Biotechnology

- Hodge, Thomas.** Identification of host genes required for viral pathogenesis. \$287,500 Hudson-Alpha Institute for Biotechnology
- Hoening, Margarethe.** Nuclear magnetic resonance, a noninvasive method to evaluate glucose and fat metabolism in the cat. \$159,809; Nestle Purina, Inc.
- Hofacre, Charles.** \$4,000; USDA-CSREES
- Hondalus, Mary K.** Vaccine potential of a riboflavin-requiring strain of *R. equi*. \$61,652; Grayson-Jockey Club Research Foundation
- Hurley, David.** Comparison of fresh and frozen colostrum in transfer of mycobacterial immunity. \$5,000; USDA-CSREES
- Jackwood, Mark.** \$4,000; USDA-CSREES
- Jaso-Friedman, Lilliana.** Expression of TNF alpha in tilapia (*Oreochromis niloticus*). \$5,000; USDA-CSREES
- Karls, Russell.** Isolation of mycobacteriophage that target *M. avium* subsp. *paratuberculosis*. \$3,708; USDA-CSREES
- Karls, Russell.** Regulation of *Mycobacterium tuberculosis* sigma factor C and identification of Sig-C transcribed genes. \$17,500; American Lung Association
- Kero, Kathy L.** Clinical evaluation of efficacy and safety of pradofloxacin tablets for treatment of lower urinary tract infections in dogs. \$7,500; Icon Clinical Research
- King, Christopher.** VCA: A monoclonal antibody toolkit for functional genomics of plant cell walls. \$92,736; NSF-National Science Foundation
- Kleven, Stanley.** Delayed serological response to *Mycoplasma synoviae*. \$45,613; U.S. Poultry and Egg Assoc.
- Kleven, Stanley.** 15th International Congress of the International Organization for Mycoplasmaology. \$10,000; USDA-CSREES
- Krunkosky, Thomas.** Acute inflammatory changes in skin and lamellar tissue during the onset of acute laminitis. \$44,950; American Quarter Horse Assoc.
- Lee, Margie.** \$3,000; USDA-CSREES
- Little, Susan.** Diagnostic assays for *Borrelia lonestari*. \$73,600; National Institutes of Health
- Maurer, John.** From egg to carcass: Tracking the entry of poultry foodborne pathogens into the food chain. \$194,135; USDA-NRI
- McCall, John.** Transfection and promoter analysis of *Brugia malayi*. \$25,000; Univ. of Alabama
- McCall, John.** Furnish *Brugia malayi* adult worms and/or *Brugia malayi* infective larvae. \$134,835; National Institutes of Health
- McCall, John.** Filariasis research reagent resource center. \$409,980; National Institutes of Health
- McCombs, Candace.** Project management and consultation. \$156,600; Sequella Inc.
- Mead, Daniel.** Transmissibility and host predilection of epidemic vesicular stomatitis New Jersey virus strains. \$315,000; USDA-NRI
- Mead, Daniel.** Avian host and vector relationships in metropolitan foci of West Nile Virus. \$49,453; SECEBT
- Miller, Doris.** BSE Surveillance. \$96,000; USDA-APHIS
- Miller, Doris.** Athens Diagnostic Laboratory. \$1,258,077; GA Dept. of Agriculture
- Miller, Doris.** BSE Surveillance. \$360,000; USDA-APHIS
- Moore, James.** Synthetic lipid A antagonists for prevention of LPS-induced effects in equine cells. \$138,456; Morris Animal Foundation
- Moore, James.** Effects of nonsteroidal antiinflammatory drugs on translocation of nuclear factor B. \$15,000; Bernice Barbour Foundation
- Moore, James.** Synthetic lipid A antagonists for prevention of LPS-induced effects in equine cells. \$138,456; Morris Animal Foundation
- Moore, Julie.** T cell memory and protection against placental malaria. \$343,061; National Institutes of Health
- Mueller, Eric.** Exogenous hyaluronan for management of equine distal limb wounds. \$6,292; USDA-CSREES
- Murray, Thomas.** Neurotoxins from marine algae and cyanobacteria. \$133,962; Oregon State Univ.
- Murray, Thomas.** Cellular activation induced by multivalent ligands. \$336,149; National Institutes of Health
- Murray, Thomas.** Cellular activation induced by multivalent ligands. \$56,144; National Institutes of Health
- Murray, Thomas.** Peptidic ligands for K-opioid receptors. \$72,408; Univ. of Kansas
- Murray, Thomas.** Affinity labels for opioid receptors. \$69,615; Univ. of Kansas
- Palmarini, Massimo.** Development of an animal model to test novel therapies for lung cancer. \$43,904; National Institutes of Health
- Palmarini, Massimo.** Development of an animal model to test novel therapies for lung cancer. \$234,441; National Institutes of Health
- Pence, Melvin.** Georgia John's Work Plan. \$15,856; GA Dept of Agriculture
- Pence, Melvin.** Georgia John's Disease demonstration herd project. \$46,656; USDA-APHIS
- Peroni, John.** Role of oxidant stress in microvascular dysfunction in equine laminitis. \$55,946; Morris Animal Foundation
- Prasse, Keith.** National network of diagnostic laboratories for animal disease monitoring and diagnosis. \$308,529; USDA-CSREES
- Quinn, Fred.** Defining the mechanism of resistance to antibiotic SQ109 by *Mycobacterium tuberculosis*. \$5,000; Sequella, Inc.

Research Contracts & Grants

- Quinn, Fred.** Crohn's disease: Potential acquisition through pasteurized milk. \$5,000; USDA-CSREES
- Reeves, David.** Manage Rogers state prison swine farm. \$72,393; GA Dept. of Corrections
- Reeves, David.** Manage Rogers state prison dairy farm \$319,612; GA Dept. of Corrections
- Ritchie, Branson.** Research Associate In Exotic/Zoo Infectious Disease And Pathology, Postgraduate program. \$40,000; Zoo Atlanta/Riverbanks Zoo
- Robertson, Thomas.** Atypical regulation of vascular tone by protein kinase C. \$184,000; National Institutes of Health
- Sanderson, Sherry.** Comparison of two dietary approaches to managing canine chronic renal failure. \$27,949; Iams Company
- Sellers, Holly.** Development of a multiplex RT-PCR to differentiate lentogenic newcastle disease virus from END. \$11,730; U.S. Poultry and Egg Assoc.
- Sellers, Holly.** \$2,000; USDA-CSREES
- Stallknecht, David.** HPAI in wild birds: Is there a potential for a wildlife reservoir? \$45,236; U.S. Poultry and Egg Assoc.
- Stallknecht, David.** West Nile virus surveillance in wild birds. \$148,340; GA Dept. of Human Resources
- Thayer, Stephen.** Diagnostic Lab. \$18,150; USDA-CSREES
- Tripp, Ralph.** Phase I: Calf study. \$22,783; Numico Research Australia Pty.
- Tripp, Ralph.** siRNA intervention strategies for respiratory syncytial virus infection (Phase 1). \$121,787; Alnylam Pharmaceuticals
- Tripp, Ralph.** siRNA intervention strategies for respiratory syncytial virus infection (Phase 2). \$174,918; Alnylam Pharmaceuticals
- Vandenplas, Michel.** Correlating MAP induced NF- κ B activation with NOD2 mutations in Johne's disease. \$5,000; USDA-CSREES
- Varela, Andrea.** Infection dynamics of *Ehrlichia chaffeensis*. \$103,482; National Institutes of Health
- Villegas, Pedro.** \$300; USDA-CSREES
- Villegas, Pedro.** Use of avian adeno-associated virus as a vector for poultry vaccines. \$26,703; U.S. Poultry and Egg Assoc.
- Wagner, John J.** Cocaine-induced metaplasticity in the hippocampus. \$147,200; National Institute of Drug Abuse
- Williams, Susan M.** \$2,000; USDA-CSREES
- Wilson, Heather.** Effects of pre-pubertal gonadectomy on adult health and behavior in Umbrella and Moluccan Cockatoos. \$110,055; Kaytee Avian Foundation
- Wilson, Heather.** Testing parameters for control of lovebird circovirus 1 & 2 infections. \$9,192; Petco Foundation
- Wilson, Heather.** Evaluation of dolphin sperm for bacterial contaminants via PCR assay. \$9,512; MGM Mirage Inc.
- Wilson, Heather.** Comparison of three methods of long bone fracture repair in rabbits. \$6,350; American Rabbit Breeders Assoc.
- Woolums, Amelia.** siRNA administration to prevent disease due to BRSV infection in cattle. \$5,000; USDA-CSREES
- Yabsley, Michael.** Reservoir potential of raccoons for tick-borne zoonoses. \$47,146; SECEBT
- Zavala, Guillermo.** \$1,400; USDA-CSREES

Grand Total: \$12,884,802

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Selected Publications

- Agnello, K.A., Trumble, T.N., Chambers, J.N., Seewald, W., and Budsberg, S.C. The effects of zoledronate on markers of bone metabolism and subchondral bone mineral density in an experimental model of canine osteoarthritis. *Am. J. Vet. Res.* 66:1487-1495, 2005.
- Agnello, K.A., Reynolds, L.R., and Budsberg, S.C. In vivo effects of tepoxalin, a dual COX/LOX inhibitor, on prostanoïd and leukotriene production in dogs with osteoarthritis. *Am. J. Vet. Res.* 66:966-972, 2005.
- Agrawal, A., Tripp, R.A., Anderson, L.J., and Nie, S. Real-time detection of virus particles and viral protein expression with two-color nanoparticle probes. *J. Virol.* 79:8625-8628, 2005.
- Alanazi, F., Fu, Z.F., and Lu, D.R. Effective transfection of rabies DNA vaccine in cell culture using an artificial lipoprotein carrier system. *Pharm. Res.* 21:675-682, 2004.
- Aldrich, J.V., Choi, H., and Murray, T.F. An affinity label for delta-opioid receptors derived from [D-Ala2]deltorphin I. *J. Peptide Res.* 63:108-115, 2004.
- Allison, A.B., Mead, D.G., Gibbs, S.E.J., Hoffman, D.M., and Stallknecht, D.E. West Nile virus viremia and wild rock pigeons. *Emerging Infectious Diseases* 10(12):2252-2255, 2004
- Alvarez, R., Jones, L.P., Seal, B., Kapczynski, D., and Tripp, R.A. Serological cross-reactivity among members of the Metapneumovirinae genus. *Virus Res.* 105:67-73, 2004.
- Alvarez, R., Harrod, K.S., Shieh, W-J, Zaki, S., and Tripp, R.A. Human metapneumovirus persists in BALB/c mice despite the presence of neutralizing antibodies. *J. Virol.* 78(24):14003-14011, 2004.
- Aragon, C.L., and Budsberg, S.C. Applications of Evidence-Based Medicine: Cranial cruciate ligament injury repair in the dog. *Vet. Surg.* 34:93-98, 2005.
- Banda, A., and P. Villegas. Genetic Characterization of Very Virulent Infectious Bursal Disease Viruses (vvIBDV) from Latin America. *Avian Dis.* 48:540-549, 2004.
- Banda, A., P. Villegas, and J. El-Attrache. Heteroduplex Mobility Assay for Genotyping Infectious Bursal Disease Virus. *Avian Dis.* 48:851-862, 2004.
- Barton, M.H., Sharma, P., LeRoy, B.E., and Howerth, E.W. Hypercalcemia and increased serum parathyroid hormone-related protein in a horse with multiple myeloma. *J. Amer. Vet. Med. Assoc.* 225:409-413, 2004.
- Bennett, M.A., Murray, T.F. and Aldrich, J.V. Structure-activity relationships of arodyn, a novel acetylated kappa opioid receptor antagonist dagger. *J. Peptide Res.* 65:322-332, 2005.
- Bennett, N., Papich, M.G., Hoenig, M., Fettman, M.J. and Lappin, M.R. Evaluation of transdermal application of glipizide in a pluronic lecithin gel to healthy cats. *Amer. J. Vet. Res.* 66: 581-588, 2005.
- Birrenkott, A.H., S.B. Wilde, J.J. Hains, J.R. Fischer, T.M. Murphy, C.P. Hope, P.G. Parnell, and W.W. Bowerman. Establishing a food-chain linkage between aquatic plant material and avian vacuolar myelinopathy in mallard ducks (*Anas platyrhynchos*). *J. of Wildlife Diseases* 40(3):485-492, 2004.
- Brennan, C.L., Hoenig, M. and Ferguson, D.C. GLUT4 but not GLUT1 expression decreases early in the development of feline obesity. *Domest. Anim. Endocrinol.* 26:291-301, 2004.

Research Publications from independent and collaborative research activities of faculty in the College of Veterinary Medicine and the Veterinary Medical Experiment Station

- Brewer, L., LaRue, R., Hering, B., Brown, C., and Njenga, M.K. Transplanting encephalomyocarditis virus-infected porcine islet cells reverses diabetes in recipient mice but also transmits the virus. *Xenotransplantation* 11:160-170, 2004
- Brown, C.A., Mathur, S., Munday, J.S., and Brown, S.A. Hypertensive encephalopathy in cats with reduced renal function. *Vet. Pathol.* 42(5):131-138, 2005.
- Buchanan, M.F., Carter, W.C., Cowgill, L.M., Hurley, D.J., Lewis, S.J., MacLeod, J.N., Melton, T.R., Moore, J.N., Pessah, I., Roberson, M., Robertson, T.P., Smith, M.L., and Vandenplas, M.L. Using 3-D Animation to teach intracellular signal transduction mechanisms: taking the arrows out of cells. *J. Vet. Med. Edu.* 32:72-78, 2005.
- Callison, S. A., D. A. Hilt, and M. W. Jackwood. Rapid differentiation of avian infectious bronchitis virus isolates by sample to residual ratiom quantitation using real-time reverse transcriptase-polymerase chain reaction. *J. Virol. Meth.* 124:183-190, 2005.
- Callison, S. A., D. A. Hilt, and M. W. Jackwood. Using DNA shuffling to create novel infectious bronchitis virus S1 genes: Implications for S1 gene recombination. *Virus Genes.* Accepted January 2005.
- Callison, S. A., D. A. Hilt, and M. W. Jackwood. In vitro analysis of a hammerhead ribozyme targeted to infectious bronchitis virus nucleocapsid mRNA. *Avian Dis.* 49:103-107, 2005.
- Chaisavanneyakorn, S., Lucchi, N., Abramowsky, C., Othoro, C., Chaiyaroj, S.C., Shi, Y.P, Nahlen, B.L., Peterson, D.S., Moore, J.M. and Udhayakumar, V. Immunohistological characterization of macrophage migration inhibitory factor (MIF) expression in malaria-infected placenta. *Infection and Immunity* 73(6):3287-3293, 2005.
- Chambers, J.N., Hanley, C.S., and Hernandez-Divers, S.J. Hip luxation in a Woodchuck (*Marmota monax*): Successful treatment by closed reduction and a modified Ehmer sling. *J. Zoo & Wildlife Med.* 35:569-571, 2004.
- Clabaugh, K., Haag, K.M., Hanley, C.S., Latimer, K.S. and Hernandez-Divers, S.J. Undifferentiated sarcoma resolved by amputation and prosthesis in a radiated tortoise (*Geochelone radiata*). *J. Zoo & Wildlife Med.* 36(1):117-120, 2005.
- Clark, H.J., R.M. Kaplan, J.B. Matthews and J.E. Hodgkinson. Isolation and characterisation of a beta tubulin isotype 2 gene from two species of cyathostomin. *Inter. J. Parasitology* 35(4):349-358, 2005.
- Cohen, N.D., Chaffin, M.K., Vandenplas, M.L., Edwards, R.F., Nevill, M., Moore, J.N., and Martens, R.J. Study of serum amyloid A concentrations as a means of achieving early diagnosis of *Rhodococcus equi* pneumonia. *Equine Veterinary J., Vol. 37, No. 3,* pp. 212-216, 2005.
- Cole, D., Drum, D.J.V., Stallknecht, D.E., White, D.G., Lee, M.D., Ayers, S., Sobsey, M. and J.J. Maurer. Free-Ranging Canada Geese as Environmental Vectors of Antimicrobial Resistance. *Emerging Infectious Diseases,* (in press), 2005.
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