VMES 2007

Science in Service to Animals SM

31st Annual Report

Avian Influenza Virus

Contents

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The University of Georgia

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July 1, 2006 to June 30, 2007

VMES Overview, Mission, and Objectives

The Veterinary Medical Experiment Station (VMES) was established as a budgetary entity by the state legislature in July 1976 following approval by the University of Georgia Board of Regents in 1973. The VMES mission is to conduct research and provide scientific training focused on the improvement of animal and human health and the elimination of animal diseases affecting the citizens of Georgia and Georgia's livestock and poultry industries.

VMES funding supports research that increases the productivity and health of Georgia's poultry and livestock, improves the quality of life for Georgia's companion animals, and defends Georgia's public health through disease surveillance. Although VMES funding is for projects that can be completed in one year, consideration is given to those investigators with long-range plans for sustainable research programs. This enhances their competitive position for extramural funding, is effective in utilizing the College of Veterinary Medicine's resources for research and most importantly, helps solve major animal health problems. In this 31st Annual VMES report we summarize research efforts for fiscal year 2007.

The objective of the VMES is to implement and support research and training programs that fulfill its mission, which addresses many issues of concern to society. These include the food we eat, the environment we live in, our physical and emotional well-being, as well as our material needs such as clothing, travel and economic stability.

Specific VMES objectives are:

- 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.

All programs and activities of the Veterinary Medical Experiment Station are conducted without regard to race, color, national origin, age, sex, or handicap.

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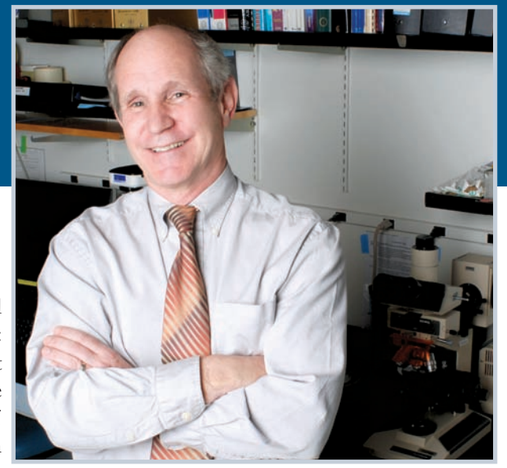
VMES



As I write this 31st Annual Report of the Veterinary Medical Experiment Station (VMES), I am reminded of the Chinese proverb: "May you be blessed to live in interesting times." There is no doubt that academic veterinary medicine faces interesting challenges as we move into the 21st century, not the least of which is positioning itself to effectively conduct research relevant to both animal and human populations. These strategic issues are of concern to the veterinary profession as a whole and are articulated and addressed in the study, Critical Needs for Research in Veterinary Medicine, which was conducted and published by the National Research Council of the National Academies of Science of the United States (National Academies Press, Washington, D.C., 2005). I quote directly from the summary of the National Academies' report: "The rich history of veterinary research, which includes studies on infectious disease and in other biomedical sciences, is replete with seminal contributions to the improvement of animal and human well-being. The many contributions of veterinary research were the results of society's recognition of its important role and society's subsequent support in the form of human, fiscal, and infrastructural resources. The current level of support for veterinary research, however, has not kept pace with the challenges posed by new and emerging threats and the nation's growing demands for knowledge in biomedicine and animal health. That society's needs are outgrowing our knowledge base is seen in examples of missed opportunities to safeguard and improve human and animal health and welfare."

Through effective strategic planning and support from the upper administration of the University, a modicum of risk-taking, and just plain hard work, the University Of Georgia College of Veterinary Medicine and the VMES are not only surviving, but thriving in the challenging fiscal environment that we now face. For example, although research funding nationwide is becoming increasingly difficult to attain, extramural dollars garnered from both federal and private grants have continued to increase in the college over the last five years and funding is now at the highest level we have ever attained (see table below). This is possible because the college has successfully recruited, and retained, excellent veterinary researchers in both clinical and basic research, who are among the most innovative and productive individuals at the University of Georgia. These faculty members in turn have attracted excellent graduate students and post-doctoral fellows to work in their laboratories. In addition, with the completion and commissioning of the new 75,000 square foot Animal Health Research Center, replete with its state-of-the-art biocontainment systems, the college now has a key infrastructural resource to position itself as a leader in infectious disease research.

Most important, of course, is the fact that the work conducted by our researchers is highly relevant to society. To showcase this, we asked Dr. David Stallknecht to write the cover article for this year's VMES Annual Report. As you will read, his work on avian influenza is cutting edge and provides important insights on the natural history and ecology of this important disease that affects both human and animal health. We have many other examples of work of this caliber in the college, some of which are also highlighted in the research abstracts on the following pages.

Veterinary research has an impact on many biomedical fields, and major support for research on animal models of human disease is available from federal agencies such as the National Institutes of Medicine. In addition, the National Science Foundation, and the United States Department of Agriculture provide funding for basic and applied animal research, respectively. Although competitive research dollars are available from some non-federal sources such as the Morris Animal Foundation, research funding that targets companion animals, including horses, is very limited. Thus, the continued commitment from the State of Georgia to support research on animal health is a critically important investment. The companion and food animal industries of the State of Georgia are a major component of the state's economy. For example, 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 continued good economic health.

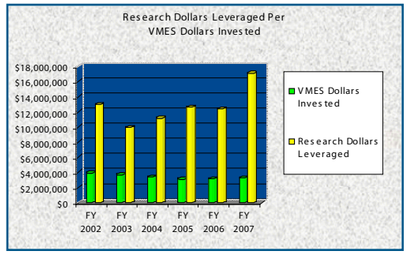
HIGHLIGHTS

The 31st VMES Annual Report provides an overview of peer-reviewed, competitive VMES-funded projects conducted during fiscal year 2007 (July 1, 2006 – June 30, 2007). 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 5.25 research dollars were leveraged for each VMES dollar invested.

I hope it is clear from my comments above, that I attribute the productivity and quality of our research programs to the hard-working and creative individuals who comprise the Veterinary Medical Experiment Station and the College of Veterinary Medicine. In this vein, I acknowledge Dr. Lari Cowgill who has been instrumental in the layout design and publication of the VMES Annual Report for 20 years and is largely responsible for the continued high quality of this publication. This year's Annual Report is dedicated to the memory of Dr. Barry Harmon, a friend, colleague, and excellent veterinary researcher and leader who died on January 19, 2007, following a brave struggle against prostate cancer. He will be missed.

Harry W. Dickerson



FUNDING SOURCE	FY02	FY03	FY04	FY05	FY06	FY07	FY08
VMES/VMAR	$3,927,297	$3,672,210	$3,380,261	$3,094,649	$3,148,784	$3,249,577	$3,384,254
Federal Grants and Contracts	$6,962,300	$4,768,808	$5,624,962	$5,746,363	$5,850,096	$8,892,304	
State Grants and Contracts	$4,563,272	$4,434,171	$3,872,763	$4,688,817	$4,482,741	$5,237,927	
Private Grants and Contracts	$1,446,110	$715,974	$1,677,282	$2,221,052	$2,075,430	$2,990,038	
TOTAL Extramural Research Dollars	$12,971,682	$9,918,953	$11,175,007	$12,656,232	$12,408,267	$17,120,269	

Avian Influenza

Understanding The Source

Historical Perspective

In 1961, a highly pathogenic avian influenza (HPAI; H5N3) virus was associated with a mass mortality event in common terns in South Africa. This event represented the first time that an avian influenza virus was isolated from a wild bird and, until 2002 when HPAI H5N1 caused wild bird mortality in Hong Kong, was the only record of an HPAI virus isolation from a wild bird. Although this HPAI H5N3 virus subsequently disappeared, its isolation led to the discovery that wild birds represent a diverse and global reservoir for type-A influenza viruses. It also led to the recognition that these viruses from wild birds represent the prototypes of all type A influenza viruses that infect domestic animal species and humans.

The movement of these viruses along the path from a wild bird reservoir to a new domestic animal or human host is a complex process that is not completely understood. While the possibility of such an event is well established, the probability is undefined. As viral adaptation to new host systems may require multiple transmission events, multiple host species, and continuous genetic changes, this probability can be affected by numerous host, agent, and environmental factors.

Natural History and Prevention

Understanding the risk of an avian influenza completing the path from a wild duck to a domestic animal or humans begins with understanding natural history. The reservoir needs to be defined along with a clear understanding of how these viruses are transmitted and maintained. Without such an understanding, transmission risks cannot be predicted and effective prevention strategies cannot be developed or implemented.

Dr. David Stalknecht
Department of Infectious Disease

Our current understanding of the natural history of avian influenza is not complete but there is an extensive body of knowledge related to the epidemiology of avian influenza viruses (AIV) in wild birds. Two primary avian reservoirs of AIV have been identified including the Anseriformes (ducks, geese, and swans) and the Charadriiformes (shorebirds and gulls). All known subtypes of AIV have been isolated from these reservoirs and, with the exception of Antarctica, infected wild birds have been detected on all continents. The epidemiology of AIV in wild bird populations is best understood in ducks where predictable temporal and spatial patterns of infection have been described. In ducks, infection peaks in late summer and early fall on the breeding grounds and transmission occurs through a fecal/oral cycle involving contaminated water. These viruses are adapted to persist in water for extended periods of time especially under cold conditions. The epidemiology of AIV in Charadriiformes is less understood but primarily involves gulls which appear to host unique AIV subtypes (H13, and H16). The viruses that are associated with wild birds normally are of low pathogenicity and are not associated with disease in wild bird populations.

When Natural History Gets Unnatural

The detection of HPAI H5N1 virus in wild birds was first reported in 2002 in Asia. Subsequent to this event, HPAI H5N1 viruses have been detected in numerous wild bird species. During 2005 and 2006 the virus spread throughout Eurasia, presumably through the movement of infected wild birds. It is widely accepted that the HPAI H5N1 evolved from low pathogenic viruses originally introduced into domestic poultry populations from wild birds. Although we know that the HPAI H5N1 subsequently spilled back into wild birds, it is unknown if this is a short-term event or if this virus can persist in wild bird populations.

Understanding the emergence and significance of this "new" virus in wild bird populations requires a solid point of reference. Our current research is based on a simple premise. That is, if you understand how native avian influenza viruses are maintained and transmitted in wild bird populations you can begin to understand and possibly predict if a specific virus (in this case HPAI H5N1) will be maintained or will subsequently disappear. The potential movement of such viruses within and between continents can also be evaluated and this is especially relevant to questions relating to the movement of this virus into or within North America via migratory wild birds. To that end, we are currently working to better understand the epidemiology of both naturally occurring AIV and HPAI (H5N1) in wild bird populations. Ongoing, research efforts include:

- AIV Transmission and the Environment: Although water represents the normal medium for AIV transmission within wild bird populations, very little research has been dedicated to environmental factors that potentially promote or limit this transmission. We have demonstrated that basic physical (temperature) and chemical (pH and salinity) characteristics can greatly affect the ability of these viruses to persist and remain infective. We also have demonstrated that individual viruses vary in their ability to persist in water. These evaluations have included several HPAI H5N1 viruses and results indicate that these viruses are not as environmentally fit as native low pathogenicity H5 or H7 viruses that are known to exist in wild bird populations. In other words, the HPAI H5N1 viruses that evolved in domestic birds systems have lost some of the characteristics that AIVs need to survive in wild bird populations. On a broader scale, we are further investigating other water quality factors that affect AIV transmission in aquatic habitats. With this information it should be possible to construct predictive models that delineate specific geographic areas and habitats where AIV transmission is likely to occur.

- Identification of Wild Avian Reservoirs: A great deal of species diversity exists within the broadly recognized AIV reservoirs (Anseriformes and Charadriiiformes) but the potential contribution of individual species to AIV transmission and maintenance is not well understood. Such differences, whether related to behavior or susceptibility, are important to determining when and where these viruses could come into contact with potential domestic animal and human hosts. In Georgia, for example, we know that the prevalence of these viruses in wild birds is generally very low and that some of our more common species, such as Canada geese, are rarely infected. Species susceptibility differences also may provide insight into the current HPAI H5N1 situation. In experimental infections of North American and other waterfowl species, we have demonstrated clear differences in species susceptibility. Many of the duck species that are traditionally associated with low pathogenicity AIV are unaffected by HPAI H5N1 and shed very little virus; shedding also is limited to a very short time period. Other species such as mute swans will die, and these birds can shed significant amounts of virus. Based on the overall results of these studies, it appears that most wild duck populations would not represent a viable reservoir for HPAI H5N1. On the other hand, infection of swans and some geese would result in mortality and may provide a mechanism for limited movement of these viruses; however, it is unlikely that the virus would persist under these conditions. Understanding these species differences also has application to improving surveillance and detection strategies directed at wild bird populations. For example, if the target of surveillance is HPAI H5N1, it would be wise to direct resources towards the detection of dead birds rather than the testing of normal birds. The opposite would apply to surveillance systems designed to detect the normal low pathogenicity AIV associated with ducks and gulls

Avian Influenza

- Providing Biological Material for Genetic and Phenotypic Studies: New problems generally demonstrate many deficiencies related to research capabilities. In the case of HPAI H5N1, a critical issue relates to the limited availability of field isolates of AIV and especially low path H5 isolates from wild birds. Since 1998, in collaboration with the USDA Southeast Poultry Research Laboratory in Athens, we have isolated over 600 AIV from North American birds to fill this gap. In collaboration with the University of Minnesota, we currently are providing similar resources to NIH. These viruses will provide the biological material to construct a comprehensive genetic data base and provide isolates for phenotypic characterization. These data are needed to address important questions related to such things as viral adaptation to non typical hosts and viral evolution.

New Alliances

The complexity of avian influenza natural history necessitates collaboration between a diversity of scientific disciplines and UGA is well suited to the task. Expertise in natural resources, ecology, avian medicine, virology, and environmental and public health all are represented in these collaborative efforts. This diversity also is reflected in our off campus collaborators and sponsors represented by CDC, USDA, USGS, NIH, the University of Minnesota, and numerous State Fish and Wildlife Agencies. In the end we hope to identify and understand many of the host, agent and environmental components that define the natural history of these viruses. Understanding natural history is the first step in defining domestic and human health risks associated with AIV and this information has immediate and long-term benefit to Georgia. HPAI H5N1 is not the first influenza problem we have faced, and it will not be the last.

In Memoriam

This year's Annual Report is dedicated to the memory of Dr. Barry Harmon, a friend, colleague, excellent veterinary researcher, and leader who died on January 19, 2007, following a brave struggle against prostate cancer. He will be missed.

Photo courtesy of UGA Public Affairs

Dr. Barry G. Harmon

1954 - 2007

Biomedical Science

Managing Intestinal Health: Exploratory Metagenome Analysis To Quantitatively Characterize Intestinal Bacterial Communities

"Little is known about the genomes and physiology of intestinal commensal bacteria"

Understanding the microbial ecology of the digestive tract is crucial to solving issues of digestive health, nutrition, and food safety. However little is known regarding the mechanisms of host/commensal interaction and the physiological contributions of commensal bacteria to host health. New molecular technologies enabling community genomic sampling (metagenomics) allows an evaluation of the metabolic and pathogenic capability of uncultivable intestinal bacteria. The long-term goal of our research is to identify the mechanisms involved in the interaction between bacterial communities and intestinal function. The hypothesis of this work is that the composite intestinal bacterial genomes (metagenome) of a host with enteritis should reflect the intestine's health status. A diseased intestine can result from the presence of pathogens but may also result from microflora imbalance, a state where symbiotic deficiencies occur because of the absence of essential commensal bacteria.

Metagenomics, the comparative analysis of the genetic information present in a community of organisms, is potentially a powerful tool to discern the contribution of complex bacterial communities on intestinal development and function. However the method has not yet been applied to evaluating the intestinal communities possessed by animals exhibiting symptoms of disease. Therefore, the Specific Aim of this research will focus on developing the tools for comparative metagenome analysis of intestinal bacterial communities from birds that exhibit intestinal disease. With the exception of a few species of bacteria, little is known about the genomes and physiology of intestinal commensal bacteria. Even less is known about the community physiology of these habitats. This approach is novel because it will enable detection of bacterial genomes that correlate with communities resulting from or causing symptoms of intestinal disease. At conclusion of these experiments we are likely to have acquired preliminary data describing the bacterial communities of the ilea of broiler chickens exhibiting runting/stunting disease. The characteristics of this community will be compared to that of a healthy community in order to assess the nature of the pathogenic community shifts.

PI: Dr. Margie Lee (leem@vet.uga.edu)

The Composition of the Bacteria Community Transferred From Breeders To Chickens

Recent evidence suggests that commercial chicks, at hatch, already have an intestinal microflora, which may interfere with the efficacy of probiotics. This bacterial community may be acquired as the chick embryo develops or upon immediate exposure to the hatchery environment once the chick pips and emerges from its egg. The objective of this research is to elucidate the source of the chick's intestinal microflora. The central hypothesis of this application is that the chick's earliest intestinal bacterial community is acquired from the breeder hen. This hypothesis is based on our published findings that *Campylobacter* can be present in the chick's intestine at hatch. Findings from our VMAR2007 confirmed the presence of bacterial DNA within the embryonating egg. Chicken embryos will be collected at different stages of development. A set of embryonating eggs will be chemically disinfected with bleach to remove surface bacteria and inactivate any bacterial DNA. We will subsequently screen the amnion, bleach-treated and untreated eggshells, yolk, and embryo's intestinal tract for the presence of bacteria by PCR using universal 16S rDNA primers. For PCR-positive samples, we will characterize the composition of the bacterial community by DGGE and 16S rDNA PCR libraries. In addition, DNA samples will be assessed for the presence of specific bacteria such as *Campylobacter* using species-specific PCR. Embryonic gastrointestinal tissue will be collected and evaluated using fluorescent labeled probes to detect total bacteria, enterococci, and clostridia. Upon completion of this specific aim, we expect to determine the composition of the intestinal microflora, and possible match between the intestinal microflora and the bacteria present on the eggshell surface.

PI: Dr. Margie Lee (leem@vet.uga.edu)
Graduate Student: Fransisco Pedroso

Influence of Polymicrobial Infection on Bovine Respiratory Epithelial Cell Responses

Bovine respiratory disease complex (BRDC) is a polymicrobial respiratory disease syndrome that has a high incidence of morbidity and mortality in U.S. cattle. BRDC is typically initiated by primary viral infection in young stressed cattle, which is closely followed by secondary bacterial infection. One of the major causes of viral respiratory disease in beef and dairy cattle worldwide is a Pneumovirus known as bovine respiratory syncytial virus (BRSV). This virus can cause severe and sometimes fatal disease alone, or it can act in concert with other viral and bacterial pathogens, such as *Mycoplasma bovis*.

The majority of research studying the immunology and pathogenesis of BRDC has focused on single agents. Evidence indicates that the mechanisms of microbial pathogenesis in multi-agent infections vary from single agent infections; however, little research is available addressing multi-agent respiratory infections and their influence on the response of the bovine airway cells. Therefore, two objectives are currently being studied in the laboratory.

The first objective is to demonstrate successful in vitro propagation and differentiation of bovine respiratory epithelial cells (BREC) from samples collected via bronchoscopy and bronchial brushing from 2-week-old colostrum-fed calves raised in isolation from birth. The second objective is to determine the differential responses in the BREC initially exposed to BRSV followed by infection with *Mycoplasma bovis*. The hypothesis of this research is that infection of BREC by BRSV leads to functional changes marked by alterations in chemokine production and surface molecule expression, and that these changes will modify epithelial cell responses to subsequent infection with *Mycoplasma bovis*.

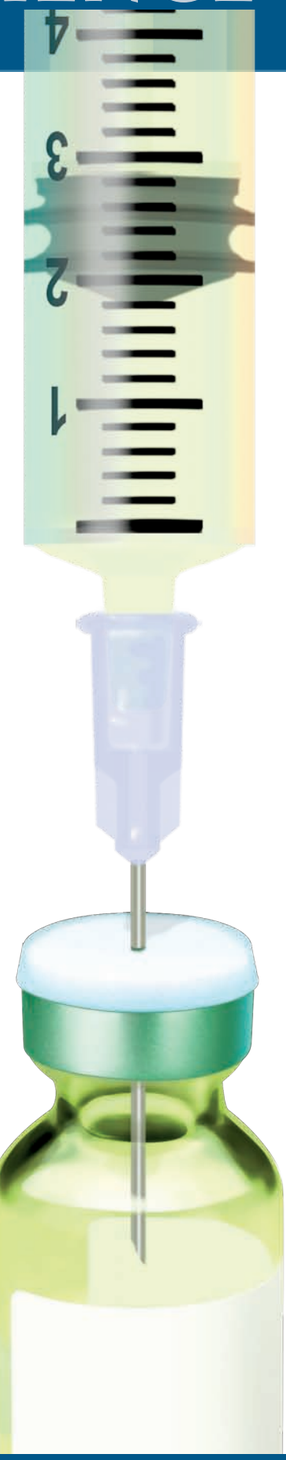
Studying the role of the airway epithelium in BRDC is very difficult in vivo; therefore, creating an in vitro culture system that enables us to dissect the response of the airway epithelium to BRSV exposure and subsequent infection with *Mycoplasma bovis* is critical in order to characterize the chemokine/adhesion molecule profile in polymicrobial disease complexes such as BRDC.

PI: Drs. Tom Krunkosky
CoPIs: Amelia Woolums and Carla Jarrett

Production of a Novel Feline Proinsulin

Diabetes is one of the most common endocrine diseases in cats. The prevalence is approximately 0.5-1% and is increasing at a rapid rate. Unfortunately, there is no early marker available to identify cats that are at risk to develop diabetes. One of the earliest markers in humans is a change in the proinsulin/insulin ratio and very specific and sensitive assays have been developed for the detection of proinsulin and insulin in people. We have cloned, expressed, and purified feline proinsulin and have developed a sensitive radioimmunoassay and ELISA for its detection in feline blood. However, there is currently no assay available that will accurately measure feline insulin. Proinsulin is the precursor of insulin. It is difficult to cleave the native feline proinsulin for production of feline insulin. We have therefore constructed a novel proinsulin which has cleavage sites that make it easier to produce feline insulin. The insulin can then be used to develop a feline specific assay or it can be used in the therapy of diabetic cats.

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



Diagnostic Science

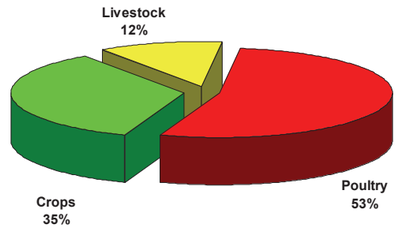
Clinical Investigations of Poultry Diseases

Two investigations of significant outbreaks of poultry diseases were performed under this project. The first involved a disease in broilers that was resulting in birds exhibiting elevated mortality and lameness. Two young broiler breeder parent flocks were identified as the only common link. Upon review of serum antibodies, it was found that these 2 breeder parent flocks had an elevation of their reovirus titers after coming into egg production. The seroconversion continued over a twelve week period. An avian reovirus was isolated from the tendons, visceral organs and intestinal contents of the broiler offspring of these 2 flocks of breeder parents. All of the virus isolates were identical to each other and were genotypically different from conventional vaccine isolates. These reovirus isolates were inoculated into SPF broilers and caused severe tenosynovitis and mortality.

The second field investigation was conducted to assist the Georgia poultry industry in understanding the sources of a *Mycoplasma gallisepticum* (MG) outbreak. Mycoplasma infections in broiler breeders are economically very costly to the poultry company resulting in declines in egg production, but more significantly, the mycoplasma is passed from the hen to the broiler offspring resulting in very high levels of mortality from secondary infections. Through the work of isolation and sequencing of highly conserved intergenic spacer region (IGSR) of the Mycoplasma DNA, we were able to determine that there were at least 3 unrelated strains of MG involved in this recent outbreak and that none of them were related to any of the commercially available live attenuated vaccines. This work gave the poultry industry the information needed to link epidemiologically related outbreaks in North Georgia.

PI: Charles Hofacre (chofacre@uga.edu)

The Largest Segment of Georgia Agriculture



2005 Farm Cash Receipts - Percent Total by Commodity

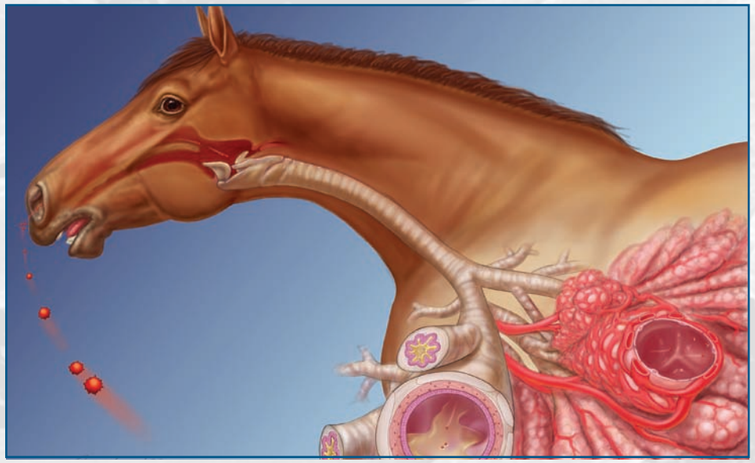
Identification and Characterization of Adhesins Expressed by Gram-negative bacterial pathogens.

Adherence to host surfaces is a key step of pathogenesis by most infectious agents as it leads to colonization. It is our hypothesis that these molecules, or fragments thereof, are good targets for vaccine development. Our laboratory studies three Gram-negative bacteria, namely *Moraxella catarrhalis*, *Burkholderia pseudomallei* and *Burkholderia mallei*. There is an urgent need to understand pathogenesis by these three organisms as well as develop vaccines. *Moraxella catarrhalis* is responsible for 15-20% of all episodes of bacterial otitis media in children under the age of three and also precipitates up to 10% of all cases of lower respiratory tract infections in patients with chronic obstructive pulmonary disease, which is the fourth leading cause of death in the United States. *Burkholderia pseudomallei* causes the human disease melioidosis and is endemic to regions bordering the equator, particularly South East Asia and Northern Australia. *Burkholderia mallei* causes glanders which primarily affects solipeds; humans are accidental hosts. Glanders and melioidosis are difficult to diagnose as well as treat and their mortality rates is unacceptably high (20-50%). *Burkholderia mallei* and *Burkholderia pseudomallei* are also particularly infectious through the aerosol route. For these reasons, these two closely related *Burkholderia* species are classified as potential agents of bioterrorism.

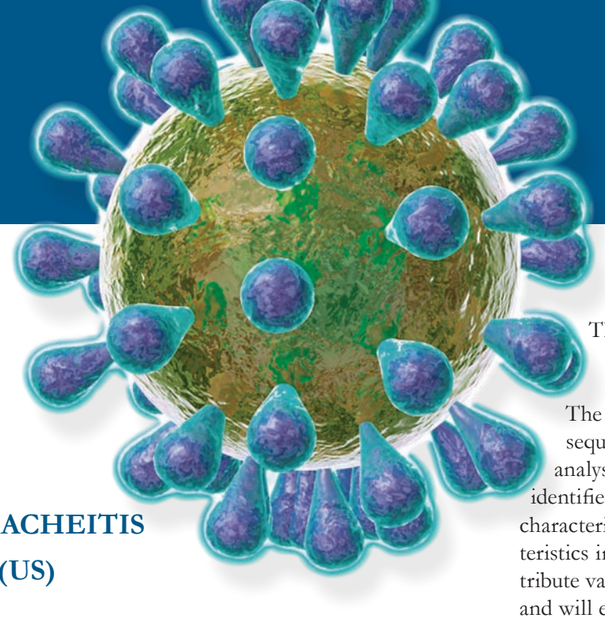
In *Moraxella catarrhalis*, we have identified the adhesins McaP, OMPCD, Hag, MhaB1 as well as MhaB2. Genetically-engineered strains lacking expression of these molecules exhibit reduced adherence to epithelial cell lines derived from the respiratory tract. These adhesins are expressed by the majority of isolates and we are in the process of identifying which portion of each protein contains the epithelial cell binding domain. Once identified, we will evaluate the vaccinogenic potential of these epithelial cell binding domains using surrogate in vitro virulence models.

In *Burkholderia pseudomallei*, we have identified the adhesins BoaA, BoaB, BoaC as well as the large surface-associated molecule BapH. We demonstrated that isogenic mutant strains lacking expression of these molecules have reduced adherence to respiratory cells. In addition, we conclusively established that BoaA, BoaB and BoaC directly mediate binding. We also discovered that *Burkholderia mallei* specify the adhesins BoaA and BoaC but not BoaB or BapH. We plan on testing whether *Burkholderia* mutants lacking expression of these adhesins exhibit reduced virulence in infection models of melioidosis and glanders. We also plan on testing the vaccinogenic potential of the *Burkholderia* adhesins in these models of infection.

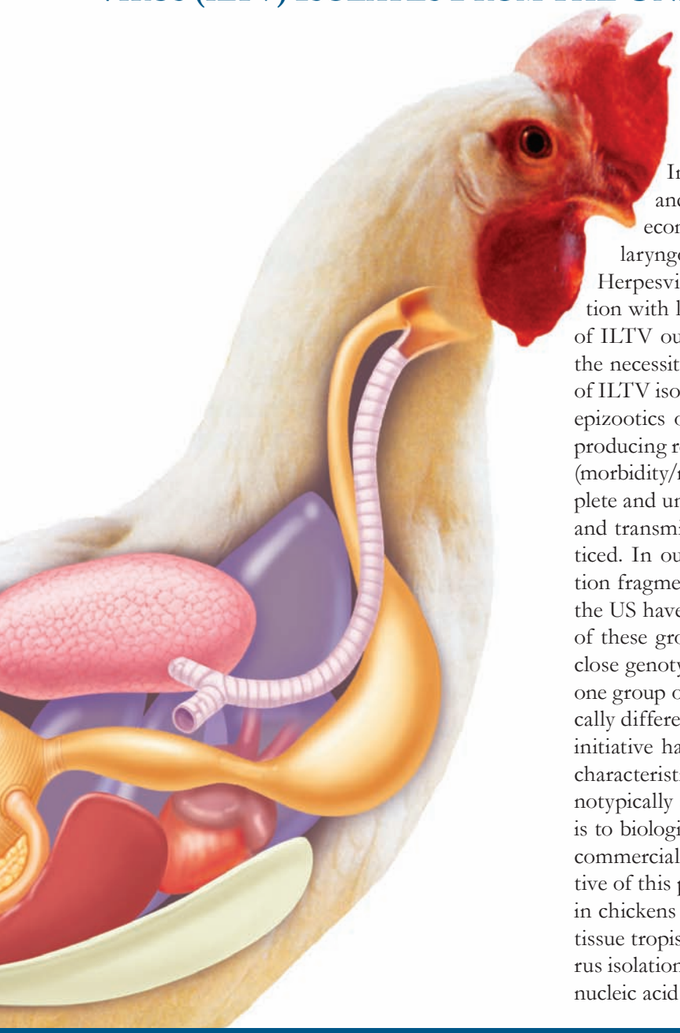
PI: Dr. Eric Lafontaine (elafon10@uga.edu)



Virology



Assessment of the Biological and Genetic Variation of Current Infectious Laryngotracheitis Virus (ILTV) Isolates From the United States (US)



Infectious laryngotracheitis (ILT) is a highly contagious and acute disease of poultry responsible for considerable economic loses to the poultry industry, caused by infectious laryngotracheitis virus (ILTV), an alpha-herpesvirus in the family Herpesviridae. Although the disease is largely controlled by vaccination with live attenuated strains of the virus, the increased incidence of ILTV outbreaks in commercial poultry in recent years emphasizes the necessity to better understand the pathogenicity and transmission of ILTV isolates currently circulating in the US. In the past three years, epizootics of the disease have been reported in all the main poultry producing regions of the US. Information from the field on the severity (morbidity/mortality) and spread of these outbreaks has been incomplete and unreliable; consequently, any differences in the pathogenicity and transmission of currently circulating ILTV isolates remain unnoticed. In our laboratory using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) ILTV isolates from the US have been classified in nine genetically different groups. Three of these groups include isolates from commercial poultry that hold a close genotype to the currently utilized live attenuated vaccines, while one group of isolates from commercial poultry was found to be genetically different to the currently utilized vaccines. The ILTV genotyping initiative has provided the framework to further study the biological characteristics (pathogenicity, tissue tropism, in vitro growth) of genotypically different ILTV isolates. The immediate goal of this study is to biologically characterize currently circulating ILTV isolates from commercial poultry as compared to the vaccine strains. The first objective of this proposal is to evaluate the pathogenicity of selected isolates in chickens by scoring clinical signs and mortality, and to evaluate the tissue tropism of current ILTV isolates and vaccine strains through virus isolation, histopathological examination, and quantification of viral nucleic acid in a diverse number of tissues.

"This research will expand the tools to better understand the origin of currently circulating viruses causing outbreaks of the disease"

The second objective is to evaluate the in vitro growth dynamics of genotypically different ILTV isolates, particularly the ability of these isolates to produce plaques in the chicken hepatoma cell line (LMH). The third objective of this proposal is to compare partial genome sequences of the different ILTV isolates and vaccine strains. Sequence analysis will target genome regions of known genetic diversity previously identified by PCR-RFLP. The long-term goal of this study is to biologically characterize current US ILTV isolates, and to correlate their biological characteristics in vitro and in vivo to their genetic diversity. This research will contribute valuable information to the future design of attenuated ILTV vaccines, and will expand the tools available for the biological and genetic characterization of ILTV isolates. A proposal including similar objectives has been submitted to the US Poultry and Egg Association (January 2007).

PI: Dr. Maricarmen Garcia (mcgarcia@uga.edu)

Pathogenesis and Virulence of a Bovine Enterovirus-1 (BEV-1) Isolate in Cattle

An initial dose-finding experiment was conducted to describe clinical signs (if any) associated with acute BEV-1 infection in calves, to characterize laboratory clinical parameter alterations (if any) associated with acute BEV-1 infection, and to characterize fecal shedding of enterovirus following challenge. Two groups of 2 male dairy calves each were inoculated intranasally with either very high (107 tissue culture infective doses (TCID-50) or high (105 TCID-50) of BEV-1 Oklahoma isolate in the form of Madin Darby bovine kidney (MDBK)-infected cell culture supernatant fluid. As a double-blinded control, each group contained an additional calf to which a placebo dose of non-infected MDBK cell culture supernatant fluid was inoculated intranasally. Calves were examined daily for signs of disease. Blood samples and fecal swabs were obtained at 7, 14, 21, and 28 days post-infection (dpi). All calves were euthanized and necropsied at 28 dpi. None of the infected calves had clinical disease. Infected calves on the very high dose group seroconverted to the virus. In each group, at least one of the infected calves shed virus through the feces. By the end of the experiment both of the placebo-inoculated calves seroconverted and one of them shed virus. There no significant gross findings observed at necropsy on any of the infected calves. In this initial experiment we conclude that the BEV-1 Oklahoma isolate is able to infect susceptible cattle and those animals are able to shed the virus through the feces. After this initial experiment, a larger experiment will follow to characterize the gross and microscopic tissue alterations (if any) associated with BEV-1 Oklahoma isolate infection, and to develop techniques, such as serum neutralization, real-time PCR, in situ hybridization, as diagnostic tests for the detection and further characterization of BEV-1.

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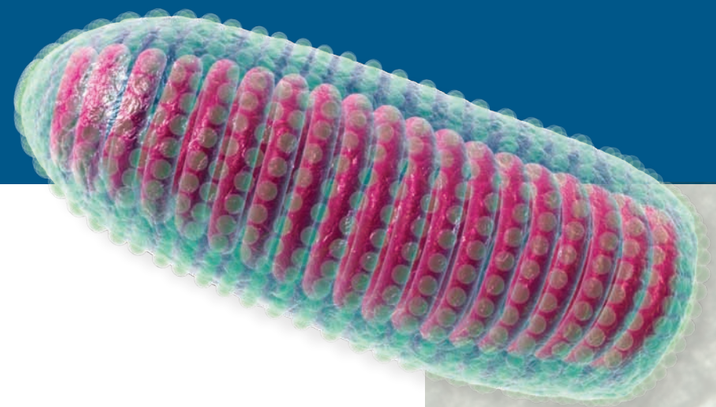

INDUCTION OF INNATE IMMUNITY AND ENHANCEMENT OF ADAPTIVE IMMUNITY FOR RECOMBINANT RABIES VIRUS VACCINES

"Our recombinant vaccines are less virulent and more immunogenic than the current armentarium"

Conventional rabies vaccines used to immunize dogs and cats are made from inactivated rabies virus (RV). Although these vaccines are safe and efficacious, multiple immunizations are required to maintain adequate immunity. Because they are inactivated vaccines, adjuvant is used, which induces unwanted side effects ranging from local skin lesions to feline sarcoma. Furthermore, these vaccines can not induce an active immune response in animals less than 3 months of age although the maternally derived antibody declined to undetectable levels. There is a period from the time of weaning of maternal antibody to the time of active immunity in which the animals may not be protected.

Live attenuated vaccines would have advantages over inactivated vaccines. They can induce long lasting immunity and requires only one injection, thus lowering the cost and associated side effects. However, such a modified live RV vaccine must be completely avirulent, particularly for humans. We have developed such vaccines by expressing immunomodulatory molecules in rabies virus genome. We have constructed recombinant rabies viruses expressing chemokine MIP-1α. The recombinant viruses were found to be completely avirulent and direct injection into the CNS did not cause any disease in mice. Vaccination of mice with such constructs induced quicker and stronger immune responses and provided better protection than parental virus against challenge with virulent rabies virus. Therefore we were able to construct recombinant vaccines that are capable of inducing innate immunity and enhancing adaptive immunity to rabies virus and reducing their virulence. Such recombinant viruses could be developed as vaccines for wildlife as well as companion animals.
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"Will *Mycoplasma gallisepticum* vaccines prevent or reduce infection?"

ABILITY OF INACTIVATED *MYCOPLASMA GALLISEPTICUM* VACCINES TO PROTECT AGAINST INFECTION

Both live and inactivated MG (*Mycoplasma gallisepticum*) vaccines are known to offer protection against clinical signs, lesions, and economic effects of infection. Studies on the ability of live MG vaccines to protect against infection or reduce populations of virulent MG strains are under way. MG bacterins have been shown to protect against clinical signs and production losses, but are not thought to offer significant protection against infection. It is not known if such bacterins will protect against infection by contact-infection or if infected birds will harbor reduced populations of virulent field strains. A fowl pox-vectored MG vaccine has been recently licensed. In the field it appears to offer some protection against infection and against severity of clinical signs, but in our hands, with severe aerosol challenge, we have not been able to demonstrate protection. Quantitative real time PCR procedures which are specific for each of the live MG strains compared with the challenge R strain have been developed. There have also been field reports of production drops and seroconversion at mid-production in flocks vaccinated with live vaccines; a combination of live vaccine + bacterin has apparently eliminated these "failures", according to testimonials of producers. The long-term goal is not only to control MG disease on such sites, but also to develop effective programs for preventing infection by wild-type strains, and eventual eradication, without the need for expensive, non-feasible depopulation efforts. The short-term goals are to determine whether inactivated MG vaccines offer demonstrable protection against contact-infections, or, if infection occurs, will a reduced population of virulent MG strain be present, thus improving chances of preventing horizontal transmission. We also wish to determine if improved protection against field challenge can be improved with a combination of live vaccine followed by bacterin.

We propose to determine if vaccination with MG bacterin or pox-vectored vaccine will prevent or delay contact infection from "seeders" challenged with virulent R strain, or if either product is capable of reducing MG populations in birds which do become infected. Quantitative real-time PCR will be used to determine presence and quantity of R strain MG in contact-challenged birds. In addition, we will evaluate the ability of live F strain vaccine followed by bacterin to prevent infection by contact challenge at the time of peak production, and again after mid-production by using quantitative real-time PCR which can specifically distinguish between F strain and the challenge R strain.

If inactivated products are able to prevent or reduce populations of virulent MG strains, vaccination programs can be designed which may allow use of the safer inactivated products instead of (or in combination with) live vaccines to prevent the inherent dangers of unintended spread of vaccine strain to neighboring flocks while offering improved resistance against field challenge.
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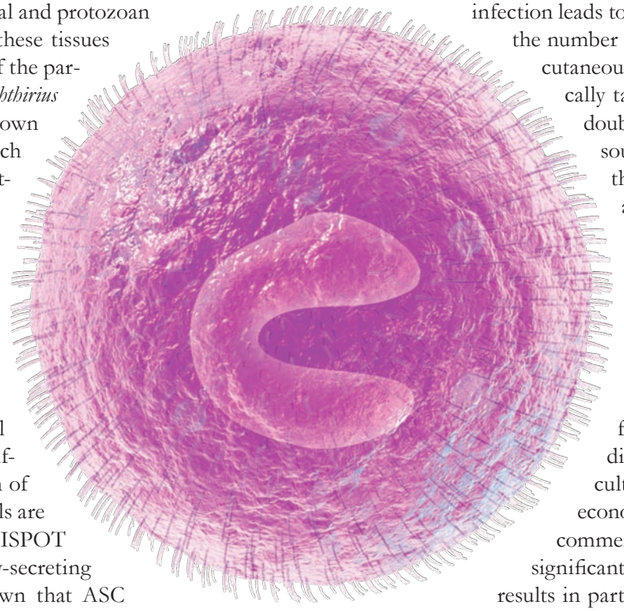
Differentiation of B Cells and Mucosal Immunity in Channel Catfish

The skin of fish serves as an anatomical and physiological barrier against the external environment, but the skin and gills also serve as the point of entry and site of infection for many bacterial and protozoan pathogens. For instance, these tissues are the sites of infection of the parasitic protozoan *Ichthyophthirius multifiliis*, commonly known as white-spot disease, which causes major, sporadic outbreaks of disease. Antibodies found in cutaneous mucus and skin play a critical role in adaptive protective immune responses against surface infections. In vitro culture of cells isolated from skin in the presence of a B cell mitogen stimulated proliferation and differentiation of B cells, showing that B cells are found in skin. Using ELISPOT assays to identify antibody-secreting cells (ASC) we have shown that ASC reside in low numbers in the skin of channel catfish, including non-replicating plasma cells. This work demonstrates that B cells and plasma cells are found in skin, as well as anterior kidney and spleen of fish.



Following immunization against the protozoan parasite *I. multifiliis,* which infects skin and gills, the number of ASC in skin increased twenty-fold, demonstrating that the number of ASC in skin is dynamic and increases in response to parasite exposure. Increased numbers of ASC in skin were detected for at least 17 weeks after exposure, showing that infection leads to long-term stable changes in the number of B cells in the skin. These cutaneous ASC include those specifically targeting *I. multifiliis*, and undoubtedly serve as the primary source of cutaneous antibodies that confer long-term humoral immunity against reinfection. Our results suggest that skin functions as an essential component of the teleost immune system.

Vaccination represents the most efficient method for preventing outbreaks of disease in commercial aquaculture that cause significant economic losses. The lack of commercial vaccines against many significant pathogens of fish, however, results in part from an incomplete understanding of the basic immunology of fish. Our work addresses fundamental questions on development and persistence of protective immunity in fish using a natural infection model. We expect that the results of this research will lead to more effective strategies for vaccine development.

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Co-PIs: Drs. H. Dickerson, J. Noe, Dr. X. Zhao

Generation of Monoclonal Antibodies Directed Against Antigenic Subtypes of IBDV

For IBDV (Infectious Bursal Disease Virus) several antigenic subtypes have been described (e.g. Al-2, E/Del, GLS, classical IBDV). They can be dantibodies (anti-D antibodies) directed against the single IBDV capsid protein VP2. Such mAb (monoclonal antibodies) were described and are available on the basis of Material Transfer Agreements for research purposes or by licensing for commercial use. Based on anti-VP2 mAb diagnostic tests can be developed and subsequently used in routine diagnostics. Due to the constant antigenic drift of field strains, currently used vaccines will fail in the future to provide protection. To discern this antigenic drift in advance tools have to be developed. To this end mAb will be established which are directed against existing antigenic subtypes of IBDV. Mice will be immunized with purified virus preparations and hybridomas will be generated. Hybridoma supernatants will be screened by ELISA (Enzyme-Linked ImmunoSorbent Assay), followed by characterization with classical and molecular virological methods (Western blot, immunoprecipitaion, virus neutralization assay, immunofluorescence). In parallel, a mixture of the above mentioned purified virus preparations will be used for immunization of rabbits to obtain anti-IBDV serum. Both reagents will be unified for the establishment of an antigen-capture ELISA for diagnostic purposes. This ELISA can be used for antigenic characterization of field isolates obtained in Georgia (later US-wide) and recommendations for the use of vaccines can be made. This will be combined with the genetic characterization of the particular viruses using reverse genetics.

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Use of Retroviral Vectors For in vitro Expression of Immunogenic Proteins of Chicken Infectious Anemia Virus (CIAV)

Chicken infectious anemia virus (CIAV or CAV) is a widely distributed immunosuppressive Circovirus that causes significant economic losses to the broiler industry. Protection of newly hatched chicks vs. CIAV relies exclusively on maternal antibodies. Thus, parent stock must be exposed to CIAV naturally or by vaccination with live virus vaccines to provide progeny protection. Killed CIAV vaccines are currently not available partially due to the difficulty of attaining high virus titers in fastidious nonadherent cell cultures. Despite availability of commercial live CIAV vaccines and regardless of the exposure method in breeders, active and passive CIAV antibody titers are usually low and poorly protective. Thus, development and production of immunogens containing a high concentration of adjuvanted CIAV proteins would be desirable. Our primary research objective is to demonstrate successful in vitro expression of immunogenic proteins of CIAV using chimeric infectious clones of avian retroviruses containing genes encoding immunogenic proteins of CIAV. Our first objective will involve developing chimeric retroviral infectious clones containing the VP1, VP2 and VP1+VP2 coding genes of CIAV. This objective will be followed by transfection of cells with the chimeric infectious clones and expression of the targeted CIAV proteins in vitro.

Detection of such proteins will be accomplished using CIAV polyclonal antibodies for indirect immunofluorescence.

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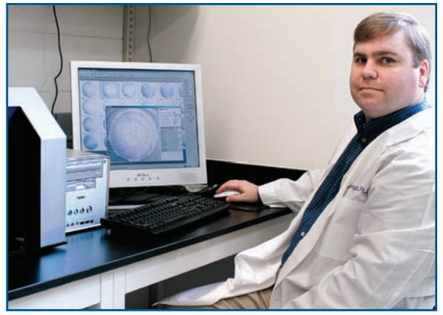
DEVELOPMENT OF NOVEL ANTIVIRAL DRUGS: INHIBITION OF AVIAN INFLUENZA BY RNAi

"If HPAI were to emerge as even a moderate pandemic strain, more than 200,000 persons could die in the United States alone"

Highly pathogenic avian influenza (HPAI) is a major threat to the United States poultry industry. In 1983, a major outbreak of HPAI in the U.S. caused the destruction of more than 17 million birds at a cost of $65 million. More than 16 outbreaks of potential HPAI strains have occurred in the U.S. since 1997. Quarantine and depopulation are the preferred means for control of outbreaks and new methods for protection of poultry flocks are needed. Moreover, several instances of human infection with HPAI have been reported, most likely caused by contact with infected poultry. If HPAI were to emerge as even a moderate pandemic strain, more that 200,000 persons could die in the United States alone and the economic cost could exceed $100 billion. Currently, there is not a vaccine available and antiviral drugs are partially effective.



RNA interference (RNAi) is an emerging technology that can specifically inhibit gene expression both in vitro and in vivo. Gene silencing is the result of sequence-specific RNA degradation and is mediated by short, 21-26 nucleotide interfering RNAs (siRNAs). A number of studies have demonstrated inhibition of replication of viruses in cell culture by RNAi. Using an established murine model of influenza virus infection, we have demonstrated that in vivo treatment with virus-specific siRNAs can effectively suppress influenza virus replication and protect animals from an otherwise lethal infection.

We have screened more than 100 candidate siRNAs for efficacy in inhibiting influenza virus replication and have identified a handful of candidates that silence a variety of gene targets, including the nucleoprotein, matrix, and polymerase genes of influenza. These siRNAs target highly conserved regions of the genome and should inhibit most influenza viruses, including low pathogenic and highly pathogenic avian influenza viruses. We found that the candidate siRNAs identified in the primary screen inhibited all influenza viruses tested, including a spectrum of avian influenza isolates. Future studies focus on development of respiratory delivery systems for nucleic acids and, pending approval, testing against highly pathogenic avian influenza viruses.

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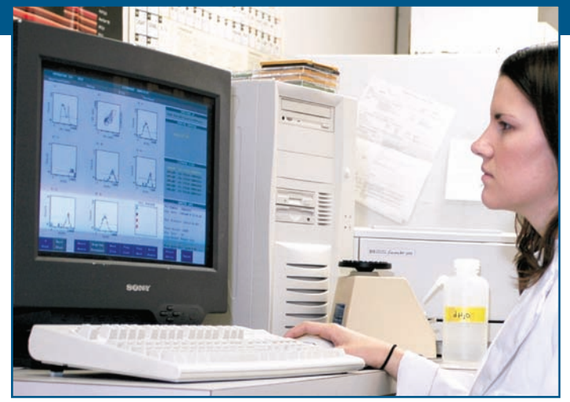

ZEBRAFISH COELOMIC EXUDATE CELLS: A MODEL TISSUE OF INNATE IMMUNITY

Use of the zebrafish (zf) as a model animal to study immune responses to infectious diseases is severely restricted due to the inability to obtain cells in sufficient quantities to determine immune functions. As a consequence the present study was designed based on the hypothesis that the zf coelomic cavity is an immune responsive tissue, and as such may provide quantities of immune competent cells to carry-out in vivo studies of infectious diseases. To accomplish this, a model was established to characterize the cellular composition of the coelomic exudate (CE) of zf; to determine whether cells in the coelomic cavity could be activated with LPS; and finally to establish a function of these cells using an assay for cell mediated cytotoxicity. First, a new colony of inbred WIK zebrafish was established by brother-sister mating. Using 4-8 month F1 generation inbred female progeny, in vivo experiments were initiated to determine the cellular responses of CE cells to intraperitoneal injections of different concentrations of LPS. Zebrafish were injected (IP) with LPS and cells were gavaged from the peritoneum at different time points post-injection. Intraperitoneal injection of 10ug LPS/zebrafish followed by cellular harvesting revealed increased cellular infiltrate into the coelomic cavity at 1-2h post-injection. Mobilized cells consisted of neutrophils and lumphocytes. Cells in the CE were also examined by Wrights stain and by flow cytometry (monoclonal antibody phenotyping). Cytotoxicity experiments were conducted using CE cells and HL-60 target cells. Maximum target cell death (86%) occurred at a 4:1 effector:target cell ratio after 4h coincubation. Data in the present study demonstrated that zf coelomic exudate cells can be activated (in vivo) with LPS and formalin killed bacteria and as such may provide sufficient quantities of leukocytes for use in in vitro cellular assays of immune functions.

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Trim, Cynthia, Professor, Large Animal Medicine, (706) 542-6318
Tripp, Ralph, Professor and Eminent Scholar, Infectious Diseases, (706) 542-1557
Uhl, Elizabeth, Asst. Professor, Pathology, (706) 583-0475
Vandenplas, Michel, Assoc. Research Scientist, (706) 542-6389
Varela-Stokes, Andrea, Asst. Research Scientist, Infectious Diseases, (706) 542-0195
Villegas, Pedro, Professor, Population Health, (706) 542-5085
Wagner, John, Assoc. Professor, Physiology and Pharmacology, (706) 542-3014
Ward, Cynthia, Assoc. Professor, Small Animal Medicine, (706) 542-6380
White, Susan, Professor, Large Animal Medicine, (706) 542-9323
Williams, Jamie, Clinical Professor, Anatomy and Radiology, (706) 542-8342
Williams, Susan, Asst. Professor, Population Health, (706) 583-0523
Williamson, Lisa, Assoc. Professor, Large Animal Medicine, (706) 542-9323
Woldemeskel, Moges, Asst. Professor, Tifton Diagnostic Lab., (229) 386-3340
Woolums, Amelia, Assoc. Professor, Large Animal Medicine, (706) 542-9329
Yabsley, Michael, Asst. Professor, Wildlife Disease Study, (706) 542-5702

Research Contracts and Grants

Allen, Sheila. *MSP/Student experiential learning: Providing opportunities for to-be-recruited USDA multicultural scholars to acquire public health training.* USDA-CSREES. $48,000.00
Allen, Sheila. *Core Animal Diagnostic Laboratory: NAHLN: GA.* USDA-CSREES. $300,000.00
Allen, Sheila. *Section 1433 Animal Health and Disease Research Funds FY2007.* USDA-CSREES. $114,583.00
Allen, Sheila. *Eidson Chair.* Georgia Research Alliance. $58,968.00
Baldwin, Charles. *Diagnostic services relative to the control, diagnosis, treatment prevention, and eradication of livestock diseases 2004.* Ga. Dept. of Agriculture. $2,160,738.00
Baldwin, Charles. *Classical swine fever surveillance.* USDA. $28,226.00
Barton, Michelle. *Relative adrenal insufficiency in critically ill neonatal foals.* American College of Veterinary Internal Medicine Foundation. $12,573.00
Barton, Michelle. *Evaluation of a new assay for the detection of endotoxin in horses with colic and foals with septicemia.* Morris Animal Foundation. $4,000.00
Berghaus, Roy. *Predicting* Salmonella *loads on broiler carcasses from pre-harvest* Salmonella *data collected in broiler houses.* North Carolina State Univ. $438,075.00
Blas-Machado, Uriel. *Pathogenesis and virulence of a bovine enterovirus-1 isolate in cattle - year 2.* USDA-CSREES. $15,000.00
Brown, Corrie. *Characterization of cells in early response to infection with vesicular stomatitis virus.* USDA-ARS. $10,000.00
Brown, Corrie. *Acquisition of goods and services; investigations into antimicrobial sensitivity.* USDA-ARS. $287.00
Brown, Corrie. *Acquisition of goods and services; investigations into antimicrobial sensitivity.* USDA-ARS. $22,624.00
Brown, Corrie. *Acquisition of goods and services; investigations into antimicrobial sensitivity.* USDA-ARS. $5,749.00
Brown, Corrie. *Detection of key factors in the development of Fibropapillomas in sea turtles; Puerto Rico.* Morris Animal Foundation. $43,200.00
Brown, Corrie. *Workshops on animal necropsy and diagnostics in collaboration with the Ministry of Agriculture.* USDA-FAS Foreign Ag. Serv. $10,322.00
Brown, Corrie. *Pathogenesis of selected Newcastle disease virus field isolates and recombinants.* USDA-ARS. $83,507.00
Brown, Corrie. *Necropsy training courses and seminar, Afghanistan.* USDA-FAS Foreign Ag. Serv. $15,401.00
Brown, Corrie. *Development of necropsy kits for pathology program in Afghanistan.* USDA-FAS Foreign Ag. Serv. $5,421.00
Brown, Scott. *A study to evaluate a nutraceutical product in cats. Ipakitine efficacy in feline renal insufficiency.* Vetoquinol USA. $41,027.00
Budsberg, Steven. *In vitro assessment of activity of N-butyryl glucosamine known as ANABUTM in modulating monocyte and neutrophil activation in dogs and horses.* Farnam Company, Inc. $53,096.00
Budsberg, Steven. *A feasibility study to investigate the efficacy of a selective glucocorticoid receptor modulator administered orally in a sodium-urage induced arthritis model with force plate analysis in dogs.* Boehringer Ingleheim. $150,875.00
Budsberg, Steven. *Assessment of the effects of nonsteroidal anti-inflammatory drugs on gastric healing in a gastric mucosal injury model.* Schering-Plough Animal Health. $165,020.00
Carmichael, Paige. *Training in clinical ophthalmology and pathology. Graduate Assistantship support for Dr. Shannon Bovelan.* Tuskegee Univ. $28,675.00
Coffield, Julie. *Neuromuscular targets of botulinum toxin.* NIH-National Institutes of Health. $341,385.00
Corn, Joseph. *Exotic arthropod surveillance in the Southeastern United States and Puerto Rico.* USDA. $200,000.00
Corn, Joseph. *Distribution of Pseudorabies virus and* Brucella suis *in feral swine.* USDA-APHIS. $103,092.00
Dickerson, Harry. *The University of Georgia 2007 Veterinary Scholars Program: A research training experience for veterinary students.* Merck-Merial Animal Health Grants Program. $20,000.00
Fischer, John. *Southeastern Cooperative Wildlife Disease Study: TN.* Fish and Wildlife Agencies. $21,080.00
Fischer, John. *Southeastern Cooperative Wildlife Disease Study: AK, VA, KY, MD, MS, MO, NC, OH, SC.* Fish and Wildlife Agencies. $200,560.00
Fischer, John. *Southeastern Cooperative Wildlife Disease Study: AL, FL, GA, WVA.* Fish and Wildlife Agencies. $171,635.00
Fischer, John. *Wildlife Services Disease Training.* USDA-APHIS. $150,000.00
Fischer, John. *Cooperative. Ag. for developing and evaluation of data relative to disease relationships that may simultaneously involve wildlife, domestic livestock and poultry.* USDA-APHIS. $350,000.00
Fischer, John. *Southeastern Cooperative Wildlife Disease Study: K.* Fish and Wildlife Agencies. $15,000.00
Fischer, John. *Southeastern Cooperative Wildlife Disease Study: PR.* Fish and Wildlife Agencies. $17,500.00
Garcia, Maricarmen. *Developing avian influenza serological tests for differentiating infected from vaccinated animals (DIVA).* Univ. of Maryland. $90,160.00
Glisson, John. *Whitefox farms.* Whitefox Farms. $150,000.00
Hensel, Patrick. *Pivotal multicentric randomized, double-blind, placebo-controlled field study of AB110 in the treatment of atopic dermatitis in dogs.* AB Science. $15,844.00
Hodge, Thomas. *Identification of host genes required for viral pathogenesis, II.* Hudson-Alpha Institute for Biotechnology. $287,500.00
Hodge, Thomas. *Determination of mechanism of action for Adam 10 and Erk 1/2 pathway in viral pathogenesis.* Avitar, Inc. $150,000.00
Hoenig, Margarethe. *Nuclear magnetic resonance, a noninvasive method to evaluate glucose and fat metabolism in the cat.* Nestle Purina, Inc. $20,250.00
Hoenig, Margarethe. *Mechanisms of insulin resistance in cats.* Nestle Purina Co. $258,030.00
Hogan, Robert J. *Production of a filovirus glycoprotein subunit vaccine component.* Western Illinois Univ. $3,688.00
Hogan, Robert J. *In vivo efficacy of novel compounds against poxvirus infection I: Optimization of viral dose.* Acute Viral Research. $25,426.00
Hogan, Robert J. *The influences of N- and O-linked glycosylation on the immunogenicity of the Ebola virus glycoprotein.* Southern Research Institute. $311,322.00
Hondalus, Mary K. *Needle-free vaccination via nanoparticle aerosols.* Harvard University. $10,353.00
Hondalus, Mary K. *Virulence of the opportunistic pathogen* Rhodococcus equi. NIH-National Institutes of Health. $264,960.00
Hurley, David. *Assessment of cell mediated and antibody mediated cytotoxicity activity induced by a DNA vaccine in dogs against tyrosinase.* Merial Limited. $60,000.00
Hurley, David. *In vitro models of cellular level processes in the early pathogenesis of black walnut induced laminitis.* American Quarter Horse Assoc. $50,890.00
Hurley, David. *Bovine clostridial transdermal: Safety and serologic response of a combination commercial bovine clostridial vaccine administered to naïve calves via the transdermal route.* Merial Limited. $41,862.00
Jackwood, Mark. *Testing Replikins' peptides in an avian vaccine challenge study using IBV.* Replikins Ltd. $12,393.00
Jackwood, Mark. *Testing Replikins' peptides in an avian vaccine challenge study using IBV.* Replikins Ltd. $29,757.00
Jackwood, Mark. *Genome sequencing and comparative analysis of coronaviruses.* NSF-National Science Foundation. $575,906.00
Jackwood, Mark. *Biological testing of low pathogenicity avian influenza virus in domestic avian spp and on farm biosecurity risks assoc with spread of avian influenza.* USDA-ARS. $410,000.00
Kaplan, Ray. Furnish Brugia malayi *adult worms and/or B. malayi infective larvae.* NIH-National Institutes of Health. $144,361.00
Kaplan, Ray. *Transfection and promoter analysis of* Brugia malayi. Univ. of Alabama. $20,000.00
Karls, Russell. *Role of* M. avium *ssp.* paratuberculosis *(MAP) as an immune modulator in the development of chronic enteropathy.* U.S. Dept. of Health and Human Services. $31,574.00
Keel, Kevin. *Disease training of wildlife biologists.* Association of Wildlife Agencies. $51,762.00
Kleven, Stanley. *Further evaluation of a potential live* Mycoplasma gallisepticum *vaccine.* Ghen Corporation. $87,331.00
Krunkosky, Thomas. *Acute inflammatory changes in skin and laminar tissue during the onset of acute laminitis.* American Quarter Horse Assoc. $47,979.00
Lafontaine, Eric. *Identification of* B. pseudomallei *and* B. mallei *adhesins.* NIH-NIAID. $288,068.00
Lafontaine, Eric. *Adherence mechanisms of* Moraxella catarrhalis. NIH-NIAID. $172,841.00
Mead, Daniel. *The role of insect vector transmission in the pathogenesis of vesicular stomatitis virus.* USDA-ARS. $40,000.00
Mead, Daniel. *Vector-borne disease surveillance - wild bird and mosquito diagnostic support.* Ga. Dept. of Human Resources. $61,012.00
Miller, Debra Lee. *Investigating blood mercury and selenium levels and protein electrophoresis in nesting female leatherback sea turtles and relationship to nest success.* Florida Sea Turtle Grants Program. $17,168.00
Miller, Doris. *BSE Surveillance – Maintenance.* USDA-APHIS $54,632.00
Miller, Doris. *BSE Surveillance.* USDA-APHIS. $25,728.00
Miller, Doris. *Athens Diagnostic Laboratory.* GA Dept. of Agriculture. $1,324,323.00
Miller, Doris. *BSE Surveillance – Maintenance.* USDA-APHIS. $30,013.00
Miller, Doris. *BSE Surveillance.* USDA-APHIS. $19,262.00
Miller, Doris. *BSE Surveillance.* USDA-APHIS. $22,735.00
Moore, James. *Elucidating structure-function relationships of lipid A: A synthetic approach.* NIH-National Institutes of Health. $109,921.00
Moore, James. *Anti-inflammatory effects of adensine A2A receptor agonists in horses.* Adenosine Therapeutics, LLC. $74,000.00
Moore, James. *Equine microarray development.* Univ. of Kentucky Res. Foundation. $18,000.00
Moore, James. *Anti-inflammatory effects of adensine A2A receptor agonists in horses.* Adenosine Therapeutics, LLC. $29,136.00
Moore, Julie. *Immunopathogenesis of severe malaria during pregnancy.* NIH-National Institutes of Health. $286,206.00
Mundt, Egbert. *Evaluation of Paramyxoviruses as a possible vector platform.* Merial Limited. $150,000.00
Mundt, Egbert. *Development of a species-independent ELISA for the detection of influenza A HA5 and HA7 antibodies.* University of Maryland. $79,375.00
Pence, Melvin. *Georgia Johne's Disease Demonstration Herd Project.* USDA. $36,100.00
Pence, Melvin. *Georgia Johne's Disease Demonstration Herd Project.* USDA. $13,285.00
Reeves, David. *Manage Rogers State Prison Dairy and Swine Farms.* GA Dept. of Corrections. $419,420.00
Ritchie, Branson. *Research Associate In Exotic/Zoo Infectious Disease and Pathology. Postgraduate program.* Zoo Atlanta/Riverbanks Zoo. $10,000.00
Robertson, Thomas. *Leukocyte and vascular function in endotoxemia and laminitis.* Grayson Jockey Club Research Foundation. $29,852.00
Robertson, Thomas. *Signal transduction pathways underlying laminar microvascular dysfunction in the early stages of acute laminitis.* USDA-NRI. $324,024.00
Saliki, Jeremiah T. *Current molecular epidemiology of canine parvovirus in the United States.* Merial Limited. $85,160.00
Saliki, Jeremiah T. *Editorial support for the Journal of Veterinary Diagnostic Investigation.* American Association of Veterinary Laboratory Diagnosticians. $93,750.00
Saliki, Jeremiah T. *Editorial support for the Journal of Veterinary Diagnostic Investigation.* American Association of Veterinary Laboratory Diagnosticians. $23,250.00
Sanchez, Susan. *Georgia Veterinary Scholars Summer Research Program.* NIH-National Institutes of Health. $39,483.00
Sellers, Holly. *Identification and charac. Of novel astro- and reoviruses assoc. with runting stunting syndrome and the develop of reagents for diagnostic detection.* U.S. Poultry and Egg Assoc. $35,190.00
Stallknecht, David. *HPAI wild birds: Potential for a new wildlife disease or dead end?* Morris Animal Foundation. $7,900.00
Stallknecht, David. *Avian influenza viruses in the environment: What is the probability of human contact and transmission?* Centers for Disease Control. $663,801.00
Stallknecht, David. *Role of aquatic environments in avian influenza virus persistence and subtype diversity.* Morris Animal Foundation. $30,787.00
Stallknecht, David. *Determination of the frequency, pathology and environmental persistence of avian influenza viruses of wild birds.* USDA-ARS. $280,000.00
Stallknecht, David. *Avian influenza viruses in the environment: What is the probability of human contact and transmission?* Centers for Disease Control. $211,165.00
Torres-Velez, Fernando. *Pathogenesis of Nipah virus in guinea pigs.* NIH-National Institutes of Health. $110,819.00
Tripp, Ralph. *siRNA intervention and delivery strategies for RSV infection.* Alnylam Pharmaceuticals. $74,641.00
Tripp, Ralph. *Antibody inhibition of respiratory syncytial virus G protein activity.* NIH-National Institutes of Health. $268,950.00
Tripp, Ralph. *Nanostructured ramen sensors for virus detections.* U.S. Army. $900,000.00
Tripp, Ralph. *NIAID Centers of excellence for influenza research and surveillance.* NIAID-National Institute of Allergy and Infectious Diseases $1,387,811.00
Tripp, Ralph. *Vaccination and therapeutic drug treatment approaches for PRRSV, SIV, and PCV2.* Murphy Brown LLC. $186,038.00
Tripp, Ralph. *Impactor testing.* Creare. $54,325.00
Tripp, Ralph. Antibody inhibition of respiratory syncytial virus G protein activity. NIH-National Institutes of Health. *$67,238.00*
Uhl, Elizabeth. *Characterization of feline immune responses to recombinant DNA vaccines against avian H5N1 influenza virus.* Winn Feline Foundation. $15,000.00
Wagner, John J. *Cocaine-induced metaplasticity in the hippocampus.* NIDA-National Inst. Drug Abuse. $139,572.00
Wagner, John J. *Cocaine-induced metaplasticity in the hippocampus.* NIDA-National Inst. Drug Abuse. $83,300.00
Ward, Cynthia R. *Environmental influences on signal transduction abnormalities in feline hyperthyroidism.* Morris Animal Foundation. $14,976.00
Williamson, Lisa. *Prevalence of anthelmintic resistant gastrointestinal nematodes in camelids.* Morris Animal Foundation. $44,495.00
Yabsley, Michael. *Natural history of* Borrelia lonestari. Oklahoma State Univ. $110,550.00

Selected Publications

Abalti, A., Bekana, M., Woldemeskel, M., and F. Lobago. Female genital tract abnormalities of zebu cattle slaughtered at Bahr-Dar town North-West Ethiopia. Trop. Anim. Health Prod., 38:505–510, 2006.

Akimana, C., and E. R. Lafontaine. The Moraxella catarrhalis outer membrane protein CD contains two distinct domains specifying adherence to human lung cells. FEMS Microbiol. Letters, 271:12-19, 2007.

Alvarado, I., Villegas, P., El-Attrache, J., and M. W. Jackwood. Detection of Massachusetts and Arkansas serotypes of Infectious Bronchitis Virus in Broilers. Avian Dis., 50:292-297, 2006.

Alvarado, I., Villegas, P., El-Attrache, J., Jensen, E., Rosales, G., Perozo, F., and L. B. Purvis. Genetic characterization, pathogenicity and protection studies with an Avian Adenovirus isolate associated with Inclusion Body Hepatitis. Avian Dis., In Press.

Alward, A., Corriher, C.A., Barton, M.H., Sellon, D.C., Blikslager A.T., and S.L. Jones. Red maple (*Acer rubrum*) leaf toxicosis in horses: a retrospective study of 32 cases. J. Vet. Intern. Med., 20:1197-1201, 2006.

Balder, R., Hassel, J., Lipski, S. and E. R. Lafontaine. The *Moraxella catarrhalis* strain O35E expresses two filamentous hemagglutinin-like proteins that mediate adherence to human epithelial cells. Infect. Immunol., 75:2765-2775, 2007.

Banet-Noach, C., Panshin A., Golender, N. Simanov, L., Rozenblut, E., Pokamunski, S., Pirak, M., Tendler, Y., García, M., Gelman, B., Pasternak, R., and S. Perk. Genetic analysis of nonstructural genes (NS1 and NS2) of H9N2 and H5N1 viruses recently isolated in Israel. Virus Genes, In Press.

Barbosa, T., Zavala, G., Cheng, S., and P. Villegas. Full genome sequence and some biological properties of reticuloendotheliosis virus strain APC-566 isolated from endangered Attwater's prairie chickens. Virus Research (Epub ahead of print), 94313:1-10, 2006.

Barbosa, T., Zavala, G., Cheng, S., and P. Villegas. Pathogenicity and transmission of Reticuloendotheliosis Virus strain APC-566 in Japanese Quail. Avian Dis., In Press.

Barbosa, T., Zavala, G., Cheng, S., Lourenco, T., and P. Villegas. Effects of Reticuloendotheliosis Virus on the viability and reproductive performance of Japanese quail. J. Appl. Poult. Res., In Press.

Barton, M. H., and B. E. LeRoy. Serum bile acids concentrations in healthy and clinically ill neonatal foals. J. Vet. Int. Med., 21(3): 508-513, 2007.

Barton, M. H. Colic in the newborn foal. In *Equine Neonatal Medicine*, First edition. Paradis MR, ed., Elsevier Saunders, Philadelphia, PA. pp 191-207, 2006.

Barton, M. H. Disseminated intravascular coagulation. In *Equine Emergency and Critical Care Medicine*, Southwood L and Wilkens P., eds., Manson Publishing, London, UK, Accepted.

Barton, M. H. Endotoxemia. In *Equine Emergency and Critical Care Medicine*. Southwood L and Wilkens P, eds., Manson Publishing, London, UK, Accepted.

Barton, M. H., Hurley, D., Norton, N., Heusner, G., Costa, L., Jones, S., Byars, D., and K. Watanabe. Serum lactoferrin and immunoglobulin G concentrations in healthy or ill neonatal foals and healthy adult horses. J. Vet. Int. Med., 20(6): 1457-1462, 2006.

Barton, M. H. Liver disease in the horse: Clinical signs and diagnostic aides. DVM: The Newsmagazine of Veterinary Medicine, 2007.

Barton, M. H. Multiple organ dysfunction syndrome. In *Equine Emergency and Critical Care Medicine*, Southwood L and Wilkens P., eds., Manson Publishing, London, UK, Accepted.

Barton, M. H. Septicemia. In *Equine Neonatal Medicine*, First edition. Paradis MR, ed., Elsevier Saunders, Philadelphia, PA. pp 75-97, 2006.

Berghaus, L. J., Corbeil, L. B., Berghaus, R. D., Kalina, W. V., Kimball, R. A., and L. J. Gershwin. Effects of dual vaccination for bovine respiratory syncytial virus and *Haemophilus somnus* on immune responses. Vaccine, 24(33-34):6018-6027, 2006.

Berghaus, R. D., Farver, T. B., Anderson, R. J., Adaska, J. M., and I. A. Gardner. Use of age and milk production data to improve the ability of enzyme-linked immunosorbent assay test results to predict *Mycobacterium avium* ssp. *paratuberculosis* fecal culture status. J. Vet. Diagn. Invest., 18:233-242, 2006.

Biffa, D., Debela, E., Beyene, F., and M. Woldemeskel. Factors associated with bovine udder infections in small holder dairy farms in Ethiopia. Bull. Anim. Hlth. Prod. Africa, 53:258-265, 2005.

Birkness, K. A., Guarner, J., Sable, S. B., Tripp, R. A., Kellar, K. L., Bartlett, J., and F. D. Quinn. An in vitro model of the leukocyte interactions associated with granuloma formation in *M. tuberculosis* infection. Immunol. Cell Biol., 85:160-168, 2006.

Boutureira, J., Trim, C. M., and K. K. Cornell. Acute pulmonary edema after diazepam-ketamine in a dog. Vet. Anaesth. Analg., online, 2007.

Braun, C., Hofmeister, E. H., Lockwood, A. A., and S. L. Parfitt. Effects of diazepam or lidocaine premedication on propofol induction and cardiovascular parameters in dogs. J. Amer. Anim. Hosp. Assoc., 43:8-13, 2007.

Braun, C., Rahn B., Fulmer, M., Steiner, A., and A. Gisep. Intra-articular calcium-phosphate cement: its fate and impact on joint tissues in a rabbit model. J. Biomed. Mater. Res., Part B: Appl. Biomater., 79B:151-158, 2006.

Brdecka, D., Rawlings, C., Howerth, E. W., Cornell, K., and K. Stiffler. A histopathologic comparison of two techniques for soft palate resection in normal dogs. J. Amer. Anim. Hosp. Assoc., 43:39-44, 2007.

Brown, C. C. Avian influenza: Virchow's reminder (commentary). Amer. J. Pathol., 168:6-8, 2006.

Brown, C. C. Risks from emerging animal diseases. Veterinaria Italiana. 42(4):305-317, 2006.

Brown, C. C., Vroegindewey, G., Thompson, S., and M. Pappaioanou. The global veterinarian: The who? The what? The how? J. Vet. Med. Educat., 33:411-415, 2006.

Brown, J. D., Keel, M. K., Yabsley, M. J., Thigpen, T., and J. C. Maerz. Clinical challenge. Skin, moderate, chronic, multifocal, histiocytic dermatitis with intralesional trombiculid mites (*Hannemania* sp.). J. Zoo Wild. Med., 37(4):571-573, 2006.

Brown, J. D., Stallknecht, D. E., Beck, J. R., Suarez, D. L., and D. E. Swayne. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerg. Inf. Dis., 12(11):1663-1670, 2006.

Bryant, C. E., Ouellette, A., Lohmann, K., Vandenplas, M., Moore, J. N., Maskell, D. J., and B. A. Farnfield. The cellular Toll-like receptor 4 antagonist E5531 can act as an agonist in horse whole blood. Vet. Immunol. Immunopathol., 116(3-4):182-189, 2007.

Bullard, B., Lipski, S. and E. R. Lafontaine. Regions important for the adhesin activity of Moraxella catarrhalis Hag. BMC Microbiology, 7:65 doi:10.1186/1471-2180-7-65, 2007.

Burke, J. M., Kaplan, R. M., Miller, J. E., Terrill, T. H., Getz, W. R., Mobini, V. S. E., Williams, M. J., Williamson, L. H., and A. F. Vatta. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. Vet. Parasitol., 147:89-95, 2007.

Burton, E. C., Miller, D. L., Styer, E. L., and M. J. Gray. Amphibian ocular malformation due to frog virus 3. Vet. J., In Press.

Callison, S. A., Hilt, D. A., Boynton, T. O., Sample, B. F., Robison, R., Swayne, D. E, and M. W. Jackwood. Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. J. Virol. Meth., 138:60-65, 2006.

Callison, S. A., Riblet, S. M., Sun, S., Ikuta, N., Hilt, D., Leiting, V., Kleven, S. H., Suarez, D. L., and M. García. Development and validation of a Real-Time Taqman® polymerase chain reaction assay for the detection of *Mycoplasma gallisepticum* in naturally infected birds. Avian Dis. 50:537-544, 2006.

Callison, S. A., Riblet, S. M., Sun, S., Zavala, G., Williams, S., Resurreccion, R., Spackman, E., and M. García. Development and validation of a Real-Time Taqman® PCR assay for the detection and quantification of infectious laryngotracheitis virus in poultry. J. Virol. Meth., 139:31-38, 2007.

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Cooke, K. M., Bernard, J. K., Wildman, C. D., West, J. W., and A. H. Parks. Performance and ruminal fermentation of dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. J. Dairy Sci., 90, 2329-2334, 2007.

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Dean, J., Latimer, K. S., Oaks, J. L., Schrenzel, M., Redig, P. T., and A. Wünschmann. Falcon adenovirus infection in breeding Taita falcons (*Falco fasciinucha*). J. Vet. Diag. Invest., 18:282-286, 2006.

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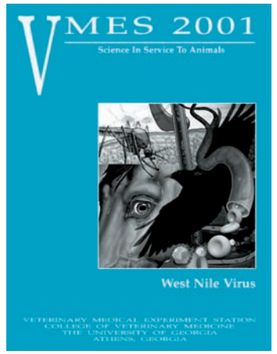

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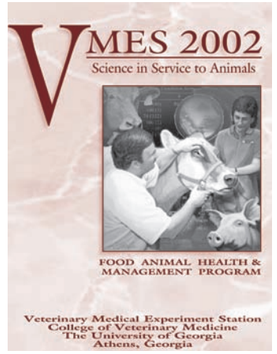

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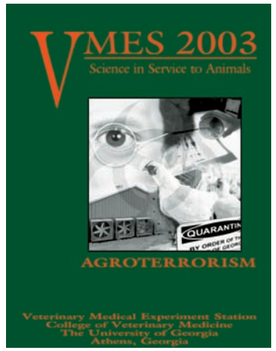

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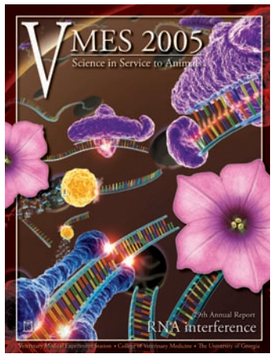

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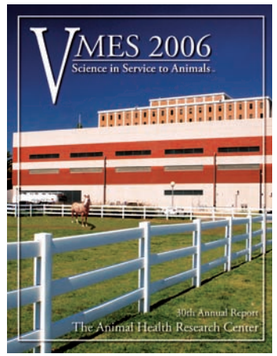

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