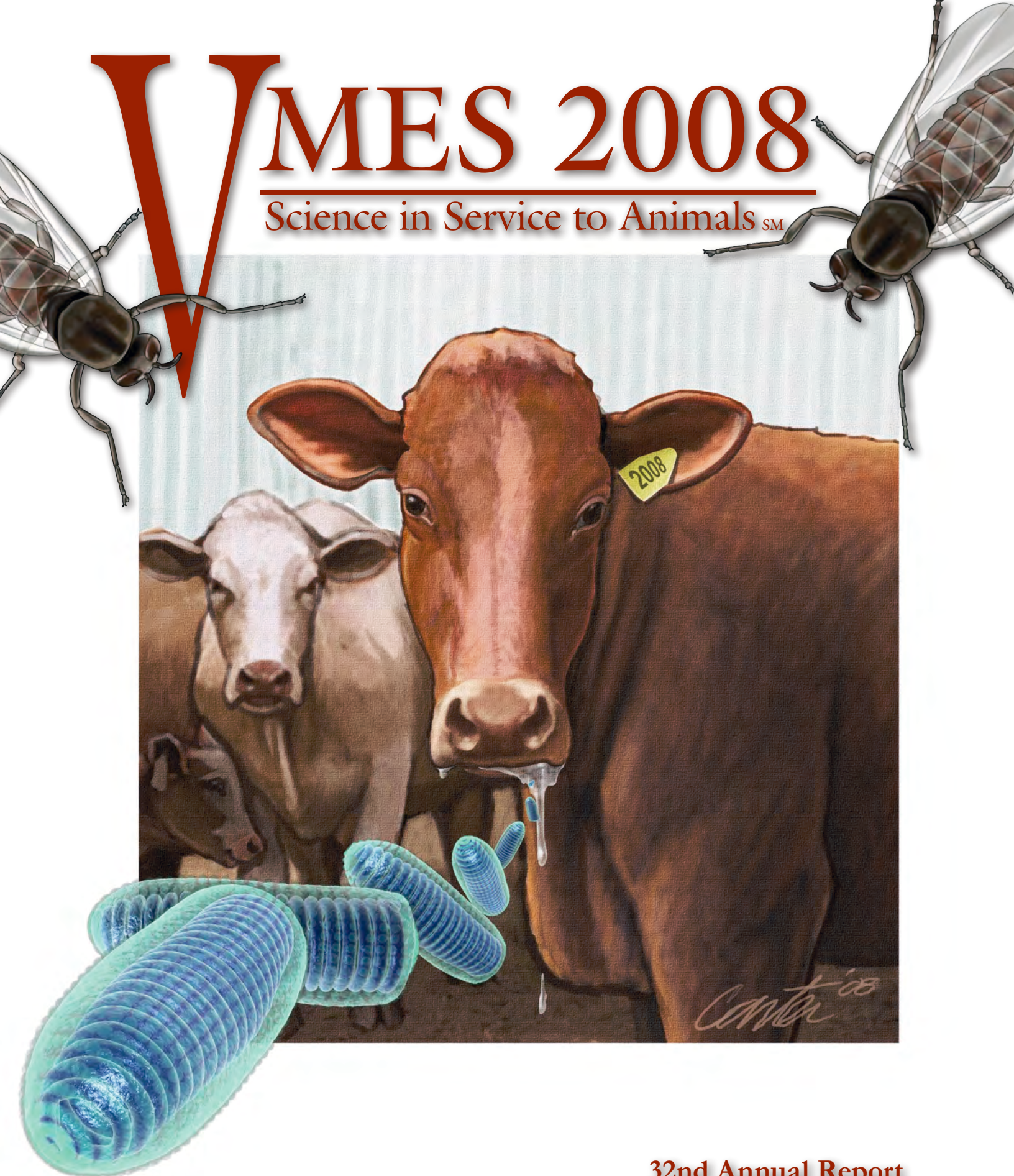


VIMES 2008

Science in Service to Animals SM



32nd Annual Report

Vesicular Stomatitis Virus

CONTENTS



VMES Overview, Mission, and Objectives	1
VMES Highlights	2
Feature Article Vesicular Stomatitis Virus	4
Last 6 Covers	7
Biomedical Science	8
Diagnostic Science	13
Immunology	14
VMES Faculty and Researchers	18
Research Contracts and Grants	19
Selected Publications	20

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The University of Georgia

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VMES 2008

Science in Service to Animals SM

32nd ANNUAL REPORT

July 1, 2007 to June 30, 2008

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 32nd Annual VMES report we summarize research efforts for fiscal year 2008.

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.*



Despite the increasing fiscal challenges imposed on the Veterinary Medical Experiment Station (VMES) by this year's troubled national and state economies, our faculty remains focused on its mission to conduct the highest quality research on diseases of animals and humans. All veterinarians take an oath to use their scientific knowledge and skills for the benefit of society through the "protection of animal health, the relief of animal suffering, the conservation of animal resources, the promotion of public health and the advancement of medical knowledge." We are deeply committed to this responsibility. The VMES motto, "Science in Service to Animals" has never been more appropriate as society recognizes that animals, humans and the environment are intricately related regarding their health and well-being.

The college's new 75,000 square foot Animal Health Research Center (AHRC), which is now completely built and commissioned, is currently going through a final, operational "phase-in." This activity included the first use of the large animal biocontainment facilities, which were put through their paces in a cattle study with vesicular stomatitis virus. This work and its significance for agriculture is highlighted in the accompanying article by Dr. Daniel Mead in which he explains the impact of his research and the critical need for highly specialized facilities that allow biocontainment work with large animal species.

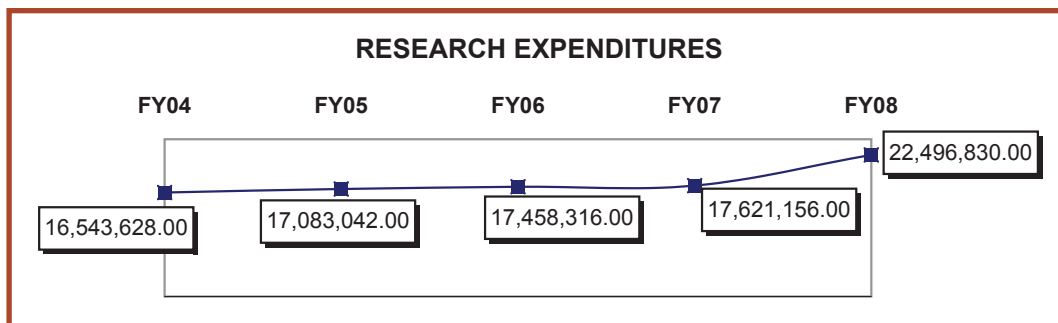
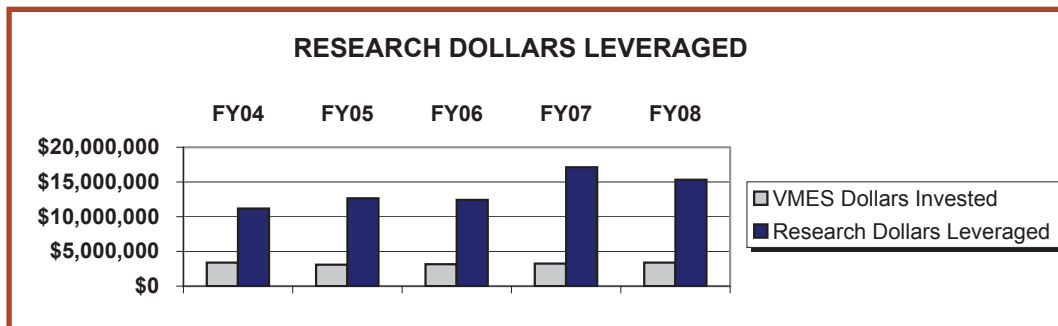
Researchers on the second floor of the AHRC have been working for over a year in state-of-the-art research laboratories, and have started many interdisciplinary research collaborations. As a direct result of these scientists and others located elsewhere in the college, many productive research relationships have been developed or strengthened. The College of Veterinary Medicine is one of the initiating partners in the Southeast Center for Emerging Biological Threats; research ties with the Centers for Disease Control and Prevention are increasing; UGA is affiliated with the Southeast Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research; and collaborations are increasing with the USDA/ARS Southeast Poultry Research Laboratory.

As I often emphasize, veterinary research has an impact on many biomedical fields, and major support for research in the college has been obtained from the National Institutes of Medicine and the United States Department of Agriculture. Although significant competitive research support was garnered by our veterinary researchers from non-federal sources including the prestigious Morris Animal Foundation, funding that targets companion animals, including horses, remains limited and more difficult to acquire than funding for research on human or food animal diseases. Thus, the continued commitment from the State of Georgia to support research focused on animal health is critically important and a smart 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. This year, approximately 4.5 research dollars were leveraged for each state dollar invested in the College. A summary of the college's research expenditures and grant support is provided below.

HIGHLIGHTS

This 32nd VMES Annual Report provides an overview of peer-reviewed, competitive VMES-funded projects conducted during fiscal year 2008 (July 1, 2007 – June 30, 2008). Additional information on any of these projects or others carried out in the College of Veterinary Medicine 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. These peer-reviewed papers represent a selection of VMES supported work and other scholarly research originating at the College of Veterinary Medicine.

Harry W. Dickerson



VESICULAR STOMATITIS VIRUS

Importance to the Livestock Industry

Vesicular stomatitis (VS) is a viral disease which primarily affects cattle, swine, and horses, causing vesicular lesions on the muzzle, tongue and oral cavity, coronary bands, and teats. Other livestock and wildlife species also can be infected. The causative agents, vesicular stomatitis viruses, are a group of antigenically related but distinct viruses of the genus *Vesiculovirus*, family *Rhabdoviridae*. The New Jersey virus serotype (VSNJV) is the serotype most often associated with epidemics in the United States. Historically, VS outbreaks have been reported throughout the United States but since the late 1970's outbreaks have only been reported from the Western U.S. During the 1982-83 epidemic, VS was diagnosed in livestock in 14 western states (673 premises). In 1985, VS was confirmed in livestock on 256 premises in Arizona, Colorado, and New Mexico. In 1995 and 1997, VS was diagnosed in livestock on 367 (6 states) and 380 premises (4 states), respectively. The most recent VS outbreak occurred over the course of 3 summers (2004 - 2006). During this time VS was diagnosed in livestock on 752 premises in 9 western states.

Vesicular stomatitis is a disease of considerable economic importance. In addition to being a cause of economic loss in beef and dairy herds, due to weight loss and a drop in milk production in affected animals, the disease is of paramount importance to animal health authorities because the clinical signs in cattle, swine, and other cloven-hoofed animals mimic those of foot-and-mouth disease (FMD), one of the most devastating and feared livestock diseases, making differential diagnosis an urgent matter. Surveillance for all vesicular diseases affecting cattle and swine has been maintained in the U.S. since the first introduction of FMD virus in 1870.

Vesicular stomatitis is classified by the World Organization for Animal Health, also known as the *Office International des Epizooties*, as a Multiple Species Disease that is reportable. Therefore, the presence of VS in livestock is seen as cause for restricting the export of livestock from an affected state or country to VS-free areas. In the U.S., all livestock with clinical signs characteristic of vesicular disease must be directly inspected by USDA:APHIS:Veterinary Services foreign animal disease-trained personnel. Suspect premises are quarantined until serological or virus isolation procedures confirm the disease or determine that it is not present. Premises with animals confirmed to have VS remain quarantined for 30 days after all lesions have healed from all livestock on the premises. The restrictions on animal movement can cause appreciable economic losses, closing rodeos, animal fairs, and sale barns. The direct cost of the disease to dairy operations during an epidemic in the Western U.S. in 1982 was between \$92 and \$253/ case. The financial impact of the 1982 epidemic on 13 dairy herds in Colorado was assessed to be \$95,752. An evaluation of two dairy herds in California during the same epidemic showed the total loss in a 2-month period to be in excess of \$225,000. During the 1995 epidemic, the economic costs of lost trade, closed sale barns, rodeos and livestock shows is estimated to be between \$50 and 100 million. The state of New Mexico reportedly lost in excess of \$14 million during the 1995 epidemic. In 1995, in efforts to protect livestock trade in the U.S., 39 states restricted animal movement from affected states by requiring certificates from accredited veterinarians that the animals being moved had not been on VS-affected premises within 30 days. Many states had more strict requirements. Animals from affected states could not be moved to Canada or the European Union. Russia and the Republic of South Africa banned importing any beef from affected states in the U.S.

Natural History

Vesicular stomatitis has been recognized in livestock (cattle and horses) for over a century, and while the causative agents have been studied intensively in the laboratory, we are only recently beginning to understand the potential transmission routes associated with these viruses in domestic animal populations. Information regarding the transmission of VSNJV during epidemics is based largely on the limited observational and entomological studies conducted during the sporadic epidemics in the western U.S. and have identified potential virus transmission routes involving insect vectors and animal-to-animal contact.

Observations that VSNJV outbreaks usually occur during warm weather, that the virus moves in concentric circles away from the site of first appearance, often following waterways, and that outbreaks are terminated by the onset of killing frosts, led to the hypothesis that VSNJV was transmitted by insects. During epidemics, VSNJV has been isolated from a variety of biting and non-biting insects including, *Culicoides* midges, mosquitoes, black flies, eye gnats, and other non-biting Diptera. However, since viremia sufficient to infect insect vectors has not been documented for any wild or domestic animal species naturally or experimentally infected with VSNJV and previous efforts to elucidate the role of biting insects in VSNJV transmission have relied on experimental models that did not include

natural livestock hosts the role of insects in transmitting the virus has remained controversial. Animal-to-animal contact transmission is thought to have been the route responsible for continued livestock infection during the winter of the 1982–83 epidemic and has been demonstrated in domestic swine in controlled experimental settings. Other aspects of the natural history of VSNJV are not well understood. For example, during outbreaks many animals are exposed to the virus, as determined by the presence of specific antibodies, yet less than 10% develop clinical disease. Additionally, the extent to which host predilection of epidemic VSNJV strains affect clinical outcome, extent and duration of virus shedding, and transmissibility following infection in different livestock hosts is not known and remains a central question.



Dr. Daniel Meade

VS Research at UGA

A long range goal of our research team is to better elucidate the epidemiology of VSNJV. Achieving this goal is challenging because research involving VSNJV and domestic animals must be conducted in specialized facilities. All work must be done following BSL-3 guidelines in a BSL-3 Ag facility. At present there are no fully functional BSL-3 Ag laboratories on the U.S. mainland. The majority of our research with VSNJV and livestock has been conducted in cooperation with USDA:ARS scientists and we relied on the facilities at the Plum Island Animal Disease Center. In studies which were supported through the USDA National Research Initiative Competitive Grants Program our research team has documented VSNJV transmission from experimentally infected black flies to domestic livestock and has shown that clinical outcome and extent and duration of virus shedding in horses and pigs following transmission by black fly bite were found to be bite site dependent. In addition, we demonstrated that black flies could become infected with VSNJV by feeding on virus rich lesions on infected livestock and by co-feeding (transfer of virus from infected to non-infected black flies feeding simultaneously on the same host). These findings were the first reports of VSNJV transmission by insects to livestock and of a biting insect becoming infected with the virus while feeding on infected livestock.

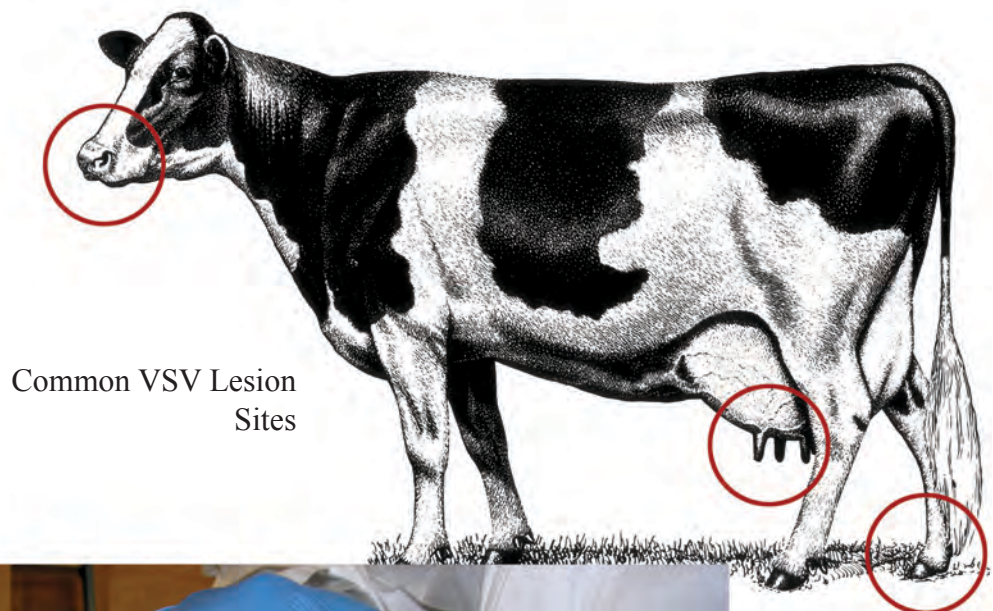
More recently, with additional funding through the USDA National Research Initiative Competitive Grants Program, we are investigating the transmissibility and host predilection of epidemic VSNJV strains and were approved to initiate this research in the College of Veterinary Medicine's Animal Health Research Center (AHRC), a BSL-3 Ag facility consisting of 16 animal rooms and support laboratories. The objectives of our research are to determine the extent to which clinical outcome and extent and source of virus shedding in VSNJV infected cattle and horses are dependent on virus strain and route of inoculation and to define the potential for virus transmission by insects and by animal-to-animal contact in relation to livestock infection with epidemic VSNJV strains.

Collectively, our research findings directly relate to the improvement and sustainability of U.S. agriculture and will lead to improved methods of VS prevention and control. For example, restrictions on animal movement would be less effective where insects such as black flies play a role in biological transmission of VSNJV. Therefore, the presence of blood-feeding insects in VS epidemic regions should be considered in the development of VS control and eradication programs. Our data also validates the need for protecting animals against insect feeding, as well as the need for basic insect control measures. In addition, our current research could provide an effective epidemiological means for the prediction and assessment of VSNJV spread during epidemics. These data are critical to the implementation of economically reasonable and effective control and prevention protocols, especially in light of the updated General Agreement for Tariffs and Trade and World Trade Organization guidelines.

New Research Opportunities at the College of Veterinary Medicine

Research involving many pathogens of veterinary and/or human importance in their natural hosts is often times limited because the specialized containment facilities needed to safely work with them are not available. This is especially true for pathogen systems that involve large animals. The College of Veterinary Medicine recognized this limitation in the 1970's and has actively pursued the construction of the AHRC. This pursuit became a reality this spring when our VSNJV project was approved by the University of Georgia's Institutional Biosafety Committee, the Institutional Animal Care and Use Committee, and the AHRC Users Committee. Final approval was granted after USDA:APHIS inspected and approved the large animal containment spaces for use. Since then, we have made tremendous progress in meeting our research objectives.

Once the AHRC is fully commissioned it will be the only large animal BSL-3Ag laboratory on the US mainland and the only one located on a university campus. The AHRC is a state of the art, technologically advanced facility that will be used to study a wide variety of infectious diseases that affect both human and animal health. The facility will enable scientists to study infectious microorganisms, such as viruses and bacteria, parasites, and toxins in an environment that is safe for the scientists and staff, animals and the public.

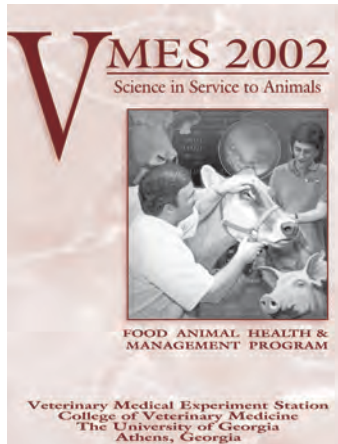


Common VSV Lesion Sites

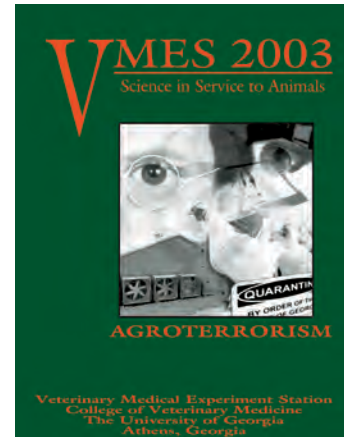


VSV lesion on lower lip

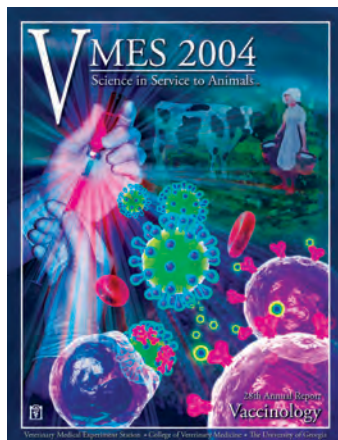
OUR LAST SIX COVERS



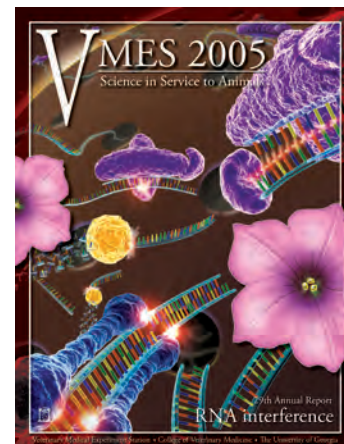
Food Animal Health & Management Program
FY 2002



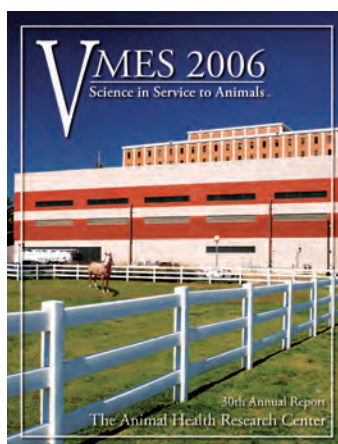
Agroterrorism
FY 2003



Vaccinology
FY 2004



RNA Interference
FY 2005



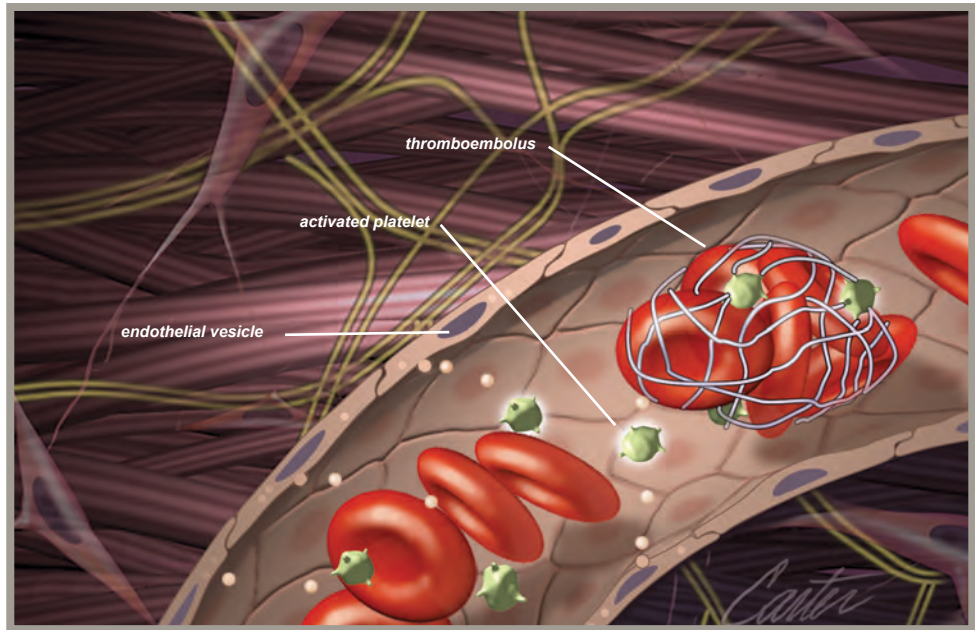
The Animal Health Research Center
FY 2006



Avian Influenza Virus
FY 2007

Identification of Endothelial Microparticles From Canine Cell Culture

“cells of the vascular endothelium are implicated in the prothrombotic state resulting from inflammation”



Critically ill animals are prone to developing abnormalities of the coagulation system. In some animals with severe inflammatory disease, this may manifest as an increased tendency to form inappropriate blood clots. These blood clots may form inside blood vessels, and travel to other parts of the body, creating thromboemboli. Patients who experience thromboembolism have a significant increase in morbidity and mortality.

The cells of the vascular endothelium are implicated in the prothrombotic state resulting from inflammation, mostly due to expression of pro-coagulant cell surface proteins. Additionally, activated endothelial cells can secrete small vesicles of membrane (endothelial microparticles) that act as circulating, activated endothelial cells. By activating the clotting cascade in inappropriate places, these small vesicles may result in the formation of thromboemboli.

This project has established a number of cell culture lines of canine endothelial cells. By stimulating these cells and evaluating the results via flow cytometry, the effect of activation on the endothelial cells can be better understood. This aspect of the project is ongoing. Once appropriate dilution and staining of the endothelial cells has been attained, these cell cultures will be used to evaluate the presence of microparticles with activation. Electron microscope evaluation of the activated endothelial cells is also pending, and will give a visual indication of the presence of microparticle formation from the cultured vascular endothelium.

Investigator: Dr. Benjamin Brainard

Co-Investigators: Dr. Elizabeth Howerth and Dr. Kenneth Latimer

Effect of Cold Ischemic Injury on Post Operative Hypertension

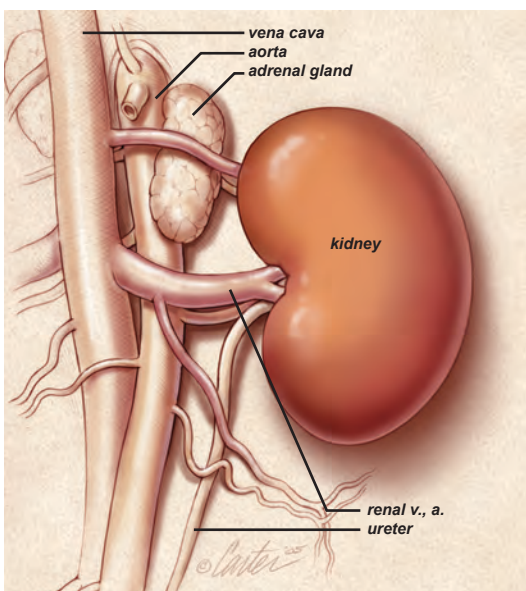
In feline patients, high blood pressure, or hypertension, during and after kidney transplantation surgery is a frequent cause of complications and may predict a worse prognosis. In people, less than 5% of renal transplantation recipients have normal blood pressure one year after surgery, and if they experience hypertension during surgery, risk for rejection of the new kidney is increased. We are trying to understand the mechanism of hypertension in these patients, especially as it relates to graft damage during storage. It is our hope that this line of investigation will identify novel therapeutic opportunities before, during, or after renal transplantation in both veterinary and human patients.

One protein of particular interest is renin. Because of its extremely low concentrations and labile nature, renin has historically been difficult to rapidly and accurately quantify. To facilitate measurement of renin in feline plasma, we are in the process of developing a novel activity assay. Preliminary work is promising and we are further optimizing, validating, and adapting this assay for use in other species. Further, we have designed and validated renin and related protein primers for RT-PCR in feline tissue samples. Using these tools as well as telemetric data from cats undergoing transplantation, we have discovered hypertension and renin plasma concentrations during transplant surgery in normal cats does not appear to be related to clinical relevant periods of organ storage. We are hoping to expand this research into clinical cases and more robust models of renal graft injury.

Investigator: Dr. Chad Schmiedt

Co-Investigators: Dr. David Hurley and Dr. Cathy Brown

“we have discovered hypertension and renin plasma concentrations during transplant surgery”





Genome-Wide Gene Expression Profile Of *Salmonella* Serovar Typhimurium In Response To Chicken Cecal Microflora

*“our objective is to characterize *Salmonella* gene expression profile in response to the chicken’s resident microflora”*

The establishment of healthy, diverse intestinal microflora is long known to reduce the susceptibility of poultry to infection by *Salmonella*. However, the genetic processes that underlie the interaction of a pathogen with the microflora are not well understood. Clear identification of *Salmonella* genes, affected in their expression relative to its interactions with the chicken’s resident microflora, will pave a new way for developing competitive exclusion products effective at eliminating food-borne pathogens from food animals, like poultry. Our long-term goal is to understand the interactions between pathogens and the chicken resident microflora so as to develop effective probiotic products to control *Salmonella* and *Campylobacter* in the poultry industry. Our objective is to characterize *Salmonella* gene expression profile in response to the chicken’s resident microflora. We have formulated this hypothesis based on the evidence that expression of specific colonization genes is affected by quorum sensing molecules. Our research goal is to understand how the interactions between *Salmonella* and microflora affect the gene expression patterns of *Salmonella*. *Salmonella* serovar Typhimurium will be either mono-cultured or co-cultured with a Nurmi-type microflora in a continuous-flow anaerobic system. RNA will be extracted from monoculture or mixed culture. The genome-wide gene expression in the presence of the microflora will be monitored on a *Salmonella*-specific microarray. Only when the genetic processes that underlie the interaction of a pathogen with the microflora are delineated can we develop defined competitive exclusion and effective probiotics to prevent the colonization and persistence of *Salmonella* in poultry.

Investigator: Dr. Ying Cheng

Co-Investigators: Dr. John J. Maurer and Dr. Margie D. Lee

Chick Embryos Possess an Intestinal Bacterial Community Within The Egg

The conventional wisdom among scientists has long been that birds acquire the intestinal bacteria that are necessary for good health from their environment, but this study finds that chickens are actually born with those bacteria. This finding could have important implications for the poultry industry and for food safety.

Understanding the microbial ecology of the developing chicken is the first step toward producing healthy birds without antibiotics. We have incubated more than 300 eggs and dipped them into a light bleach solution before extracting the embryos using sterile tools. DNA analysis revealed a diverse community of bacteria within the intestines of the developing embryos. We hypothesize that the bacteria penetrate the surface of the shell to the egg white, which is then ingested by the developing embryo.

The finding could lead to better methods for promoting growth in poultry and for reducing the risk of food-borne illness. As the poultry industry has moved away from the use of growth-promoting antibiotics in recent years, it increasingly relies on administering probiotics (beneficial intestinal bacteria) to newly hatched chicks. Establishing a community of healthy bacteria in the birds is thought to make it more difficult for pathogenic bacteria to establish themselves, but studies on the effectiveness of probiotics have shown mixed results. It appears now that the timing of probiotic administration is important.

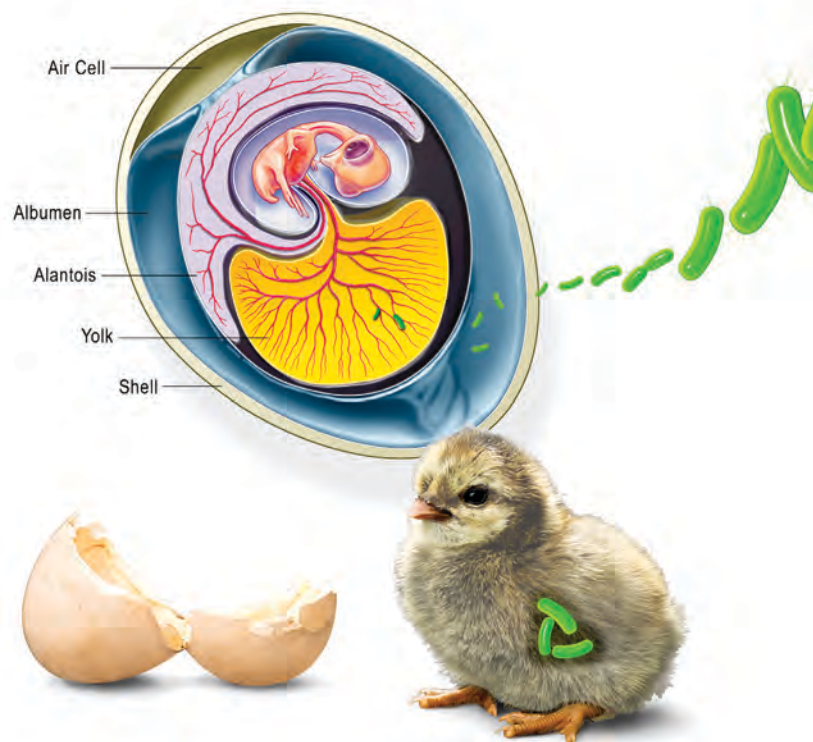
Most probiotics are administered after the chicks have hatched. Our study suggests we might need to administer probiotics in ovo (in the egg) to get better results. The idea that embryos are sterile in the egg and that chicks acquire their intestinal bacteria after hatching goes back to the 1960s, when early experiments using bacterial cultures, often Petri dishes with a growth medium, failed to grow any bacteria. Newer DNA techniques such as those used in this study are much more sensitive, however, and aren't influenced by how well a bacterium grows in a dish.

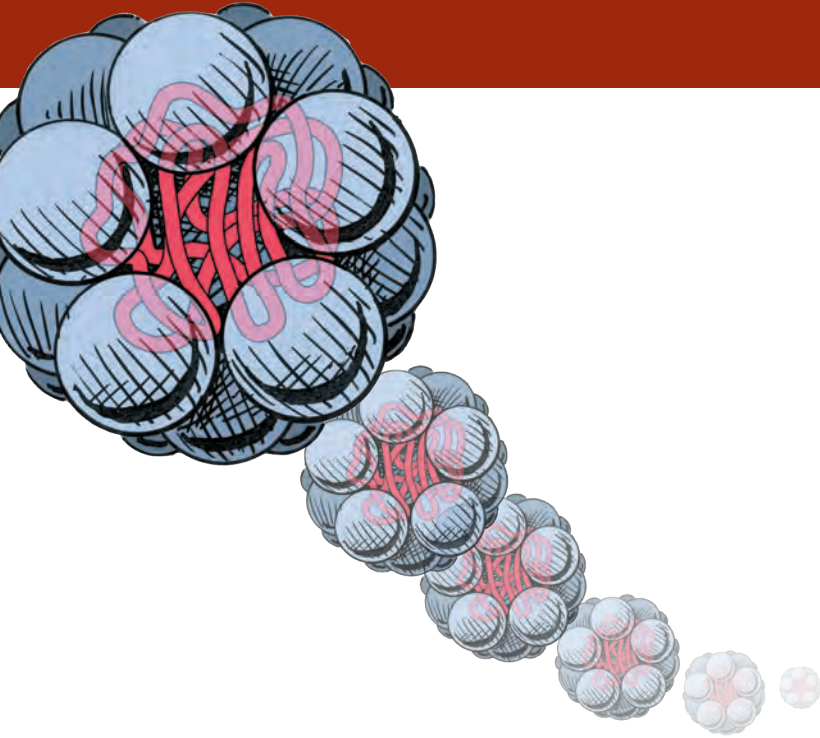
Previous assumptions were based on the use of cell cultures but we now know that only 1 percent of bacteria in the biosphere can be cultured.

Investigator: Dr. Adriana Pedrosa

Co-Investigators: Dr. Margie Lee and Dr. John Maurer

*“the first step
toward producing
healthy birds
without
antibiotics”*





Genetic and Antigenic Characterization of a New Variant of Canine Parvovirus Isolated in the United States

Canine parvovirus (CPV) is one of the most devastating infectious diseases of dogs and causes severe disease and a high death rate, especially in puppies younger than 6 months. The virus emerged in 1978, apparently through a mutation from the cat parvovirus that causes feline panleukopenia. It has become established in dog populations all over the world. Although the availability of effective vaccines has greatly reduced the death rate initially caused by CPV when it emerged, parvoviral disease remains highly prevalent in puppies because of a phenomenon known as the “window of susceptibility.” This describes a period during which immunity-conferring antibodies derived from a puppy’s mother are capable of neutralizing the vaccine, but not capable of protecting the puppy from infection by field virus.

Since its emergence in 1978, CPV has continued to change, resulting in two sub-types, CPV-2a and CPV-2b, which have completely replaced the initial virus since 1984. Recently, a new sub-type, CPV-2c, emerged in Italy and has been reported in Spain and Vietnam. This research project was aimed at determining the occurrence, distribution, and properties of CPV-2c in the US.

We described the occurrence of CPV-2c in the United States for the first time in 2007 and determined that the new strain was widespread in the US. We also determined that two separate sub-strains of CPV-2c are currently circulating in the US dog population. Efforts are ongoing to further characterize these strains by sequencing the entire genome. The information generated in this study will be useful to pharmaceutical companies in determining if the current vaccine formulations remain broadly protective against all current strains of canine parvovirus. The information will also be used to determine if currently used DNA diagnostic tests work well on the new strain of CPV.

Investigator: Dr. Jeremiah T. Saliki

Co-Investigator: Dr. Susan Sanchez

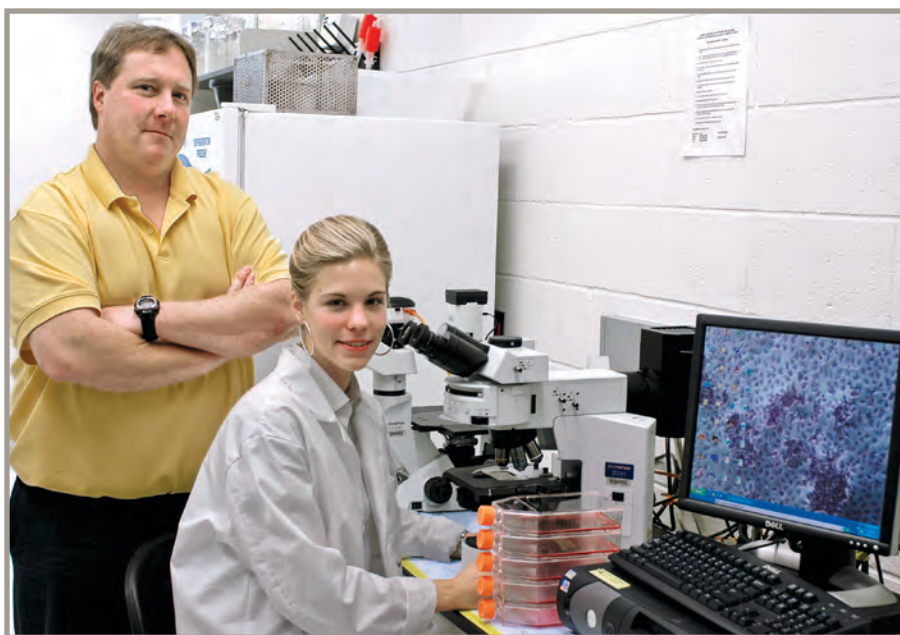
“two separate sub-strains of CPV-2c are circulating in the US dog population”

Detection and Identification of Mycoplasmas by Surface-Enhanced Raman Spectroscopy (SERS)

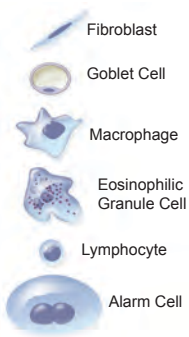
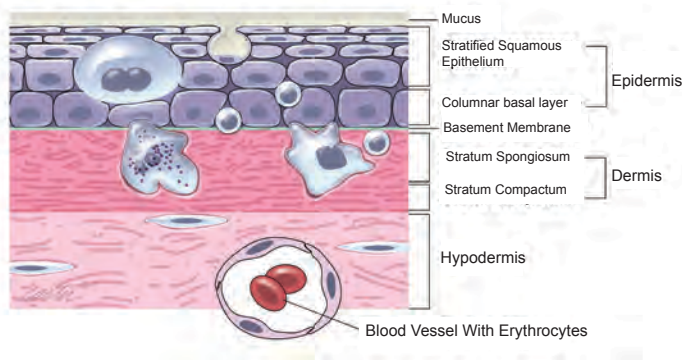
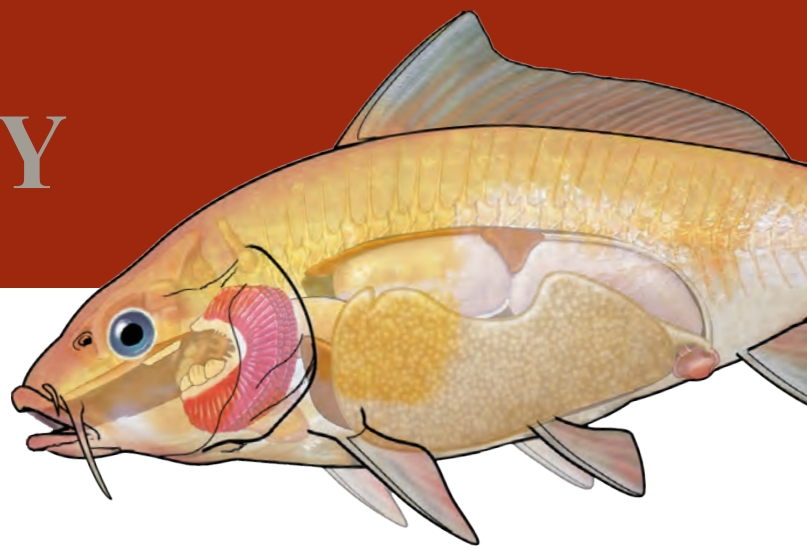
“rapid and reliable diagnostics for mycoplasma infections are non-existent”

Mycoplasma species cause chronic respiratory disease in animals and in humans. Due to the chronic nature of infection, *Mycoplasma* species such as *M. gallisepticum* mediate substantial disease burden in poultry impacting weight and egg production, while other *Mycoplasma* species similarly impact feed animals including cattle and pigs. Rapid and reliable diagnostics for mycoplasma infections are non-existent. For humans, primary care physicians typically diagnose based on physical findings after ruling out other possible causes, and in food animals, existing diagnostic tests often yield a high percentage of false-positives which require confirmation by direct culture which takes weeks and at great cost to the producer. Our research group has developed a novel nanofabrication technique that produces silver nanorod substrates that vastly improves the sensitivity of surface-enhanced Raman scattering (SERS) biosensing. Using this method and SERS to detect a variety of important *Mycoplasma* species, we show that SERS can rapidly (near real-time) detect extremely low levels (sub-attomolar) of *Mycoplasma* species without the need for amplification or species-specific reagents, and show that the chemical makeup of individual *Mycoplasma* species translate to unique SERS spectra that can be analyzed using chemometric multivariate statistical techniques to provide “molecular fingerprints” of the bacteria that can be used to detect multiple *Mycoplasma* species in mixed cultures. Thus, SERS biosensing offers a powerful platform for rapid and sensitive detection and identification of important human and animal *Mycoplasmas*, and will facilitate disease intervention strategies.

Investigators: Dr. Ralph A. Tripp, Dr. Stan Kleven, Dr. Jeremy Driskell, Dr. Rich Dluhy, Dr. Yiping Zhao and Dr. Duncan Krause



Dr. Ralph A. Tripp in his laboratory



Cutaneous Antibody Secreting Cells and B Cells in a Teleost Fish

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 infects all fresh water fish and can cause major, sporadic outbreaks of disease in commercial aquaculture operations. Immunity against *I. multifiliis* is elicited within weeks following infection of the skin and gills, or by vaccination with major surface proteins from *I. multifiliis*, which are referred to as immobilization antigens (I-antigens). However, the duration of the immune response following infection has not been established.

The adaptive protective immune responses generated in response to *I. multifiliis* infection are primarily antibody mediated and antibodies found in cutaneous mucus and skin play a critical role in controlling surface infections. I-antigen specific antibodies are present in the skin and cutaneous mucus, presumably secreted by the antibody-secreting cells (ASC) we have shown are present in skin of channel catfish. Using ELISPOT (enzyme-linked immunospot) analysis we have now demonstrated that channel catfish immunized by surface infection over two years previously respond to a challenge infection by increasing total ASC and I-antigen specific ASC in skin at days 7, 14 and 21 following re-infection. The number of I-antigen specific ASC also increases in head kidney at days 14 and 21. This shows that I-antigen specific ASC are found in skin for at least two years after infection. These fish remain protected against parasite challenge and we hypothesize that cutaneous antibodies produced by ASC in the skin are responsible for protection.

Infections caused by bacteria and protozoan parasites result in significant economic losses in commercial aquaculture. Vaccination represents the most efficient method for preventing these outbreaks. 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.

Investigator: Dr. Robert C. Findly

Co-Investigators: Dr. Harry Dickerson, Jane Noe and Dr. Xiguang Zhao

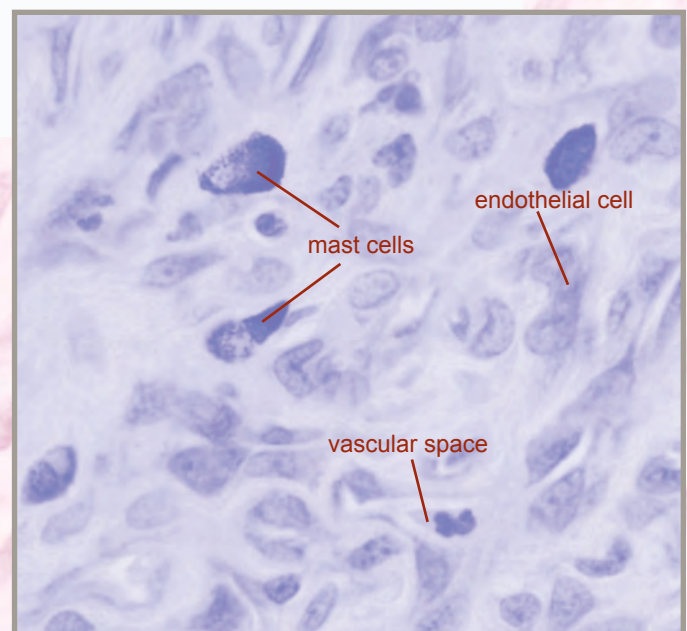
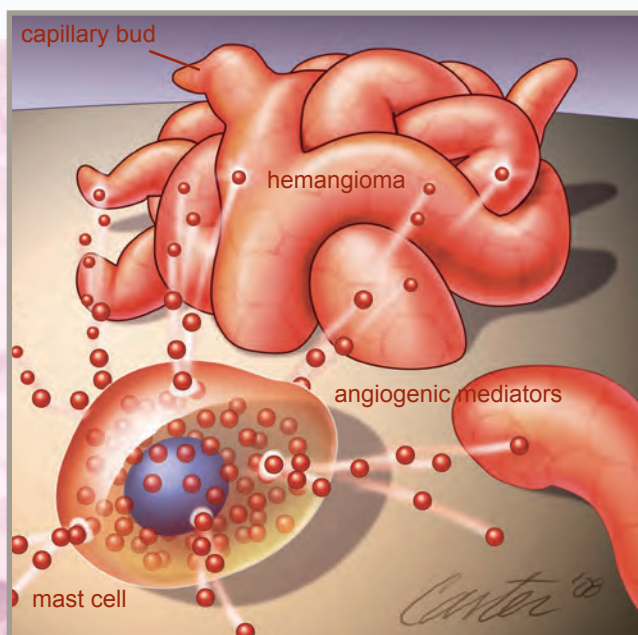
“antibodies found in cutaneous mucus and skin play a critical role in controlling surface infections”

“several mast cell mediators are reported to be angiogenic and regulate endothelial cell proliferation”

Role of Mast Cells in Neoplasm Angiogenesis and Intestinal Inflammation/Infections in Domestic Animals

Mast cells are important effector cells providing granule and membrane mediators as well as cytokines in allergic and inflammatory diseases. They play an important role in gastrointestinal pathology especially in the intestinal response to bacterial infections and in antigen presentation to T cells. Furthermore, several mast cell mediators are reported to be angiogenic and regulate endothelial cell proliferation. In human medicine, it is reported that mast cells play an important role in tumor progression and angiogenesis. The objective of the research work is to determine and assess the role of mast cells in inflammation and neoplastic progression and angiogenesis in domestic animals. Formalin fixed specimens submitted to and specimens collected from necropsy cases at Tifton Veterinary Diagnostic and Investigational Laboratory were evaluated. The specimens were processed for routine histopathology and stained with H&E (hematoxylin and eosin stain), special stains for mast cells and further evaluated by immunohistochemistry. The preliminary findings indicate that mast cells are involved in some neoplastic lesions such as canine hemangioma and hemangiosarcoma and intestinal inflammations. Further histopathological examination of inflammatory and neoplastic lesions supported with special stains and immunohistochemical investigation is in progress.

Investigator: Dr. Moges Woldemeskel



Cutaneous hemangiosarcoma in a dog. Mast cells are interspersed among variably sized vascular spaces lined by plump endothelial cells.



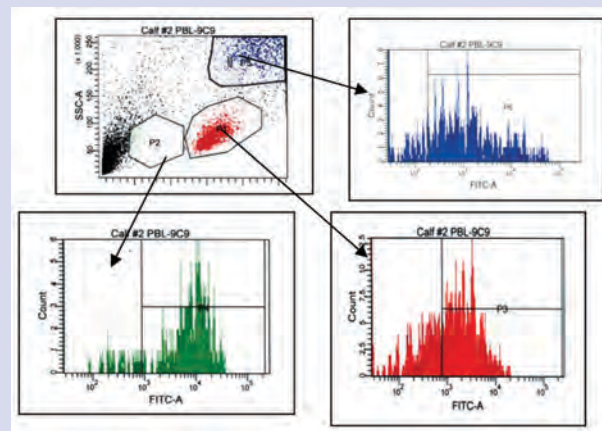
Expression of a Novel Pattern Recognition Receptor on Bovine Leukocytes

“we have found a novel molecule that acts as a PRR and as such may activate innate immunity in response to bacterial infections”

One of the principal host mechanisms of pathogen recognition is initiated when receptors on cells of the host innate immune system bind to molecular patterns of microorganisms. These proteins have been collectively called pattern recognition receptors (PRR) due to their ability to bind to repeat sequences expressed in bacterial products. The importance of PRR in animal health is well documented and their stimulation through specific ligands has been crucial in vaccine formulations. In all species studied, PRR participate directly in immune functions and in the activation of responses such as inflammation and initiation of adaptive immunity. The molecular identification of new PRR and their responses following in vivo activation will provide an invaluable tool for vaccine development and could also lead to the elucidation of mechanisms of innate and adaptive immunity not yet understood in food animals. To address these issues, recent advances in our laboratories have led to the identification of a new class of pattern recognition receptors (PRR) on nonspecific cytotoxic cells (NCC) of catfish that we refer to as NCC cationic antimicrobial protein-1 (Ncamp-1). Ncamp-1 recognizes bacterial DNA, oligodeoxynucleotides (ODNs), and polyguanosine motifs and its expression appears to be up-regulated in response to this stimulation. Unlike other PRR, Ncamp-1 in soluble form is a potent antimicrobial protein. Thus although the similarities of Ncamp-1 to other PRR are compelling, studies are needed to identify the mechanisms of activation of innate responses that lead to the production and secretion of this novel protein. In the present study, we proposed to characterize the expression and activation of Ncamp-1 in bovine leukocytes. Our data show that an orthologue of Ncamp-1 is expressed on PBL (peripheral blood leukocytes) in calves and that recombinant Ncamp-1 is bactericidal against E.coli from bovine mastitis isolates. Future studies are directed to identify specific cell populations from bovine PBL that express this novel PRR. Cattle represent an important food animal for the State of Georgia (1.3 million head of cattle grown in all 159 counties, worth \$262 million). Bacterial infections cause pain, distress and economic loss to the cattle industry. We have found a novel molecule that acts as a PRR and as such may activate innate immunity in response to bacterial infections. Activation responses to this PRR are likely to result in the production of inflammatory cytokines and secretion of soluble (bactericidal) Ncamp-1. This added property of Ncamp-1 as a natural bactericidal protein produced in food animals may provide an ideal therapeutic agent in cattle. The study of Ncamp-1 expression by cells of the bovine immune system will add to the body of knowledge of this important food animal.

Investigators: Dr. Liliana Jaso-Friedmann and Dr. Amelia Woolums

PBL from 1 horse and 2 calves [calf 1 and 2 tested on 2 different days, a) & b)] were loaded on Histopaque cushions and stained with anti-Ncamp-1 Mab 9C9. The top left panel shows the scatter profiles of calf PBL (size vs. granularity) of one representative experiment. The blue population most likely corresponds to PMN, while the red is monocytes and the green lymphocytes. The fluorescence histograms with anti-Ncamp-1 mAb of each of these populations are shown in matching colors. An isotype control mAb was used as a negative control in all experiments.



“runting and stunting syndrome continues to be a major problem for broiler companies across the United States”

Evaluation Of Immunization Strategies in Broiler Breeders for Reducing the Economic Impact of RSS on Commercial Broilers

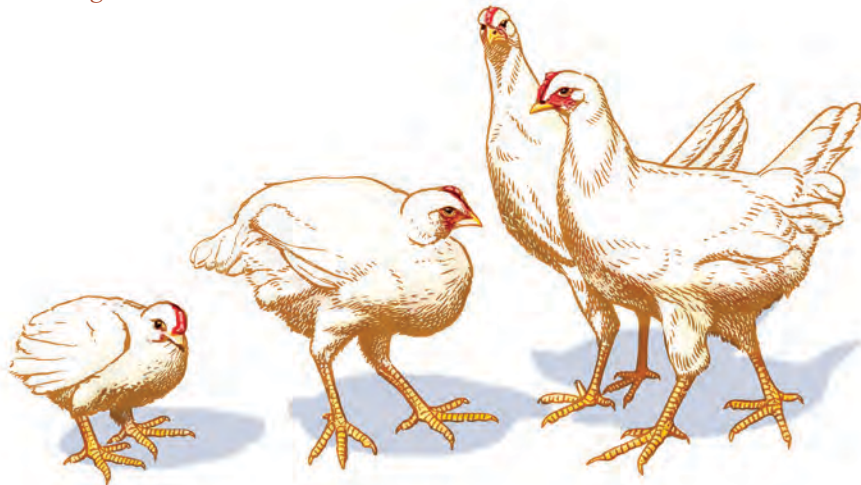
Runting and stunting syndrome continues to be a major problem for broiler companies across the United States. In the field, disease problems are on the rise with implications of immunosuppression and emergence of variant infectious bronchitis viruses. The immunosuppressive role of RSS has also been questioned. In an effort to provide the poultry industry with tools to help mitigate the effects of RSS, we propose to pursue vaccination strategies using tools developed at PDRC.

The long term goal of this research is to control the economic impact of RSS. The specific goals of this project are to determine if maternal derived immunity induced with any one of four vaccines can be used by the industry to overcome the health and economic effects of RSS. Our specific research objectives for this project are to:

1. Determine if maternal derived immunity in RSS-challenged progeny from breeders vaccinated with purified recombinant protein is protective.
2. Determine if maternal derived immunity in RSS-challenged progeny from breeders vaccinated with purified, inactivated embryo-passaged virus preparations is protective.
3. Prepare inactivated non-enveloped virus vaccine to immunize hens. Progeny will be challenged to determine if maternal derived immunity is provided to RSS-challenged day-old broilers.
4. Determine vaccination of breeder hens with a commercial inactivated enteric reovirus vaccine or an autogenous reovirus vaccine provides any protection in RSS-challenged day-old broilers.

Data generated from this project will provide much needed information for directing future research on RSS, as well as, provide a potential means of mitigating the significant economic effects of RSS for the poultry industry.

Investigators: Dr. Holly Sellers and Dr. Egbert Mundt



Broilers with RSS next to normal chickens.

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RESEARCH CONTRACTS AND GRANTS

- Allen, Doug.** Targeting 5-HT in equine laminitis. Grayson-Jockey Club Research Foundation, Inc. \$26,266
- Allen, Sheila.** Core Animal Diagnostic Laboratory: NAHLN: GA. USDA-CSREES. \$300,000
- Allen, Sheila.** Section 1433 Animal Health and Disease Research Funds 1st and 2nd Quarter FY2008. USDA-CSREES. \$57,291
- Baldwin, Charles.** Diagnostic services relative to the control, diagnosis, treatment prevention, and eradication of livestock diseases 2008. Ga. Dept. of Agriculture. \$2,190,737
- Baldwin, Charles.** Diagnostic service relative to the control, diagnosis, treatment, prevention and eradication of livestock diseases 2009. Georgia Department of Agriculture. \$2,189,856
- Barton, Michelle.** Hydrocortisone replacement therapy in septic foals. Grayson-Jockey Club Research Foundation, Inc. \$27,056
- Berghaus, Roy.** Management practices for *Salmonella* reduction on broiler breeder farms. USDA-CSREES. \$304,157
- Brainard, Benjamin.** Pharmacokinetic and pharmacodynamic study of clopidogrel in dogs. Morris Animal Foundation. \$12,540
- Brown, Corrie.** Acquisition of goods and services; investigations into antimicrobial sensitivity. USDA-ARS. \$25,467
- Brown, Corrie.** Characterization of cells in early response to infection with vesicular stomatitis virus. USDA-ARS. \$10,000
- Brown, Corrie.** Development of a Field Manual for Collection of Specimens to Enhance Diagnosis of Animal Diseases in Afghanistan. USDA-FAS-ICD. \$18,425
- Brown, Corrie.** Development of Illustrated Manual for Diseases of Livestock of Afghanistan. USDA-FAS-ICD. \$11,817
- Brown, Corrie.** Producing an Illustrated Handbook of Animal Diseases of Afghanistan. Fort Valley State University. \$12,540
- Budberg, Steven.** Efficacy and safety of tramadol HCl extended release for the control of pain associated with osteoarthritis in dogs under clinical conditions. Farnam Companies. \$53,150
- Budberg, Steven.** A feasibility screening study of a phosphatidylinositol 3-kinase gamma inhibitor. Boehringer Ingelheim, Inc. \$169,659
- Budberg, Steven.** In-vivo and ex-vivo effect of meloxicam on chondrocyte metabolism in osteoarthritic canine cartilage. Boehringer Ingelheim, Inc. \$243,024
- Crowell-Davis, Sharon.** Evaluation of the efficacy of a synthetic pheromone analogue in the treatment of stromphobia in dogs. CEVA Sainte Animals. \$75,000
- Dickerson, Harry.** The University of Georgia 2008 Veterinary Scholars Program: A research training experience for veterinary students. Merck & Co., The Merck Company Foundation, and Merial Grants Program. \$20,400
- Epstein, Kira.** Effect of synovitis on amikacin concentrations following intravenous regional limb perfusion. American College of Veterinary Surgeons. \$9,868
- Fischer, John.** Southeastern Cooperative Wildlife Disease Study. Various Other States. \$385,122
- Fu, Zhen.** Developing avirulent rabies virus vaccines. NIH-National Institutes of Health. \$295,000
- Garcia, Maricarmen.** Validation of avian influenza serological tests for differentiating infected from vaccinated animals (DIVA). USDA (University of Maryland). \$86,968
- Hogan, Robert J.** Determining SARS-CoV host gene requirements by mutagenesis and RNA interference. NIH-National Institutes of Health. \$248,463
- Hogan, Robert J.** Generation of mumps virus-specific monoclonal antibodies. Centers for Disease Control. \$2,500
- Hogan, Robert J.** In vivo efficacy of novel compounds against poxvirus infection I: Optimization of viral dose. Acute Viral Research. \$2,500
- Hogan, Robert J.** The influences of N- and O-linked glycosylation on the immunogenicity of the Ebola virus glycoprotein. Southern Research Institute. \$295,486
- Hollett, Bruce.** Animal evacuation rescue and shelter training. GEMA & Dept. of Homeland Security. \$50,000
- Hondalus, Mary K.** Needle-free vaccination via nanoparticle aerosols. Harvard University. \$75,000
- Hondalus, Mary K.** Virulence of the opportunistic pathogen *Rhodococcus equi*. NIH-National Institutes of Health. \$315,485
- Hurley, David.** Correlation between circulating endotoxin and inflammatory activation following a standard exercise stressor. American Quarter Horse Assoc. \$58,296
- Jackwood, Mark.** Multiplex detection of avian influenza HA and NA types using microsphere assay. USDA-CREES. \$88,675
- Jackwood, Mark.** Testing of Replikins' Synthetic Vaccines in an avian vaccine challenge study using IBV: Trial #2. Replikins Ltd. \$12,393
- Johnston, Spencer.** A multi-center clinical efficacy and safety study of 0.2 mg/ml recombinant human bone morphogenetic protein-2 (rhBMP-2) on Absorbable Collagen Sponges (ACS) implanted in dogs with diaphyseal fractures. Fort Dodge Animal Health. \$42,413
- Kaplan, Ray.** Characterization of anthelmintic resistance in small ruminant gastrointestinal nematodes in the mid-Atlantic US. Delaware St. Univ. \$32,500
- Kaplan, Ray.** Furnish *Brugia malayi* adult worms and/or *B. malayi* infective larvae. NIH-National Institutes of Health. \$149,405
- Kaplan, Ray.** Transfection and Analysis of Transcription in *Brugia malayi*. NIH-National Institutes of Health. \$26,445
- Karls, Russell.** Testing a novel tuberculosis mucosal vaccine. American Lung Association. \$40,000
- King, Christopher.** VCA: A monoclonal antibody toolkit for functional genomics of plant cell walls. NSF-National Science Foundation. \$78,768
- Latimer, Kenneth.** *Cytosuxoon felis*: Assessing genetic variability in an emerging feline infectious disease. Morris Animal Foundation. \$33,620
- Mead, Daniel.** The Role of Insect Vector Transmission in the Pathogenesis of Vesicular Stomatitis Virus Specific Cooperative Agreement. USDA-ARS. \$25,000
- Miller, Doris.** BSE Surveillance. USDA-APHIS. \$7,940
- Miller, Doris.** Diagnostic services relative to the control, diagnosis, treatment prevention, and eradication of livestock diseases 2008. Georgia Dept. of Agriculture. \$1,460,492
- Moore, James.** Assessment of the capacity of differently adjuvanted equine vaccines to induce systemic inflammation. Merial Limited. \$188,471
- Moore, James.** Elucidating structure-function relationships of lipid A: A synthetic approach. NIH-National Institutes of Health. \$108,386
- Moore, James.** Equine microarray development. Univ. of Kentucky Res. Foundation. \$9,000
- Moore, James.** Role of toll-like receptors in activation of equine monocytes by microbial ligands. Morris Animal Foundation. \$37,800
- Moore, Julie.** Immunopathogenesis of severe malaria during pregnancy. NIH-National Institutes of Health. \$575,178
- Mundt, Egbert.** Antigenic characterization of field isolates using the reverse genetics system of infectious bursal disease virus (IBDV). U.S. Poultry and Egg Assoc. \$94,278
- Mundt, Egbert.** Proposal for research and development of intervention strategies to reduce or prevent economic losses from Runting-Stunting-Syndrome in chicken. GA Poultry Federation. \$39,000
- Northrup, Nicole.** Maddie's Shelter Medicine Externship at University of Georgia College of Veterinary Medicine. Maddie's Fund Foundation. \$6,000
- Pence, Melvin.** Memorandum of Agreement - Voluntary Bovine John's Disease Control Program/Tifton. USDA via GDA. \$9,360
- Pence, Melvin.** Memorandum of Agreement Voluntary Bovine John's Disease Control Program/ Food Animal. Georgia Dept of Agriculture. \$6,911
- Peterson, David.** *Cytosuxoon felis*: Assessing genetic variability in an emerging feline infectious disease. Morris Animal Foundation. \$14,024
- Peterson, David.** The Development of an In Vitro Cell Culture System for *Cytosuxoon felis*. Morris Animal Foundation. \$4,000
- Platt, Simon.** Syringomyelia in the Brussels Griffon (Griffon Bruxellois): Magnetic resonance imaging findings, clinicopathology, and prevalence. American Kennel Club - Canine Health Foundation. \$140,509
- Ritchie, Branson.** Research Associate in Exotic/Zoo Infectious Disease and Pathology Postgraduate Program. Zoo Atlanta. \$23,000
- Robertson, Thomas.** Leukocyte and vascular function in endotoxemia and laminitis. Grayson-Jockey Club Research Foundation. \$29,852
- Robertson, Thomas.** Vagal and glossopharyngeal afferent cell bodies as biological sensors. University of Virginia. \$257,246
- Saba, Corey.** Phase II clinical evaluation of lomustine chemotherapy for feline vaccine-associated sarcomas. American Association of Feline Practitioners. \$30,000
- Saliki, Jeremiah T.** BSE surveillance testing. USDA/NVSL. \$132,876
- Saliki, Jeremiah T.** Current molecular epidemiology of canine parvovirus in the United States. Merial Limited. \$89,400
- Saliki, Jeremiah T.** Diagnostic services relative to the control, diagnosis, treatment, prevention and eradication of livestock 2009. Georgia Department of Agriculture. \$1,585,757
- Sanchez, Susan.** Georgia Veterinary Scholars Summer Research Program. NIH-National Institutes of Health. \$42,033
- Schatzberg, Scott.** Polymerase chain reaction for viral, bacterial and rickettsial nucleic acid detection in dogs with meningoencephalitis. Morris Animal Foundation. \$221,612
- Sreekumari Rajeev.** Leptospira Infection and its role in infertility in dairy cows. University of Florida. \$21,794
- Stallknecht, David.** Avian influenza viruses in the environment; What is the probability of human contact and transmission? Centers for Disease Control. \$874,996
- Stallknecht, David.** Role of aquatic environments in avian influenza virus persistence and subtype diversity. Morris Animal Foundation. \$30,787
- Torres-Velez, Fernando.** Pathogenesis of Nipah virus in guinea pigs. NIH-National Institutes of Health. \$113,495
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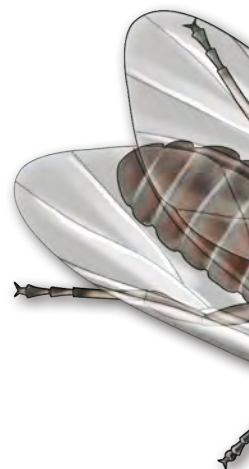
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