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VMES WORKING FOR GEORGIA
COVER ILLUSTRATIONS AND LEAD ARTICLES
2002-2005

Food Animal Health and Management Program
2002

Agroterrorism
2003

Vaccinology
2004

RNA interference
2005

www.vet.uga.edu/research/vmes/
The Veterinary Medical Experiment Station supports a wide range of research that impacts on many aspects of our lives; the food we eat and the clothes we wear, our physical, emotional, and economic health, and the quality of our environment. VMES research includes efforts to improve the productivity and health of poultry and livestock, to better the quality of life for companion animals, and to improve public health through disease surveillance. This year’s research is profiled in our 2005 - 2006 VMES annual report.

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

Our objectives are as follows:

• To improve the health and productivity of domestic livestock, poultry, fish, and other income-producing animals and wildlife through research;

• To assist in preventing disease epidemics by providing laboratory resources and highly skilled scientific personnel;

• To assist in protecting human health through the control of animal diseases transmissible to man;

• To improve the health of companion animals, which serve to enrich the lives of humankind;

• To train new scientists in animal health research in order to provide continuity and growth in this vital area of veterinary medicine.

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

Enhancing animal production, profitability, and well-being by improving animal health.
In this, the 30th Annual Report of the Veterinary Medical Experiment Station (VMES), I am pleased to introduce the Animal Health Research Center, which was opened for occupancy in 2006. As you will read in the accompanying article, the road from conception to construction and finally to commissioning and occupancy has been long. It was well worth the wait and effort, however, as the AHRC provides the College of Veterinary Medicine and the University of Georgia with an unprecedented capacity to conduct research on infectious pathogens of humans and animals in state-of-the-art biocontainment facilities. The AHRC now houses the research laboratories of Dr. Ralph Tripp, Georgia Research Alliance Eminent Scholar in Animal Health Vaccine Development, as well as the laboratories of the infectious disease researchers Drs. Mark Tompkins, Jeff Hogan, and Thomas Hodge. This team of researchers is developing vaccines and therapeutics for a number of important viral diseases including influenza, severe acute respiratory syndrome (SARS) corona virus, and AIDS. The containment facilities for animals on the first floor are scheduled for completion and commissioning in 2007.

In addition to funding basic and applied research projects, the VMES budget continues to support research and training related to both animal and human health. 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.

The 30th VMES Annual Report provides an overview of peer-reviewed, competitive VMES-funded projects conducted during fiscal year 2006 (July 1, 2005 – June 30, 2006). Additional information on any of these projects can be requested by contacting the VMES office by phone, email or website, or directly from the investigators themselves. A list of publications is provided as well. These peer-reviewed papers represent a selection of VMES supported work and other scholarly research originating at the College of Veterinary Medicine.

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

Harry W. Dickerson
The Animal Health Research Center (AHRC) at the College of Veterinary Medicine is a 75,000 sq. ft. free-standing building designed for the study of highly infectious diseases of animals, animal models of human disease, and hazardous chemical problems. The laboratories and animal holding capacities will accommodate interdisciplinary work among colleges. The completion of a high containment facility in the academic environment is very timely and long overdue considering the recent spate of emerging diseases and looming threats of bioterrorism.

As early as 1978, the faculty of the College of Veterinary Medicine recognized this need for a biocontainment laboratory and multispecies animal holding facility for research and training in the investigation of emerging and re-emerging biological threats to human and animal health. Dr. John Bowen, Associate Dean for Research at the college, planned and secured initial funding for the building through the 1980’s and early 1990’s. Construction took place from 1996 through 1998 with $16 million secured from state and federal sources. Nevertheless, due to funding shortfalls and design and construction deficiencies, the building remained unfinished and unoccupied. No progress toward completion of the facility was made until 2000, at which time the Georgia State Finance and Investment Commission (GSFIC) contracted with the consulting firm Boyken International, Inc. to do a cost analysis for completing and commissioning the facility. An end-user steering committee comprised of UGA researchers, staff, and administrators was assembled to critically review renovation and construction concepts. In December, 2002, GSFIC hired a design/build team to validate the plan and to carry out the required design and construction. The design-build team, Gilbane Inc. and CUH2A-Smith Carter, with extensive experience in complex biomedical research laboratory design and construction, including high-level containment facilities, was hired to construct the facility with a stated cost-limitation of $35 million from various state and University sources. The new design addressed current containment requirements, original deficiencies, and new programmatic requirements of the College. Most of the original mechanical systems were removed and replaced with up-to-date technology.

This structure is a high-containment laboratory and animal holding facility consisting of 8 animal rooms (2 large and 6 smaller) capable of holding multiple species of animals including rodents, poultry, and livestock. On a scale of 1 to 4 recognized biosafety levels, these spaces are categorized as Biosafety Level 3 Agriculture (BSL-3 Ag), one step below the highest containment category of BSL-4. An additional 6 animal rooms for rodents, caged animals and penned animals are rated at a lesser classification of Animal BSL-3. There is complete support space for the animal facility such as cage and rack washing and sterilization equipment, and a pathology suite designed for large and small animals. Carcass disposal is handled with a 500 lb/hr pathological waste incinerator and a tissue digester whose effluent is recycled via composting. All liquid waste effluent is sterilized on-site in a waste water treatment plant before being discharged to the sanitary sewer.
Containment of infectious organisms is ensured by a series of engineering designs including sterilizing high efficiency particulate air filters (HEPA) for the supply air and double HEPA-filtered exhaust air; directional air flows; differential pressures within containment zones; special elastomeric wall coatings that are 2.25 mm thick (vs. 0.18 mm for typical epoxy paint) ensuring virtually air-tight construction; shower-out facilities for each animal room; decontamination and sterilization procedures for equipment and solid/liquid waste streams. Extensive commissioning and validation of systems are undertaken to assure the operational capabilities of the facility. The building is equipped with multiple security devices throughout.

The facility also boasts a BSL-3 enhanced laboratory suite composed of five laboratories (1 large, 4 small) for work with highly infectious animal pathogens. In addition, the second floor has seven BSL-3 laboratories to study infectious diseases in addition to the five standard BSL-2 laboratories located there. The second floor of the building also houses a vaccine development laboratory for small scale development of animal vaccines.

When completely commissioned, the Animal Health Research Center at The University of Georgia will rank as a world class facility meeting or exceeding the biocontainment guidelines promulgated by NIH and the USDA/ARS.

Research in the Animal Health Research Center

The majority of emerging infectious diseases (EID) are of zoonotic origin, i.e. transmissible between humans and animals causing infection in both species. Many of the most dangerous and easily transmitted of these agents are viruses and bacteria. These agents impact global security by affecting food for an increasing world population, access to international trade and economic growth, and raise concerns for potential use as pathogens in bioterrorism. Biodefense is at the extreme end of the spectrum of serious disease threats. However, the emergence of new agents, the resurgence of old diseases, the appearance of resistant forms, and the recognition of viral and bacterial agents in the etiology of chronic diseases all support the need for an integrated program that can address both short- and long-term needs. These needs will involve basic and applied public health research, will be multidisciplinary in nature, and will utilize modern and robust molecular and quantitative tools and facilities.

Some progress has been made in identifying disease-causing agents of zoonotic origin, such as rodent-borne viruses like the hantaviruses and arenaviruses. Nevertheless, there is an acute need for a comprehensive approach to identifying, preventing and controlling viral and bacterial zoonotic EID. In the past few years the world has had to respond to SARS-associated coronavirus identified in domestic and wildlife species, influenza viruses from birds, the West Nile virus from birds via mosquitoes, *E. coli* O157:H7 from spinach contaminated with animal or human feces and a variety of multi-drug resistant bacteria acquired from food animals and wildlife. It is imperative that those in human, animal, agricultural and environmental sciences work together to address EID threats that will undoubtedly continue to pose a significant threat to human and animal health in the future. Thus, it is an important time to open the AHRC to initiate studies.
The AHRC will serve as a multi-disciplinary platform to facilitate viral and bacterial EID, select agent research, and product development among investigators at UGA, from outside academic and public health institutions, as well as by private companies. Personnel affiliated with the AHRC will consist of veterinarians, virologists, bacteriologists, immunologists, cell biologists, biochemists, pharmacists, physicists, chemists, epidemiologists, statisticians, graduate students and laboratory technologists from UGA, as well as adjunct faculty and collaborators from neighboring organizations. The purpose of the AHRC will be to draw on UGA's strengths, commitments and resources to create a world-class research organization for disease intervention strategies, provide a means to capture extramural funding opportunities, facilitate collaborations that lead to new program projects, grants, and contracts, and provide a conduit for development of small businesses from the research findings. Furthermore, development of AHRC research programs will provide unparalleled expertise and facilities, and position UGA to take a leadership role in this critically important public health area.

A Plethora of Opportunities

- The AHRC will assist investigators working on the national public health agenda which is to expand research on the biology, diagnostics, therapeutics and prevention of diseases caused by infectious organisms. The emergence and reemergence of infectious diseases in animals and man (zoonoses) indicates this agenda will continue into the foreseeable future.
- Increases are expected in extramural research support for all academic units that use this facility.
- The AHRC will expand capabilities for ongoing work at the University of Georgia including research on SARS, avian influenza, avian Newcastle, West Nile virus, tuberculosis, glanders, tularemia, botulism, encephalitic viruses, and other pathogens of public health importance.
- The prospect of working in this facility is attracting infectious disease researchers of the highest quality.
- The AHRC has unique capabilities to support research on agricultural diseases and to provide emergency response capabilities for foreign animal disease outbreaks such as exotic Newcastle disease of poultry and foot and mouth disease of hoofed animals.
- The extramural dollar flow generated by research in the AHRC will augment Georgia's economy at local and state levels.
- The research in this facility will result in new, commercialized biological products such as diagnostic reagents, vaccines, and drugs. For instance, one of the largest generators of royalty income to the University is a drug for the treatment of dry-eye disease that was created through research at the College of Veterinary Medicine. We expect these sorts of contributions to expand through research conducted at the AHRC.
- A primary focus of the programs in the AHRC will be the training of scientists and forging of industrial collaborations for the dissemination of laboratory research. The program already has in place agreements for research and exchange with 19 industrial partners that include Merck-Merial, Numico Research, Pty., Dharmacon, Nomadic, Protiva, Trellis Bio, Ventria, Virax, MedImmune, Aerovectrx, Sporian, Smithfield Foods, Protiva, nGimat, Millennium, Lentigen., BD Biosciences, Creare, and Alnylam Pharmaceuticals.
- The AHRC will facilitate interdisciplinary collaborations among regional biomedical research institutions. As a direct result of the AHRC, the following relationships have evolved: the College 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.
- The AHRC will be a critical resource for a direct response against bioterrorism and other emergencies involving infectious organisms.
- Researchers at the College are involved in the development of novel vaccines and therapies against diseases of state and national importance, namely tuberculosis, SARS, avian influenza, RSV and glanders. The AHRC is essential for this to occur.
Clinical Investigation of Poultry Diseases

Two studies were completed under this project. The first was to determine if there is a potential interaction between turkey coronavirus and reticuloendotheliosis virus. The objective of this experiment was to co-infect SPF turkeys with turkey coronavirus and reticuloendotheliosis virus obtained from field cases to determine if there is a potential interaction between the two. Body weights of the experimental groups were compared to controls to determine weight reduction after infection. The digestive tract and immune system tissues were examined microscopically for lesions. RT-PCR was performed on intestinal contents of individual turkeys to detect the presence of TCV RNA and to assess virus persistence in the gut. Virus isolation was performed from plasma to demonstrate viremia induced by REV. The combination REV-infected and TCoV-infected birds suffered more severe weight depression than the control birds or the birds infected with REV or TCoV alone. Viral persistence in the gut was variable.

The second study involved the investigation of alternative routes (oral, eye drop or wing web) of vaccination in poultry. Broiler breeder pullet vaccination for fowl cholera via wing web route is costly due to labor and can result in stress and injuries to vaccinated birds. Pullets vaccinated with live vaccine by wing web route also risk acquiring the disease contributing to joint problems, morbidity, and death. Therefore in 2004, in an effort to investigate alternative routes of vaccination, Bruzual et al., evaluated the use of a commercial Pasteurella multocida PM-1 vaccine administered by the drinking water in broiler breeder pullets versus administration by wing web. It was concluded that there was no protection by administration of a 6X dose of PM-1 orally in pullets whereas those given the vaccine by the w.w. route were protected. In this investigation, oral, eye drop, and wing web vaccination will be evaluated using the less attenuated CU (Clemson University) strain of Pasteurella multocida. Both post-vaccination mortality and post-challenge mortality after inoculation with a highly virulent serotype 1 Pasteurella multocida will be compared between treatment groups vaccinated orally, by eye drop, and via wing web with the CU strain and a group vaccinated with a commercial PM-1 strain via wing web application.
During the first trial, it was concluded that CU vaccine at commercial titer levels would not provide protection in birds vaccinated orally or by eyedrop. In the second trial, it was concluded that there was an improvement in the amount of protection from eyedrop administration of CU vaccine (54% protection), however, the vaccine administered was contaminated and therefore, the exact titer level that provided that protection could not be determined.

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CO-PI: Dr. Guillermo Zavala and Dr. Stephen Collett

**A molecular ecology approach for the design of effective probiotic formulations**

The goal of this project was to use an ecological approach to create a strategy for identifying and utilizing putative probiotic strains. The central hypothesis is that by assessing the development of the bacterial community structure of the small intestine we can identify relevant symbiotic bacteria that may improve intestinal health and feed conversion.

Objective 1. Identify and isolate candidate probiotic strains of *Lactobacillus* and avian-specific anaerobes from the bacterial community of healthy broiler chickens.

Objective 2. Characterize the development of the small intestinal bacterial community resulting from oral administration of candidate probiotic formulations.

We performed several culture-based isolations of avian anaerobes from the ileum of broiler chickens in order to isolate clostridial species related to clusters IV, XI and XIV. We had detected the 16S rDNA sequences of these species from birds fed growth-promotants and in a commercial probiotic and had hypothesized that they may function as potential intestinal symbionts. The bacteria were cultured on 3 different media (blood agar, reinforced clostridium medium, and Wilkins-Chalgren agar) and incubated under several different atmospheres (80% nitrogen/20% CO2 or 100%CO2). Individual colonies were subcultured and characterized by 16S rDNA sequencing in order to determine their identity.

We detected bacterial species such as lactobacilli, enterococci, and cluster I clostridia. We also cultured an isolate with high similarity to *Clostridium bifermentens*, cluster XI. We did not detect isolates with similarity to clusters IV and XIV although we DNA-sequenced nearly 100 isolates.

During these studies we identified several significant hurdles to screening the bacterial isolates that would also affect the distribution of bacteria detected from the cultures. The most significant hurdle was the logistical challenge of performing the cultures and subcultures in a timely manner. We couldn’t always access the anaerobe chambers in Dr. Whitman’s lab when we needed them because of the number of people performing studies at the same time. We found that some cultures did not grow upon subculture, probably due to the extended time of incubation. We tended to isolate sporeforming clostridia (such as *Clostridium sporogenes* cluster I) because these organisms are very hardy. We attempted to perform cultivations at night and on weekends, when the chambers were more available, and discovered that the growth conditions were not as optimal as we had hoped.

PI: Dr. Margie Lee (leem@vet.uga.edu)
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Development of a Culture-Independent Tool for Reporting Bacterial Growth Rate

Bacterial growth rate is monitored by traditional techniques, and these methods have their limitations. As an alternative we can use the growth-rate dependent promoter, rRNA fused to the jellyfish green fluorescent protein (GFP) to monitor bacterial cell growth. Our hypothesis is based on the growth-rate dependent nature of this promoter and direct correlation between bacteria growth rate and rRNA levels within bacterial cell. We developed a plasmid-borne GFP reporter fused to rRNA-promoter and demonstrated growth-rate dependence of our GFP reporter. However, the plasmid was not stably maintained without antibiotic pressure. We created a second GFP-reporter, using group II intron to specifically direct insertion of our GFP reporter into a universally conserved region of rRNA operon, and introduced it into Salmonella enterica Typhimurium. Our GFP reporter was stably maintained in the absence of antibiotic selection pressure. There was an inverse relationship between fluorescence and growth rate for our GFP-marked S. Typhimurium grown in complex media at 25C, 30C and 37C. Similar results were observed when bacterial cells were grown in different nutritional conditions, including egg white, defined minimal media with glucose or glycerol as carbon source, and nutrient rich, complex media. Fluorescence for cells grown in egg whites was low, comparable to signal produced in a nutrient-poor medium. Our new growth rate reporter can be theoretically introduced into any bacterial species, stably maintained, and report back the organism’s growth rate in vitro and in vivo. This is especially important in assessing the potential of foods, food processes and cooking/refrigeration temperatures to support microbial growth.

Jellyfish green fluorescent protein (GFP) for monitoring growth of pathogens like Salmonella in foods and animals. The promoter-less GFP gene was cloned into a group II, self-splicing intron. This intron was re-engineered to identify sequence unique to rRNA gene in bacteria. The intron, borne on a plasmid, inserted itself and the GFP into the bacterial chromosome of Salmonella. When the bacteria are grown in nutrient poor media, they have a slow growth rate and this is manifested in weak transcription AND low fluorescence. Salmonella grown in rich media have a “fast” growth rate, which translates into strong transcription AND high fluorescence.

PI: Dr. John J. Maurer  (jmaurer@vet.uga.edu)
Co-PI: Adriana A. Pedroso, Andrea P. Oliveira, Margie D. Lee, Marie Maier, Paulina Cruz-Venegas, Dana Cole, Susan Sanchez, and Charles Hofacre
*P. falciparum* cytoadherence: Modulation of host cell function.

Malaria is an enormous, global public health problem. A staggering 40% of the world’s population lives in areas where malaria transmission occurs, resulting annually in an estimated 500-600 million cases with 2.7 to 3.5 million deaths. The most severely affected are young children, pregnant women, and their unborn infants. Symptoms of severe malaria commonly include anemia, cerebral malaria (malaria-induced coma) and placental malaria (a condition where numerous parasite infected red blood cells are found in the placenta). It is significant that complications in the lung are commonly seen in association with these severe symptoms, and are considered an important contributor to malaria mortality. The malaria parasite is thought to induce tissue damage in organs through the ability of parasite-infected red blood cells to bind to endothelial cells lining the circulatory system. While the parasite proteins and host cell receptors that mediate this binding have been well-studied, relatively little is known about how this binding impacts the function of the bound endothelial cell. Our proposed investigation of the effects of this binding to endothelial cells has important implications for the disease process in severe syndromes such as cerebral malaria and acute respiratory distress. We believe that a detailed understanding of the effects of this binding will lead to novel drugs and vaccines that would disrupt the disease process.

The long-range goal of our research is to understand how the binding of infected red blood cells to host endothelial cells contributes to severe disease. The objective of this application is to determine the responses of lung microvascular endothelial cells to the binding of malaria infected red blood cells. The central hypothesis for the proposed research is that signaling induced by binding of malaria-infected red blood cells will have a profound and specific effect on these microvascular endothelial cells. Preliminary work in an in vitro system that we have developed has demonstrated that binding of infected red blood cells to placental cells leads to modification of several proteins in the host cells. The expression of genes which encode immune factors is also increased, and a specific immune factor, macrophage migration inhibitory factor, is specifically secreted in a time-dependent manner. The rationale for the proposed research is that several of the endothelial cell receptors bound by malaria binding proteins function in initiating cell signaling events. Thus, it is likely that binding of infected red blood cells activates signaling pathways within these endothelial cells resulting in changes in gene expression. This could then serve to favorably modify the local environment for parasite survival, manipulate the host immune response, and in the case of lung endothelium directly affect barrier function.

PI: David Peterson  (Peterson@vet.uga.edu)
**Isolation of mycobacteriophage that target *Mycobacterium avium* ssp. paratuberculosis**

*Mycobacterium avium* subsp. paratuberculosis (Map), the causative agent of Johne’s disease in cattle, is an emerging pathogen. Johne’s disease is a progressive disease that becomes evident 2-5 years after a Map infection of the intestinal ileum primarily in calves in the first year of life. Following infection, Map spreads throughout the infected organ causing chronic inflammation and scarring of the tissue. The disease manifests itself in the form of progressively more severe diarrhea and wasting until the death of the animal. Map is shed in the manure and contaminates the udders of cattle, which can lead to infection of suckling calves or contamination of milk fed to calves. As pathogenic mycobacterial infections require administration of multiple antibiotics for prolonged periods (years), this is not an economically viable option for the cattle industry. Various vaccines have been produced; however, each offers only partial protection. Moreover, vaccination often results in positive antibody tests for Map or *M. bovis*. Therefore, a critical need exists to develop alternate approaches to stem the transmission of Map.

The goal of this proposal was to isolate phages (viruses that only infect bacteria) that target mycobacteria (mycobacteriophages), specifically Map. The long-term goal of this research is to develop the mycobacteriophages into an intervention strategy to prevent transmission of Johne’s disease. Soil and manure samples from various Johne’s positive farms in the state of Georgia were examined. These included both beef and dairy operations.

A screen for identification of mycobacteriophages was developed. Basically, samples are suspended in buffer and subjected to a series of filtration steps to sequentially remove large debris, intermediate-sized debris, and finally bacteria, leaving submicroscopic particles that include any mycobacteriophages present. The resulting filtrates are spotted onto Petri dishes containing rapidly-growing, non-pathogenic, *Mycobacterium smegmatis* in soft agar. This enables the mycobacteriophages to infect the bacteria, replicate inside and lyse the bacteria, and spread to adjacent bacteria. Bacteriophages produce small zones of clearing (plaques) among the smooth bacterial “lawn”.

Based on differences in plaque morphologies, at least three different mycobacteriophages were isolated. The mycobacteriophages were tested for the ability to form plaques on the slow-growing, pathogenic Johne’s bacillus. One of the mycobacteriophages does indeed generate plaques on Map. Efforts are underway to study the Map-infecting mycobacteriophage. By deleting the region of the mycobacteriophage DNA that under some conditions prevents the phage genes from being expressed inside the host, we anticipate that the modified phage will only grow uncontrollably upon entering Map resulting in bacterial lysis every time. Farm soil and manure samples are continuing to be screened for additional mycobacteriophages that target Map. By combining different mycobacteriophages to treat Map contamination, it is more likely to produce sterilizing effects due to reduced likelihood that the bacteria will be able to mutate multiple surfaces to evade attack by mycobacteriophages that attach and infect through different receptors on the Map.

This research demonstrates that mycobacteriophages can be isolated from Georgia Johne’s positive farms. These mycobacteriophages have potential to be developed into an antibiotic-free intervention strategy to be used to help halt the spread of Johne’s disease.

**PI:** Russell Karls, PhD  (rkarls@vet.uga.edu)
**Co PI:** Mel Pence, DVM MS PAS Diplomate
**Staphylococci in companion animals and characterization of virulence factors**

The global rise in antibiotic resistance in both human and animal bacterial pathogens is reflected in the epidemic spread of clonal clusters of *S. aureus* possessing multidrug-resistance, methicillin resistance, and more recently, vancomycin resistance. There is some evidence that the same phenomenon may be occurring in animal-associated *Staphylococcus* species, and this prompts concern regarding treatment failure of the severe bacterial infections that both human-associated and animal-associated *Staphylococcus* species cause in companion animals. In a recent review of the literature, the evolutionary development of human Methicillin Resistant *S. aureus* (MRSA) strains was hypothesized to have occurred via frequent horizontal transfers of virulence gene clusters, resulting in five global clonal clusters of MRSA that arose independently. It is not known how animal-associated MRSA and Methicillin susceptible *S. aureus* (MSSA) may be related to these human epidemic clonal clusters, or if the prevalence and types of toxin and super-antigen genes is similar. Additionally, it is not known if the frequency of carriage and types of toxin and super-antigen genes in other animal-associated *Staphylococcus* species is similar to those *Staphylococci* commonly causing disease in humans. The objectives of this proposal was to examine the genetics of *Staphylococci* species affecting companion animals in the state of Georgia, and determine the carriage of selected virulence genes (toxins, superantigens) which are known to contribute to the severity of disease caused by these organisms. As well as, to examine the genetic relatedness and carriage of virulence genes of *S. aureus* isolates from companion animals with those causing disease in humans. The central hypothesis of this project is that the *S. aureus* isolates from animal disease are of human origin (reverse zoonosis), and carriage of certain virulence genes contributes to severity of disease seen in companion animals. Thirty nine MSSA as well as 10 MRSA canine isolates were included in the study. Rep-PCR was used to create a fingerprint for each isolate and generate a dendrogram. Standard format PCR was used to determine the presence of eight different super-antigen genes eta, etb, sea, seb, sec, sed and see. Twenty four bacterial isolates were also tested for the presence of seg, she, sei and sej. Our studies show that *S. aureus* isolated from dogs are a genetically very diverse group and not related to the most common MRSA clones isolated in the US. The super-antigen gene distribution in MSSA was as follows 0% sea, 8.3% seb, 4.2% sec, 0% sed, 0% see, 4.8% seg, 23.8% seh, 17.4% sei, 13% sej, 16.7% tsst-1, 0% eta, and 0% etb. The distribution of super-antigens in MRSA is as follows 0% sea, 10% seb, 0% sec, 70% sed, 0% see, 0% eta, and 0% etb. Super-antigen gene carriage seems to be different between MSSA and MRSA with a high prevalence of sed and no sec in MRSA. Further work needs to be carried out to understand the genetic link between mecA and some super antigens i.e.: sed. The undergraduate student working on this project will be presenting this work at the next CURO symposium and ASM.

**Abortifacient potential of eastern tent caterpillar in domestic goats**

Late instar Eastern tent caterpillar (ETC, *Malacosoma americanum*), were administered at the dose of 50g/day by oral gavage to six late term pregnant goats until they aborted or for 10 days. Late instar autoclaved Eastern tent caterpillar were administered to six late term pregnant goats which served as negative controls. No abortions were observed in the test and control groups. All animals were euthanized at 5-10 days post administration of the last dose. No significant gross findings were observed during necropsy. No significant alterations in blood chemistry and complete blood counts were observed except for few animals having mild eosinophilia. Histopathological examination of the tissues showed multiple granulomas in the abomasum and intestinal tract of both control and test group animals with setae (hair of ETC) in the center of these granulomas. Similar granulomas with setae were observed in rat GI tract and no abortions were observed in rats. The experiment indicate that ETC or its setae as such cannot induce abortions in pregnant goats and abortifacient potential of ETC is species specific, similar to the findings in pregnant mice and rats in which no abortions were observed.

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**CoI:** Mimi Healy and Brooke Wheeler

**Abortifacient potential of eastern tent caterpillar in domestic goats**

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**PI:** M. M. Sebastian  (msebasti@uga.edu)

**CoI:** ME. Pence, ME. Hines II, and C. Watson
Identification of immunodominant proteins in *Mycobacterium avium* ssp. paratuberculosis using proteomics.

Johne’s disease (JD) caused by *Mycobacterium avium* subsp. paratuberculosis (MAP) is an economically significant animal health problem that results in an annual economic loss of ~220 million dollars per year to the dairy cattle industry in USA. This agent is also implicated as an etiology of Crohn’s Disease in human beings. Control and eradication of JD has been hampered by the lack of rapid and unambiguous diagnostic tests. Currently used diagnostic methods such as ELISA and culture have variable levels of sensitivity and specificity and do not allow early diagnosis of this disease. The Scientists at Veterinary Diagnostic and Investigational Laboratory, Tifton are constantly striving to advance the methods for diagnosis and prevention of JD. Currently we are approved by National Veterinary Services Laboratory for MAP culture and serology. We actively participate in Georgia’s JD demonstration herd programs and voluntary JD control programs. We use an automated broth culture system (ESP para JEM system by Trek Diagnostics) to culture MAP and confirmation is done by Polymerase Chain Reaction. This method offers faster (6 weeks vs. 16 weeks with the conventional methodology) and accurate detection of MAP from clinical samples. The VDIL is equipped to culture 6700 samples per year. We are constantly striving to develop faster, cost effective and sensitive method to diagnose true infection status of an animal. We are concentrating on identifying immunodominant antigens present in MAP by proteomic technology. Identified antigens will be used for developing antibody based diagnostic assays such as ELISA, Dot blot, and Western blot. We have made considerable improvements in optimizing the detection of immunodominant antigens in MAP. Other projects at VDIL include development of vaccines to prevent JD, development of improved procedures for organism and antibody detection. Our long-term goal is to develop rapid, sensitive and specific diagnostic tests and vaccines which will facilitate the control of this chronic and problematic disease in cattle.

**PI:** Sreekumari Rajeev ([srajeev@uga.edu](mailto:srajeev@uga.edu))

**Diagnostic Detection, Isolation, and Characterization of Avian Viruses**

The mission of the diagnostic virology laboratory is to provide accurate and timely diagnostic virology services for the U.S. poultry industry, conduct applied research on current avian disease isolates from the field, and improve detection and isolation methods for monitoring avian viruses. The diagnostic virology laboratory provides a vital function in the overall services offered by the diagnostic facility at the PDRC. During 2005-2006, the diagnostic virology isolated 175 viruses. No virus was isolated from 81 samples. Two hundred and seventy whole blood samples were submitted for leukosis virus isolation with subsequent LL antigen capture ELISA. Seventy six serum samples were submitted for virus neutralization assays.

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

**Investigation of Natural Disease Outbreaks**

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

Activity is represented by farm visits by Faculty and students. Clinical investigations may include disease outbreaks, farm management problems, hatchery and feed mill issues, or problems associated with condemnations at processing. Extensive serology test, bacterial, virus, or Mycoplasma isolation and PCR test often reveals a particular disease agent as a causative agent mismanagement,
equipment failures, vaccination failures or other issues. Modification of current practices often brings the problem under control. The professional staff and students often investigate more long term problems on farms within the region. These are typically multi-faceted problems. Many times these are assigned as student projects under direct supervision of a clinician. These investigations bring recommendations and changes in vaccination programs and management practices which allow that grower to become competitive again.

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

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

Diagnostic Services/Teaching Laboratory activity is represented by 9,154 accessions, 33,170 bacterial procedures, 91 antimicrobial susceptibilities, 143,564 ELISA tests, 46,569 IBV-HI tests, 196,459 total serological tests, 2,687 diagnostic PCR tests, 6,439 histopathology slides, and 1,267 necropsies.

PIs: Stephan G. Thayer (sthayer@uga.edu)

Diagnostic Avian Mycoplasmosis

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

In 2005 we conducted 3148 diagnostic cultures for mycoplasmas, 4693 HI tests for MG, 5488 HI tests for MS, and 645 HI tests for MM, in addition to RAPD analysis from 151 cases and mgc2 and vlhA.

PI: Dr. S. H. Kleven (skleven@uga.edu)
Co-PI: V. Leiting

Development of Monoclonal Antibodies as Diagnostic Reagent for Reticuloendotheliosis Virus

The primary objective of this research was to develop antibodies against reticuloendotheliosis virus (REV) with the intention of using them as a diagnostic reagent. Our laboratory isolated multiple field REVs, sequenced several of them partially, and also resolved the full genome sequence of one such field isolate. The entire gag gene was PCR-amplified and cloned into a suitable vector but expression was unsuccessful. As an alternative method, the gag region coding for the CA protein known as p30 was also cloned into an expression vector. This protein was successfully expressed but efficiency of expression was extremely poor and inconsistent as determined in Western blots. Meanwhile, polyclonal antibodies against p30 were used successfully in indirect immunofluorescence assays (IFA) in infected cells in culture. IFA was useful, and relatively efficient and practical, albeit not very sensitive. We are still attempting expression of gag and p30 specifically, but have considered changing our strategy to use a baculovirus expression system, still with the ultimate goal of developing monoclonal antibodies vs. p30 in REV.

PI: Guillermo Zavala (gzavala@uga.edu)
Co-PI: Sunny Cheng and Taylor Barbosa
Altering semen extender and glycerol concentration to optimize results after cryopreservation of equine spermatozoa

After cryopreservation, spermatozoa from many stallions may have a lower capacity to fertilize an oocyte than fresh or cooled semen. The aim of this study was to evaluate a standard panel of semen extenders and varied concentrations of the cryoprotective agent (glycerol) to optimize sperm survival rates after cryopreservation. Semen was collected from Quarter Horse stallions (n=3) from March to May 2006 (6 collections per stallion). Semen was filtered immediately after collection and sample volume and sperm concentration were measured. A drop of raw semen was placed on two prewarmed slides to estimate the percentage of progressively motile sperm. The semen sample was then diluted to 100 x 106 spermatozoa/ml with a dried skim milk glucose extender (EZ Mixin Original Formula, ARS, Chino, CA) or a chemically defined, milk-free diluent (INRA 96, IMV, Maple Grove, MN). After 1 h slow cooling and equilibration to 4°C, semen samples were centrifuged for 10 min at 400xg. A defined volume of supernatant was removed, so that a concentration of 1000 x 106 spermatozoa/ml was obtained after resuspension of the sperm pellet. 150 µl of semen was then added to the same semen extender used after semen collection and cryopreservation medium (Cryoguard, Minitube, Verona, WI) to obtain a final glycerol concentration of 2, 3 and 4%, respectively. This also gave a concentration of 100 x 106 spermatozoa/ml. After equilibrating samples for 1 h at 4°C, spermatozoa were loaded into 0.5 ml straws and frozen in liquid nitrogen vapor. After 10 min, straws were plunged into liquid nitrogen. Semen was thawed at 37°C for 30 sec and evaluated as prior to cryopreservation. Mean total semen volumes were 56, 11, and 60 ml in the 3 stallions. Their respective mean sperm concentrations were 124 x 106, 505 x 106 and 161 x 106 sperm/ml. Mean percentages of progressively motile sperm prior to cryopreservation were 64, 89 and 72%. With the paired Students t-test, percentages of progressively motile sperm after cryopreservation were evaluated with respect to semen extender and concentration of glycerol used. Mean overall progressive motility of spermatozoa after cryopreservation differed significantly between the two extenders and was 46% for INRA 96 and 35% for EZ Mixin OF (p < 0.001). Using EZ Mixin OF as semen extender, best mean post thaw progressive motility was achieved with 4% glycerol (39%) and differed significantly from 2% glycerol (32%; p<0.01). When INRA 96 was used (49% for 4% and 42% for 2% glycerol), there was no difference. These results provide evidence, that, during freezing of equine spermatozoa, there is a significant effect of the semen extender and the concentration of the cryoprotectant on post thaw sperm motility. We therefore suggest mini-freezing trials prior to freezing large numbers of sperm to find the semen extender and glycerol concentration, which provides optimal survival rates.

Col: M Aceves, J Scherzer, L Ray, G Heusner and R A Fayrer-Hosken (jscherzer@vet.uga.edu)
Wound management of the distal limb in the horse using topically applied esterified hyaluronic acid

Wounds to the distal limb are commonly encountered in equine practice. The severity of the wound and paucity of available skin most often preclude primary suture closure of these wounds, leaving healing by second intention as the only available treatment. Unfortunately second intention healing of wounds of the distal limbs of horses is almost always complicated by the development of excessive granulation tissue, leading to prolonged convalescence, loss of athletic ability, inferior cosmesis, increased morbidity, and increased expense and frustration to the horse owner.

Wound healing is largely regulated through a multitude of transforming growth factors (TGF-β 1 and 3) released from cells in response to injury. These factors in turn alter the expression of enzymes such as matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMP) which regulate collagen synthesis and degradation.

Exogenous hyaluronan (HA), a non-sulfated glycosaminoglycan has been used extensively for intra-articular treatment of synovitis and degenerative joint disease, adhesiolysis and adhesion prevention, and various intra-ocular conditions. More recently, hyaluronan has been shown to significantly increase the rate and quality of wound healing in people. The exact mechanism by which hyaluronan enhances wound healing is not clear, but likely involves the regulation of cellular proliferation and signaling.

Samples taken, at regular intervals from the equine lower limb, following topical application of an esterified hyaluronic acid (Hyalofill) are currently being processed. Preliminary results assessing the effect that topical hyaluronic acid might have on the rate of closure of a wound through contraction and epithelialisation do not show an appreciable difference to the control situation.

Histological evaluation of the influence that topical hyaluronic acid has on rate of blood vessel ingrowth (angiogenesis) and degree of inflammatory response is underway. Immunocytochemical determination of the concentrations of the growth factor TNFa and in situ hybridization techniques assessing TGF-β 3 and Collagen III levels is also being performed. Looking at these factors will give some indication as to how the topical product influences the wound environment. In collaboration with the Department of Pharmacology and Toxicology at the School of Veterinary Medicine, in Hannover, Germany, frozen tissue samples are also being run in order to determine levels of the aforementioned MMPs.

While it appears that, under the conditions of this study and using the esterified hyaluronic acid product, there might be no immediate indication for its use alone, in promoting wound healing, the results of the study will provide valuable baseline information on the safety of this product for use in horses. This product has been used as a vehicle in a plethora of recent studies in the human field. It has proven valuable in providing a matrix for deposition of keratinocytes, osteoblasts, chondrocytes, mesenchymal stem cells and hepatocytes at specified target locations. Similar indications may warrant its future use in equine medicine.

PI: PO Eric Mueller (emueller@vet.uga.edu)
**Development of novel antiviral drugs: inhibition of avian influenza by RNAi**

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 than 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, to date. Future studies focus on testing lead candidates for efficacy against HPAI in culture and testing for efficacy in animal challenge models. Candidate siRNAs from these screens could be developed as a novel therapeutics and prophylactics for poultry flocks and workers to protect against HPAI infection.

**PI: S. Mark Tompkins**

**The Effect of *Mycoplasma synoviae* (MS) challenge at the onset of lay, on performance of table egg layers and typing of MS and *M. Gallisepticum* (MG) isolates from field cases.**

This project was initiated because of concerns that in many cases seroconversion for MS appeared to be delayed, thereby complicating the early detection of outbreaks. The objectives were as follows:

1. To determine if delayed or negative serological responses to MS by agglutination, HI, or ELISA can be reproduced by upper respiratory tract challenge with very low numbers of organisms, or if there is something unique about MS strains which have been isolated from flocks exhibiting poor seroconversion.

2. To determine, in such cases, whether ELISA or HI testing can be relied upon as screening serological tests to substitute for agglutination, or if such monitoring will need to be conducted by PCR and/or culture.

3. To fully characterize, by DNA fingerprinting, virulence and antigenic differences, such field isolates found to be atypical or unique with regard to poor serological responses.

Chickens were challenged by intratracheal administration of 10-fold dilutions of two challenge organisms (F10-2AS, a commonly used challenge strain, and K5664, an isolate from a field case with atypical serological results) to determine if low challenge doses readily infect chickens and if they seroconvert normally. There were 10 principals and 2 contact controls per group, and there was one group of unchallenged controls. Chickens were sampled weekly until 8 weeks post challenge and tested by culture, serum plate agglutination, HI (with standard WVU 1853 antigen, and antigens prepared from F10-2AS, and K5664), and IDEXX and Synbiotics ELISA. The results can be summarized as follows.
1. Challenge with as few as 76 color change units (ccu) of K5664 and 24 ccu of F10-2AS readily infected chickens – the majority were culture positive at 7 days post-challenge.

2. Infection spread readily to the in-contact controls. With F10-2AS, contact controls were positive by culture by 1 week post-challenge, and with K5664, they were positive by 2 weeks post-challenge.

3. The unchallenged controls remained negative by culture throughout the study, even though they were maintained in floor pens in the same house as the challenged groups. There were 2 empty pens between the controls and an infected group.

4. All groups of challenged chickens responded normally by all serological tests. Groups with the lowest challenge groups seroconverted about 1 week behind all of the other groups, but then developed a normal serological response.

5. Antigenic differences among strains were clearly detected by using homologous and heterologous HI antigens. The homologous antigen generally detected an earlier and stronger antibody response than did the heterologous antigens. Nevertheless, all HI antigens detected a positive antibody response.

6. All serological tests exhibited adequate sensitivity and specificity. The groups challenged with the lowest numbers of organisms generally had a weaker antibody response with the IDEXX ELISA kits than with the Synbiotics kits with the lowest challenge levels with both strains.

7. There was no evidence that strain K5664 induces an atypical antibody response.

To summarize, chickens were readily infected with very low challenge doses of MS, and seroconversion was delayed only about a week in those groups. We do not consider this to be abnormally slow. Infection spread readily to contact controls, but unchallenged controls in the same house remained uninfected, suggesting rapid transmission by contact, but no evidence of infection by aerosol. Only the IDEXX ELISA kits were insensitive in detecting antibody with the lowest challenge doses. There was evidence of serological variability among strains with the HI test, but not to the extent that HI antigens made by any of the strains tested would have been inadequate in detecting seroconversion.

PI: Dr. S.H. Kleven (skleven@uga.edu)
C0-PI: Dr. Ziv Raviv and Naola Ferguson
**Virology**

**Cellular activation induced by FIV vaccine strain viruses**

Feline immunodeficiency virus (FIV) naturally infects cats causing AIDS similar to that induced by HIV in humans. Critical to development of an effective commercial FIV vaccine was the identification of which FIV viruses to include in the vaccine; however, the mechanisms underlying why certain FIV isolates could be used to make a protective vaccine while others could not are not well understood. The central hypothesis for these studies was that strains of FIV that are effective vaccine immunogens induce characteristic patterns of cellular activation in feline T-cells.

The specific aim was to identify unique cellular transcription factor activation patterns for FIV vaccine isolates. The experimental approach characterized virus-induced changes in transcription factor activation profiles, using a feline T-lymphocyte cell line inoculated with different FIV isolates, including those used in the FIV vaccine. In addition, the transcription factor activation profiles of FIV-infected cells treated with a protein known to enhance FIV replication were also determined. Transcription factor activation profiles were detected using protein/DNA arrays that can simultaneously assess activation of multiple transcription factors.

Our results indicate that variations in pathogenicity and ability to infect and replicate in cultured feline T-cells between FIV isolates is associated with differences in transcription factor activation. Further studies are currently underway to confirm and compare these differences. The results of these studies were used as preliminary data for several grant applications, including one that was submitted to the National Institute of Health, and will be included in a scientific paper that is currently in preparation. Future studies will build on these results in order to provide a better understanding of protective immunity against FIV. A better understanding of these mechanisms will improve our knowledge of AIDS pathogenesis and provide information important for development of diagnostic tests that can distinguish between vaccinated cats and those naturally infected with FIV- a major problem with the current antibody-based diagnostic FIV tests.

**PI: Elizabeth W. Uhl (euhl@vet.uga.edu)**

**siRNA Molecules for Disease Intervention Against Poxviruses**

Poxviruses have been studied for more than 200 years. From the time of Edward Jenner’s pioneering vaccination experiments in 1796 to the present, poxviruses that infect nearly every vertebrate animal on the planet, from crocodiles to humans, have been identified. In addition, smallpox was the first disease to be eradicated by man.

While nearly 30 years have passed since smallpox was declared eradicated, poxviruses continue to plague both animals and man as a zoonotic pathogen. For example, in 2003, 71 people in the United States became infected with monkeypox virus after interaction with prairie dogs. Based on analyses of sera for poxvirus-specific antibodies, a wide variety of animals including rats, rabbits, prairie dogs, and squirrels may also be potential reservoirs of poxviruses. Given the potential impact on both animals of agricultural importance and human health, new treatments for acute poxvirus disease are needed.

As a result of the termination of the smallpox vaccination program in 1972, approximately 42% of the current U.S. population has no immunity to poxviruses. Furthermore, there are no antiviral drugs approved by the F.D.A. or U.S.D.A. for the treatment of poxvirus infection in humans or animal. To bridge this unmet need, cutting-edge technology based on RNA interference (RNAi) was used as a therapeutic approach to inhibit poxvirus replication. RNAi is an evolutionarily conserved, gene-silencing mechanism in which small (19-23 nt) double-stranded RNA molecules, or small interfering RNAs (siRNA), target RNAs for destruction with exquisite potency and selectivity, causing gene silencing at the post-transcriptional stage before proteins can be made.

Using DNA sequence information from a variety of different poxvirus genomes, 88 candidate siRNA molecules specific for 30 different poxvirus genes were designed and synthesized. The resulting siRNAs were tested for their abilities to inhibit the replication of both
Vaccinia virus and Cowpox virus in vitro. siRNA molecules specific for 9 different viral genes were able to efficiently block poxvirus replication in vitro. These studies revealed that: 1) RNAsi can be used to inhibit the replication of poxviruses, and 2) transfection of cells with siRNAs can dramatically reduce the ability of both Vaccinia virus and Cowpox virus to replicate. These data suggest that poxvirus-specific siRNAs may be useful for the treatment of animal and human infection, and can be used to identify viral genes that may be targets for other potentially useful therapies such as small molecule inhibitors.

PI: Jeff Hogan (jhogan@vet.uga.edu)

Production of polyclonal antibodies against ILTV glycoprotein E using Synthetic Peptides

Infectious laryngotracheitis virus (ILTV) is an alphaherpesvirus worldwide distributed and causes acute respiratory disease in chickens with high morbidity, decreased growth and egg production and causes moderate mortality. The herpesviruses have glycoproteins located on the viral envelop, which play essential roles in viral attachment, penetration, assembly and egress, and also in viral replication and cell-to-cell. Polyclonal antibodies against ILTV glycoproteins are an important tool to study ILTV the glycoproteins functions, as well as to detect viral replication and protein expression. This study describes the use of synthetic peptides to produce specific ILTV glycoprotein (gE) antibodies. A peptide of 18 amino acids was selected to immunize mice against generated polyclonal serum. The antibodies generated reacted with ILTV infected cells and gE transfected cells by immunofluorescent assay (IFA) and immunoblot assay. The gE peptide sequence showed to be useful to produce specific ILTV gE antibodies, which can be used to study the expression of this ILTV essential protein during in vitro propagation of the virus, and in vivo during different stages of infection.

PIs: Ivomar Oldoni, Sylva Riblet and Maricarmen Garcia (mcgarcia@uga.edu)

Runting and Stunting (AV-111)

In late 2004-present, broiler companies throughout the United States had increasing cases of a runting and stunting syndrome (RSS), particularly in the winter and spring. Currently, the few broiler companies in the Southeast that were spared encounters with RSS have reported severe outbreaks in several of their complexes. RSS is a transmissible disease affecting young broilers between 1-2 weeks of age. Most notably, RSS causes severe weight suppression during the first few weeks of age, lack of flock uniformity, diarrhea, distention of the intestines, and a significant increase in feed conversion. Cystic enteropathy is consistently observed by histopathological examination of small intestine samples from affected flocks. The economic effect of this disease hits both the broiler company and contract growers. Although descriptions of RSS date back to the 1970s, the etiologic agent(s) has yet to be identified. All evidence suggests this is a multifactorial disease.

Virological studies conducted on RSS at PDRC over the past year have been aimed at isolating potential pathogens. The disease is reliably reproduced using filtered intestinal homogenates from clinically affected broilers, thus implying viral etiologic agents. In addition, our in vivo studies indicate that heat treatment does mitigate some of the affects of RSS. We have isolated and characterized many viruses from field cases of RSS. As expected, numerous virus isolates were of vaccine origin or not considered to play a role. However, novel reoviruses and chicken astroviruses were isolated and characterized. Molecular characterization of the S1 gene revealed novel reoviruses that are <60% similar at the amino acid level to any vaccine used in the U.S. Specifically, the reoviruses isolated from cases of RSS were phylogenetically most similar at the amino acid level to malabsorption isolates from Europe. In vivo studies in day of age broilers and SPF's revealed a significant weight suppression in the novel reovirus challenged birds. Molecular characterization of the capsid gene of chicken astroviruses (CAstV) isolated from RSS flocks reveals approximately 85% amino acid similarity to avian nephritis virus, a recently classified astrovirus. Continued studies are aimed at determining the pathogenicities of single and dual challenge with the CAstV and Reo in an effort to identify etiologic agent(s) involved with RSS.

PI: Dr. Holly Sellers (hsellers@uga.edu)
Co-PI: Dr. Guillermo Zavala
Allen, Sheila. Core Animal Diagnostic Laboratory: NAHLN: GA. USDA-CSREES. $300,834
Allen, Sheila. Section 1433 Animal Health and Disease Research Funds FY2006. USDA-CSREES. $102,880
Allen, Sheila. Promoting cultural diversity in the veterinary workforce. USDA-CSREES. $120,000
Barton, Michelle. Relative adrenal insufficiency in critically ill neonatal foals. American College of Veterinary Internal Medicine Foundation. $9,485
Blas-Machado, Uriel. Pathogenesis and virulence of a bovine enterovirus-1 isolate in cattle - year 2. USDA-CSREES. $15,000
Brown, Corrie. Delivering training on necropsy procedures and coordinating national planning for animal health Afghanistan. USDA-FAS Foreign Ag. Serv. $13,202
Brown, Corrie. USDA Research Support. USDA. $3,850
Brown, Corrie. USDA Research Support. USDA. $15,450
Brown, Corrie. Technical consultant animal health infrastructure Afghanistan, Standard Cooperative Agreement. USDA-APHIS. $7,784
Brown, Corrie. Detection of key factors in the development of Fibropapillomas in sea turtles; Puerto Rico. Morris Animal Foundation. $28,620
Brown, Corrie. Development of a histopath research platform at the research foundation in tropical diseases - Cameroon. Mectizan Donation Program. $37,180
Budsberg, Steven. A placebo-controlled study to investigate the efficacy of a tachykinin receptor antagonist in a urate induced arthritis model. Boehringer Ingleheim. $198,929
Budsberg, Steven. In vivo protocol for testing the effects of Firocoxib on whole blood, gastric mucosal and osteoarthritic synovial fluid prostaglandin and leukotriene. Merial Limited. $126,000
Budsberg, Steven. The analgesic effects of EAA-129 (WAY-209129) and PLA-902 (WAY-195902) in a canine model of osteoarthritis. Fort Dodge Animal Health. $54,158
Budsberg, Steven. Clinical efficacy and safety of an extended release formulation of tramadol HCl in dogs: a pilot trial. Farnam Companies, Inc. $71,492
Budsberg, Steven. Cyclo-oxygenase selectivity of nonsteroidal anti-inflammatory drugs in feline blood. Merial Limited. $18,397
Carmichael, Paige. Training in clinical ophthalmology and pathology. Graduate Assistantship support for Dr. Shannon Boveland. Tuskegee Univ. $28,675
Coffield, Julie. Neuromuscular targets of botulinum toxin. NIH-National Institutes of Health. $349,600
Corn, Joseph. The third reservoir of infection: M. paratuberculosis in the environment and protozoal amplification. USDA-APHIS. $75,183
Corn, Joseph. Distribution of Pseudorabies virus and Brucella suis in feral swine. USDA-APHIS. $87,500
Corn, Joseph. Exotic tick surveillance in the southeastern United States and Puerto Rico FY2005. USDA. $200,000
Corn, Joseph. Exotic arthropod surveillance in the Southeastern U.S. and Puerto Rico FY2006. USDA. $200,000
Corn, Joseph. Pseudorabies virus and Brucella suis in transitional and feral swine. USDA-Veterinary Services. $25,000
Dickerson, Harry. Clinical vaccine trail to test Tetrahymena as a protective vaccine against Ichthyophthirius in Koi: I. Vaccination. Tetragenetics, Inc. $10,000
Dickerson, Harry. Clinical vaccine trail to test Tetrahymena as a protective vaccine against Ichthyophthirius in Koi: II. Immobilization. Tetragenetics, Inc. $7,500
Dickerson, Harry. UGA 2006 Veterinary Scholars Program: A research training experience for veterinary students. Merck Merial Animal Health Grants Program. $20,400
Dickerson, Harry. Emerging and re-emerging infectious disease residency/PhD program. Merial Limited. $240,000
Dietrich, Ursula. Identification of ocular tissue MMPs within the aqueous humor outflow pathway in the canine eye. ACVO Vision for Animal Foundation. $3,000
Ferguson, Naola M. Evaluation of Mycoplasma gallisepticum vaccines in chickens. Fort Dodge Animal Health. $17,093
Fischer, John. Wildlife services disease training. USDA-APHIS. $150,000
Fischer, John. Cooperative agreement for development and evaluation of data relative to disease relationships that may simultaneously involve wildlife, domestic livestock and poultry. USDA-APHIS. $350,000
Fischer, John. Southeastern Cooperative Wildlife Disease Study. Fish and Wildlife Agencies. $182,280
Fu, Zhen. Personnel training. Changchun Changsheng Life Sciences Ltd. $6,000
Garcia, Maricarmen. Developing avian influenza serological tests for differentiating infected from vaccinated animals (DIVA). Univ. of Maryland. $126,584
Garcia, Maricarmen. Avian influenza virus H9N2: Characterization and control strategies. BARD - Binational Agric. Research and Dev. Fund. $50,000
Halper, Jaroslava. Degenerative suspensory ligament desmitis in horses. U.S. Equestrian Federation, Inc. $25,000
Hodge, Thomas. Identification of host genes required for viral pathogenesis, II. Hudson-Alpha Institute for Biotechnology. $287,500
Hoenig, Margarethe. Nuclear magnetic resonance, a noninvasive method to evaluate glucose and fat metabolism in the cat. Nestle Purina, Inc. $30,125

www.vet.uga.edu/research/vmes/
Hofacre, Charles. Anti-inflammatory effects of muscadine grape products on gastrointestinal diseases in chickens and rats. Paulk Vineyards/USDA. $96,500

Hofacre, Charles. Reducing Salmonella prevalence in the processing plant by breaking vertical transmission through vaccination of affected broiler-breeder flocks. U.S. Poultry and Egg Assoc. $64,576

Hogan, Robert J. Identification of Francisella tularensis-spectivid human T cell epitopes. SECEBT. $49,997

Hogan, Robert J. The influences of N- and O-linked glycosylation on the immunogenicity of the Ebola virus glycoprotein. Southern Research Institute. $311,322

Hondalas, Mary K. Virulence of the opportunistic pathogen Rhodococcus equi. NIH-National Institutes of Health. $331,200

Hondalas, Mary K. Needle-free vaccination via nanoparticle aerosols. Harvard University. $119,779


Jaso-Friedman, Liliana. A novel telost pattern recognition receptor and its role in antimicrobial innate immunity. USDA-NRI. $300,000

Kaplan, Ray. Sustainable control of gastrointestinal nematodes in small ruminants. Fort Valley State University. $22,482

Kaplan, Ray. Use of condensed tannin-containing plants to control gastrointestinal nematodes in sheep and goats in the southern USA. Fort Valley State University. $50,000

Kaplan, Ray. Maintenance of gastrointestinal nematodes for in vitro drug efficacy testing. Divergence, Inc. $2,996

Keel, Kevin. Epizootiology of latent and active duck plague virus infection in free-flying waterfowl. Morris Animal Foundation. $98,660

King, Christopher. VCA: A monoclonal antibody toolkit for functional genomics of plant cell walls. NSF-National Science Foundation. $92,736


Maurer, John. A molecular approach for creating Haemophilus paragallinarum vaccine strain thru site-directed mutagenesis. Fort Dodge Animal Health. $53,750

McCall, John. Furnish Brugia malayi adult worms and/or Brugia malayi infective larvae. NIH-National Institutes of Health. $139,499

McCall, John. Filariasis research reagent resource center. NIH-National Institutes of Health. $422,240

McCall, John. Transfection and promoter analysis of Brugia malayi. Univ. of Alabama. $20,000

Miller, Doris. BSE Surveillance. USDA-APHIS. $372,264

Miller, Doris. Classical swine fever surveillance. USDA. $208,793

Miller, Doris. Athens Diagnostic Laboratory. GA Dept. of Agriculture. $1,324,323

Moore, James. leukocyte activation in the developmental period of equine laminitis. Ohio State University. $133,820

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Rawlings, Clarence. Comparison of carbon dioxide laser and 4 MHz radiofrequency for feline onychectomy: Histologic comparison. Ellman International, Inc. $23,017

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Ritchie, Branson. Research Associate In Exotic/Zoo Infectious Disease And Pathology Postgraduate program. Zoo Atlanta/Riverbanks Zoo. $13,000

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Sum, Steffen. Determination of the role of rickettsial pathogens in naturally infected dogs. IDEXX, Inc. $8,000

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Tripp, Ralph. Phase I: Calf study. Numico Research Australia Pty. $16,065

Vandenplas, Michel. Use of microarrays to characterize endotoxemia in vivo. Grayson-Jockey Club Research Foundation. $22,745

Varella-Stokes, Andrea. Infection dynamics of Ehrlichia chaffeensis. NIH-National Institutes of Health. $105,939

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Yabsley, Michael. Molecular and biological characterization of Trypanosoma cruzi from United States. NIH-National Institutes of Health. $221,250
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