VMES 2009

Science in Service to Animals℠

Swine Flu

The 2009-2010 Pandemic

33rd Annual Report

Veterinary Medical Experiment Station • College of Veterinary Medicine • The University of Georgia

Contents

Director: Dr. Harry W. Dickerson
Managing Editor: Dr. Lari M. Cowgill
Associate Editor: Renita Anthony
Designer: Brad Gilleland
Illustrators: Brad Gilleland, Kip Carter
Photographer: Christopher Herron

The University of Georgia

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33rd Annual Report

July 1, 2009 to June 30, 2010

Overview, Mission, & 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.

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

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

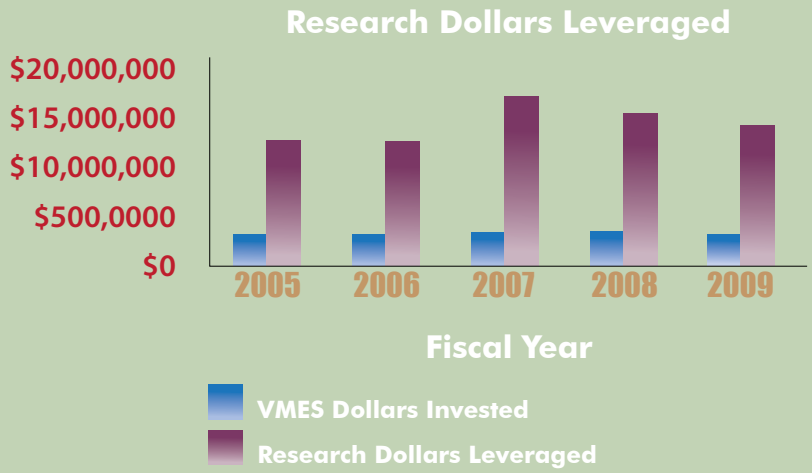
Report of the Director

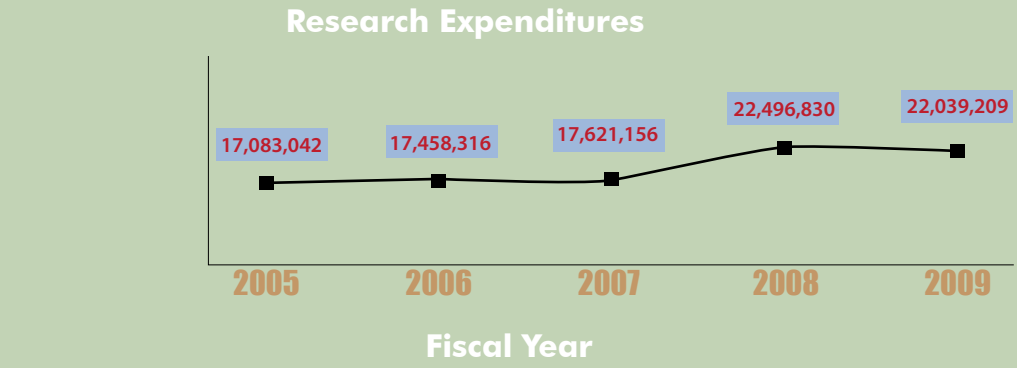
One of the most gratifying characteristics of veterinary research is that scientific investigation with animals often directly benefits the animals themselves. "Science in Service to Animals" has always been a guiding principle for the University of Georgia Veterinary Medical Experiment Station. The concept of research for animals is important in light of the role that all colleges of veterinary medicine play in training veterinarians and future veterinary researchers. And yet, as states struggle with diminishing budgets, state-supported veterinary colleges must look more and more to federal and private funding sources for the conduct of biomedical research, the majority of which is targeted to humans and not animals.

How can colleges of veterinary medicine compete for extramural biomedical research dollars against much larger and more numerous medical colleges and research institutes, and at the same time sustain a focus on animal health? The answer is to recruit scientists who can integrate human and animal health research, provide those investigators with a stimulating work environment, and support their creativity and productivity.

The merging of human and animal research is actually becoming more common in post-genomic biomedical research, which increasingly recognizes the "one health-one medicine" holistic relationship of humans, animals and the environment. The swine flu H1N1 pandemic that is highlighted in this year's VMES Annual Report is an excellent and timely example. This year's cover story by Drs. Tripp and Tompkins provides insights into the transmission of virus from animal to human populations and strategies toward disease control. The UGA College of Veterinary Medicine is in a unique strategic position to address basic and applied biological questions such as swine flu at the molecular/cellular, organism, and population/environmental levels.

The VMES supports research that directly impacts the health of animals and livestock in the State of Georgia. VMES non-personal funds also are used to initiate extramurally funded (federal or private) animal health research programs at the University of Georgia, College of Veterinary Medicine. Over the past five years we have managed to directly leverage $575,000 of VMES project support into over $17 million in extramural research awards. Projects supported by VMES are reviewed by veterinary scientists to ensure quality of the science and guarantee that they address important disease problems. The research must be innovative, applicable to animal health, and solve current health problems that affect animals in Georgia.





A summary of the College's research funding is provided above. Over the past year approximately four research dollars were leveraged for each VMES dollar invested. Expenditures are from all sources including State Appropriations, Extramural Research Funding, Donations - Includes all expenditures including personnel costs.

Veterinary medicine is an indispensable component of Georgia's public health system. Veterinary researchers protect animal and human health by detecting, preventing and controlling infectious diseases, and their research ensures the safety and security of our food supply. The continued commitment by the State of Georgia to support basic and applied research on animal health of immediate concern to the population is a critically important investment. The food animal industries of Georgia are valued at well over $3 billion and sales of livestock, poultry and their products account for more than half of Georgia's annual farm income. Protection of these resources by detection and elimination of new and emerging diseases is paramount to the growth of the State's animal-based economy. Through support of basic and applied studies by new and established veterinary researchers and the transfer of new animal health technology to the production unit, the Veterinary Medical Experiment Station plays an important role in sustaining and enhancing profitability in food animal production while maintaining reasonable costs to consumers for animal products.

Swine Flu:
The 2009-2010 H1N1 Pandemic

Seasonal influenza epidemics affect up to 15% of the global population annually, and are responsible for up to 3–5 million cases of severe disease and 500,000 deaths/year [1]. While influenza (flu) occurs among all age groups, the young, elderly, and those with underlying chronic conditions are especially vulnerable. Over the course of a flu season occurring typically from October through May in the United States [2], different flu types (A & B) and subtypes of flu A circulate and can cause epidemic illness. Flu frequently undergoes minor mutations that lead to gradual changes in its surface proteins, hemagglutinin (H) and neuraminidase (N), a process called "antigenic drift". Antigenic drift produces new virus strains that may not be recognized by the body's immune system which is why we can be repeatedly infected throughout our lives, and why we need to develop new vaccines to match the currently circulating strains. Another type of mutation that infrequently occurs in flu A viruses is called "antigenic shift", which is a sudden and major change in H and/or N surface protein expression. When this occurs, most people have little or no protection against the new virus, which generally leads to pandemics.

The world has experienced at least three flu pandemics in the 20th century, occurring in 1918, 1957 and 1968 caused by H1N1 (Spanish flu), H2N2 (Asian flu) and H3N2 (Hong Kong flu), respectively [3]. As we look back in history a repeating pattern can be observed, i.e. a limited wave of infections in the first year followed by global spread in the following year. Pandemic flu causes considerable morbidity, mortality and economic disruption when it emerges. In late March/early April 2009, new swine-origin flu A (S-OIV) H1N1 virus emerged in Mexico and the southwestern United States [4]. In a matter of weeks, the virus spread worldwide by human-to-human transmission causing the World Health Organization to declare a pandemic alert on June 11, 2009 confirming S-OIV H1N1 as the first flu pandemic for the 21st century.

Studies with one of the first S-OIV H1N1 isolates (A/California/04/09; CA/09) indicated that it originated from a triple-reassortant swine flu A virus that had been circulating in North American pig herds since the end of the 1990s [5]. The CA/09 H1N1 isolate was shown to be triple-reassortant flu A virus that contained an H gene derived from the 1918 swine flu virus, and other genes from human, avian, and Eurasian swine flu viruses. Clinically, it was shown to behave similarly to seasonal flu with the only differentiating characteristic attributed to vomiting and diarrhea in a quarter of infected patients which is rare in seasonal flu infections [6]. Another unique feature of S-OIV H1N1 is the unusual robustness for emerging outside the normal flu season causing disease in the summer months of the northern hemisphere. Many questions await answers, including the clinical impact of the pandemic as the second wave approaches, vaccine efficacy and availability, and the future destiny of the virus.

Despite the potential impact of S-OIV H1N1, and the possibility that mutations and/or acquisition of genes derived from other human or animal flu viruses could make the virus more virulent, there is strong evidence that that a serious pandemic is not imminent. The S-OIV H1N1 virus lacks a key molecular signature of virulent flu strains (e.g. 1918 flu virus), i.e. the PB1-F2 protein which has been shown to contribute to pathogenesis [7]. Further, recent evidence indicates that a substantial portion of the population likely has some cross-reactive immunity against the virus from previous exposure to H1N1 viruses or via vaccination [8]. This cross-protective "herd immunity" most likely will limit the magnitude of clinical disease attributed to S-OIV H1N1 virus. In addition, most S-OIV H1N1 virus still remain sensitive to neuraminidase inhibitors, so drugs like Tamiflu™ are generally effective.

Our government has also taken major steps to address flu and as an example has initiated five Centers of Excellence for Influenza Research and Surveillance (CEIRS) across the country. Two CEIRS centers have faculty members in the College of Veterinary Medicine, and one is a CEIRS consortium between the university and Emory University in Atlanta. Goals of the UGA CEIRS are to work with the government, industry and academics to determine the mechanisms of flu transmission, disease pathogenesis, and to evaluate vaccines and anti-viral therapeutics in a broad effort to address the H1N1 pandemic. These goals aim to diminish the future impact of flu on the health of our citizens and the economy of our country.

Ralph A. Tripp and S. Mark Tompkins

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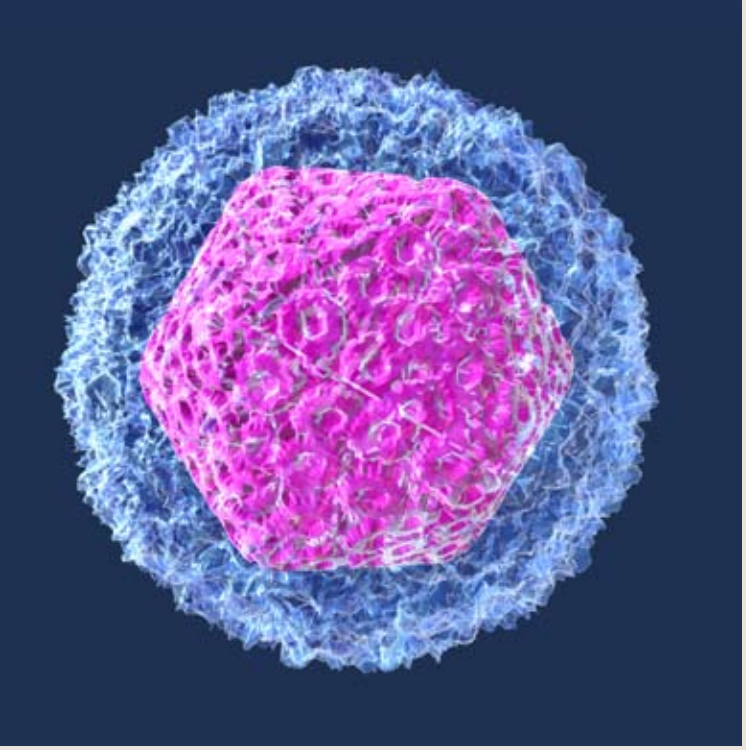
Mycoplasma Research

Strain differentiation. More poultry producers are turning to vaccination as an approach to the control of Mycoplasma gallisepticum (MG). Vaccination does not completely prevent infection with virulent wild-type MG. It is very difficult to identify wild-type MG in vaccinated flocks using the currently available techniques because these procedures do not have the sensitivity required to detect mixed MG infections. We have developed real-time PCR protocols using restriction enzymes to identify concurrent infections with wild-type MG in ts-11-vaccinated poultry. We defined a unique restriction enzyme site within the MGA0319 gene of ts-11. The site allows differentiation of ts-11 from other MG strains by restriction enzyme digest using the Taq I enzyme. Results show specificity, sensitivity, and the ability to detect ts-11 even in the presence of wild-type MG strains.

Ovarian regression. It is often necessary to challenge poultry with MG strains under laboratory conditions to characterize the strains and evaluate the efficacy of vaccines. Although most of the MG vaccines are administered to layers and breeders to protect the reproductive as well as respiratory system, many of the currently accepted challenge techniques involve the evaluation of pathology in the tracheas and air sacs of young chickens. Ovarian regression (follicle atresia) was observed in a previous study in groups of chickens challenged with MG. We propose to use the incidence of ovarian regression by gross evaluation as a simple method to evaluate the effect of MG challenge on the reproductive system of chickens in production. The study is ongoing.

Vertically transmitted ts-11? MG vaccines have been used in the field for many years with little evidence of vertical transmission of the vaccine to progeny. We have identified a "ts-11-like" MG isolate from broilers who were the progeny of breeders vaccinated with ts-11. We attempted to differentiate the isolate from ts-11 by RAPD (a type of PCR procedure) and sequencing of 4 target genes. We have cloned the isolate and determined that it does not possess the temperature-sensitive phenotype of the ts-11 vaccine; this characteristic is frequently absent in re-isolates from vaccinated chickens. We are in the process of determining the pathogenicity of the isolate.

Principal Ivestigator: Naola Ferguson



ILTV

Towards the Development of Genetically Engineered Live Attenuated Infectious Laryngotracheitis Virus (ILTV) Strains: "Knock out / Knock in" System to Genetically Manipulate the Viral Genome

Infectious laryngotracheitis (ILT) continues to be a recurring problem in poultry in many parts of the world including the United States. Recently, recombinant vaccines using fowl poxvirus (FPV) and herpesvirus of turkey (HVT) as vectors carrying Infectious laryngotracheitis virus (ILTV) genes administered to broilers flocks are achieving inconsistent results in the field. A vaccination strategy that has not been critically considered by the industry is the use of ILTV mutants generated by the direct deletion of viral genes. Gene deletion mutants have been successfully constructed from the genome of virulent European and Australian ILTV strains. Some of these mutants grew efficiently in vitro and showed different degrees of attenuation in vivo indicating their potential as live attenuated marker vaccines. The long-term goal of this study is to establish the tools for the genetic manipulation of the ILTV genomes of United States isolates and to provide the framework to design ILT viruses expressing antigens of other important respiratory poultry pathogens. We have rescued a glycoprotein J deleted mutant of the USDA ILTV challenge strain. The attenuation of this mutant in chickens will be evaluated. We have also developed an assay to identify serological responses to specific viral glycoproteins that will facilitate the detection of infected vs. vaccinated animals using deletion mutants as marker vaccines to control the disease.

PrincipaI Investigator: Maricarmen García:
Co-Principal Investigators: Egbert Mundt and Alice Mundt

Clinical Investigation of Poultry Diseases

The objectives of this project were to investigate poultry health issues and determine solutions for poultry producers. The work is also done in conjunction with the training of the Master of Avian Medicine (MAM) students. There were several farms that local poultry companies have designated as "problem farms." These are farms that do not have an obvious disease or husbandry issue. A clinician and two MAM students were able to identify issues on these farms as primarily husbandry and help correct the situation. Research studies were completed by the MAM students resulting in 4 presentations at state and national veterinary medical meetings. Some of the health issues that were addressed this past year were an epidemiological investigation of several mycoplasma disease outbreaks in broiler breeders and an outbreak of mortality and tenosynovitis in broiler chickens that were found to result from a transovial transmission of a particularly virulent reovirus infection.

Finally, work was done to elucidate the causative agent or agents in a disease of broilers called gangrenous dermatitis. There were two papers presented by MAM candidates at the Georgia Veterinary Medical Association entitled "Epidemiology of MA and MG Infection in Georgia" by Dr. Michelle Andersen and "Field Investigation of Adenovirus Infection in Broilers" by Dr. Jeff Courtney. The diseases caused by Mycoplasma synoviae (MS) and Mycoplasma gallisepticum (MG) are both respiratory diseases that can be egg transmitted from hen to chick. When chicks receive these organisms from their parents, they will be very highly susceptible to death or suffering from respiratory disease. Understanding the source of mycoplasma from hen flocks helps the poultry industry to prevent the introduction into any additional flocks. This work by Dr. Andersen and Dr. Zavala helped the Georgia poultry industry to begin to eliminate these infections that can cost them millions of dollars. The second major investigation by the MAM candidate, Dr. Courtney, into the source of an adenovirus infection for a company in Georgia and Mississippi helped this company identify the source of this viral infection that was causing the deaths of thousands of broiler chickens.

PrincipaI Investigator: Dr. Charles Hofacre
Co-Investigators: Dr. Steve Collett and Dr. Guillermo Zavala

Evaluation of natural plant products as anthelmintic agents

Gastrointestinal parasitism is recognized as the single most important health concern for small ruminants, and this problem has recently been magnified by escalating levels of multiple-drug resistance in these parasites. In a recent study we published examining the prevalence of anthelmintic resistance on sheep and goat farms in the southern US, multiple-anthelmintic resistance to all three major classes of anthelmintics was seen on 48% of farms, and total anthelmintic failure (resistance to all three major drug classes plus moxidectin) was seen on 17% of farms. Thus, it is imperative that anthelmintic drugs be used in a more limited and intelligent manner, and that novel non-chemical approaches to parasite control be developed and implemented.

One such approach is the use of natural plant products/extracts that demonstrate anti-parasitic activity. Two plants that are of special interest to our laboratory are pumpkin seed and pineapple leaf. Both of these are readily available inexpensive by-products that have demonstrated anti-parasitic activity in some studies. For the evaluation of in vivo efficacy of ground pumpkin seeds and pineapple leaves against gastrointestinal nematode parasites of goats, 30 goats were stratified by body weight and fecal egg count (FEC), and assigned randomly within block to 1 of 3 treatment groups: pumpkin seed, pineapple leaf, and untreated controls. Goats were treated once per week for 4 weeks. The ground pumpkin seed and ground pineapple leaves were administered orally at the rate of 1 g/kg and 300 mg/kg, respectively. Body weight, FEC, packed cell volume (PCV), and FAMACHA eye scores were measured once per week starting two weeks before the initiation of treatment and were continued weekly throughout the treatment period and for two weeks after the last treatment.

During the course of the study four animals (two controls, 1 pineapple, and 1 pumpkin seed) became clinically anemic (PCV <15%), and were treated with moxidectin and removed from the study. Average FEC over the course of the study were 1025, 851, and 702 eggs per gram of feces (EPG) for the pumpkin seed, pineapple leaf, and control groups, respectively. An un-stacked one way ANOVA statistical analysis of the weekly average fecal egg count for all groups was performed using Minitab 15 statistical software. The comparison yielded a p value of 0.233 indicating there was no statistically significant difference between the weekly average FEC for the three groups over the course of the study.

Though these results were disappointing, it may be a product of the experimental design used. The optimal dose rate and treatment frequency for these plant products is unknown, so it is still possible that these products do have antiparasitic activity, but that we failed to attain adequate levels of the active compounds in our test animals. The treatment frequency (1X/week) was selected since this is a typical interval used with natural worm remedies that are commonly sold (none of which have been proven to be effective). We hope to repeat this study using a daily treatment regimen instead of a weekly one. Such a design will permit a definitive evaluation of the potential usefulness of these products as natural/novel antiparasitic agents. Multiple-drug resistance in gastrointestinal nematode parasites currently threatens the viability of the growing small ruminant industry in the southeastern U.S. Thus, it is imperative that research into novel non-chemical approaches to parasite control be continued.

Principal Investigator: Ray M. Kaplan, DVM, PhD, DEVPC, Associate Professor
Co-Investigators: Dr. Lisa Williamson, Sue B. Howell, Bob Storey

Intranasal administration of a novel midazolam gel formulation for the treatment of seizures in dogs

Persistent seizure activity lasting longer than 5 minutes is called status epilepticus (SE). Status epilepticus is a medical emergency requiring immediate therapy and benzodiazepines, such as midazolam, form the cornerstone of such therapy. Midazolam has a marked anticonvulsive effect in humans and animals, being more effective than comparable doses of diazepam. Some dogs may experience multiple episodes of SE during their life, which is both a major health issue for the patient and has a significant financial impact on the owner. Reports on the mortality of SE range from a few percent to, in one study, over 50% in humans. The human mortality associated with SE depends on the age of the patient, the cause of the seizures and the duration of SE. Similar studies are limited in veterinary medicine but death or euthanasia associated with SE in dogs has been reported to be approximately 25%. Rapidly effective treatment options for SE therefore need to be available to both the veterinarian and the owners of dogs which exhibit this devastating disease.

Since intravenous (IV) and intramuscular (IM) injections are impractical to administer during SE by a veterinarian or dog owner, other routes of administration have recently been investigated. Intranasal (IN) anticonvulsants have been used successfully in human SE. Rapid absorption and attainment of therapeutic levels have been demonstrated for IN diazepam and midazolam in dogs; however, the parenteral formulations (formulations administered outside the alimentary tract) are impractical to use in large patients because of safety and dosing issues. These problems could be avoided by the use of midazolam formulated as a bioadhesive gel. Bioadhesive gel formulations, which utilize unique polymers such as carbopol, have been shown to improve adherence time, transmucosal absorption and bioavailability of drugs when administered IN. We hypothesized that Intranasal administration of a bioadhesive midazolam gel formulation will have improved pharmacokinetic properties to that of IN-administered parenteral midazolam solution. Our objectives were to compare the pharmacokinetics of a novel carbopol midazolam gel formulation administered IN to the pharmacokinetics of parenteral midazolam when administered IN and IV in dogs.

Preliminary findings in this study confirm that IN administration of the novel midazolam gel has comparable pharmacokinetics to intravenous administration and superior results to intranasal administration of the parenteral formulation. This carbopol gel formulation of midazolam is anticipated to have a large impact on the treatment of canine SE by veterinarians and pet owners, reducing the complications associated with prolonged seizures.

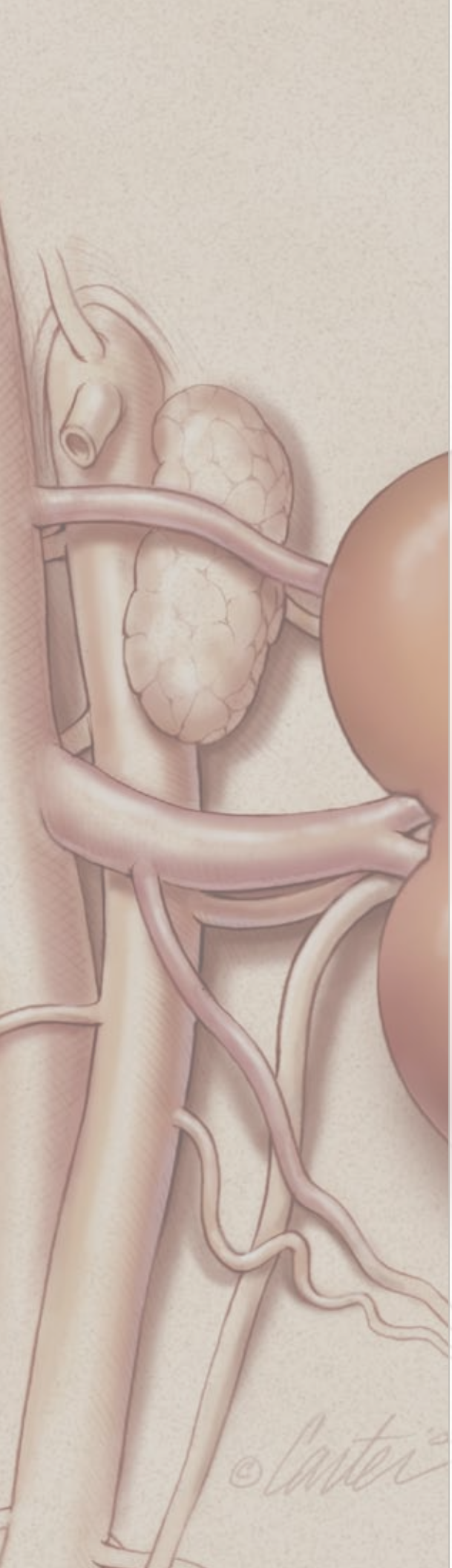
Principal Investigator: Simon Platt BVM&S MRCVS
Dipl. ACVIM (Neurology) Dipl. ECVN Associate Professor Neurology Service
Co-Investigators: Marc Kent, Scott Schatzberg

Genetic and Functional Differences Between Human and Animal MRSA Isolates

Infections caused by Methicillin-resistant Staphylococcus aureus (MRSA) are a growing public health problem in the United States, affecting thousands of people annually. More information is needed on the epidemiology of MRSA, not only in people but also in companion animals, in order to develop effective MRSA prevention practices, exposure control plans, and treatment regimens. MRSA in the US has shifted in the past 8 years from being an infrequent hospital-acquired infection to an infection that is now spreading through the community, being routinely encountered at gymnasiums and schools, disproportionately affecting our young, elderly, and the financially disadvantaged. It was estimated that 94,360 invasive MRSA cases (the most serious kind of bacterial infection) occurred in the US in 2005, with 18,650, or about 1 in 5, of those cases resulting in death. In this same study, only one Georgia hospital participated: Grady Memorial Hospital in Atlanta. The number of cases per 100,000 at Grady was an alarming 33 - the second highest of all 8 states participating in the study. The GA-Public Health Office confirms that there were 13 deaths due to invasive MRSA in 2005, eight of them involving young previously healthy individuals. Most interestingly, our investigations have shown that this increase in MRSA among people in Georgia was mirrored by an increase in MRSA in its animal population. Five years ago, only one MRSA isolate per year was identified in Georgia animals; currently, two isolates per week are reported by the State Veterinary Diagnostic Laboratory in Athens. Reports from Canada and Denmark are similar. In studies of MRSA carriage (the bacteria are present, although not causing disease) in veterinarians and veterinary students, different MRSA strains were associated with different types of clinical practice. Despite evidence such as this, there is very little work carried out to determine virulence factors associated with animal isolates of CA-MRSA, or to relate the advance of the epidemic of CA-MRSA in humans to that in animals. There are significant "one world-one health" questions that must be answered about the nexus of MRSA, people, and animals: Are our animals a reservoir of MRSAs that can be transmitted to people? Do these animals harbor MRSA and perpetuate its presence within the community? To what extent is MRSA encountered in different animals as an adapted subpopulation and not as virulent?

We identified 4823 coagulase-positive Staphylococcal isolates over a seven-year period from specimens submitted by clinicians from sick animals. 117 isolates were phenotypically MRSA and 75 of these non-nosocomial isolates were stored and tested. Additionally, 71 MRSA human isolates were randomly obtained from two local hospitals over a similar time period. In total, 146 isolates were studied. When compared with humans and horses, the prevalence of SCCmecII in dogs was significantly higher. Human isolates were more likely than equine isolates to contain SCCmecII, though this difference is not statistically significant. Subtyping of SCCmecIV isolates revealed that humans were much more likely to carry SCCmecIVa, while SCCmecIV Non-A subtypes characterized the majority of equine isolates. These two subtypes were found with nearly equal frequency in dogs. Furthermore, we described a new SCCmec type with two variants that we named SCCmecVIIGA in MRSA isolates from people in our state and in methicillin resistant Staphylococcus pseudointermedius and S. schleiferi subsp. coagulans, isolated from dogs with disease from the same region. It appears that both companion animals and people may be infected by the same strains of MRSA and that different MRSA strains vary in their ability to cause disease in different animal hosts. In addition, the resistance patterns observed by species and SCCmec type are conserved, SCCmecIV-nonA isolates are characterized by high frequencies of resistance to gentamycin, tetracycline, and trimethoprim/sulfamethoxazole in all host species, because this SCCmec type is rarer in humans but frequent in horses, the question of interaction between an MRSA patient and an equine environment may indicate which antibiotic treatment would be most efficacious earlier during infection. In conclusion, the overall increase in the prevalence of MRSA-infected animals over this study period demonstrates the need of continued research in this field. We have seen a shift in the prevalence of SCCmec types in humans from SCCmec II to IV, but not animal MRSA; that SCCmecII is more prevalent in dogs and that SCCmecIV-nonA are more prevalent in horses.

Principal Investigator: Dr. Susan Sanchez
Co-Investigator: Dr. Jeff Hogan



Aldosterone Inhibition and the Severity of Chronic Allograft Nephropathy

This study evaluated the effect of aldosterone inhibition on chronic allograft nephropathy using a rodent renal transplantation model. Chronic allograft nephropathy (CAN) is an extremely common condition in human and feline renal transplant recipients. In fact, it is the most common cause of allograft failure in people. There are many immunologic and nonimmunologic causes for CAN; however, regardless of the cause, the typical pathologic lesions are always similar and involve thickening of blood vessel walls, fibrosis of the transplanted kidney, and tubular atrophy. CAN is also typified by progressive loss of transplant function, which presents a substantial problem for human and veterinary transplant recipients. Fortunately, to study this condition, CAN can be recreated in a rodent interstrain transplantation model. A kidney from one strain of rat (Fisher F344) is implanted into another closely related rat strain (Lewis). These strains are similar enough to avoid overt rejection, but different enough to cause some immunologic damage. The result is a kidney which looks and functions like a kidney with CAN in a relatively short period of time.

Aldosterone is a hormone in the body that contributes to CAN as it has significant profibrotic effects and can contribute to renal injury in other models of renal injury. However, the role of aldosterone following renal transplantation has never been demonstrated. We evaluated the effect of an aldosterone receptor blocker called spironolactone in rats after renal transplantation. To delineate the effect of spironolactone on CAN we evaluated 1) functional changes of the transplanted kidney (serum creatinine concentration and urine protein levels) and 2) pro-inflammatory and profibrotic gene expression within the renal cortex of the transplanted kidney with and without daily spironolactone administration. In total, four groups were evaluated: 2 experimental groups and 2 control groups. The experimental groups received oral spironolactone or water daily for 4 months. The control groups underwent a one-sided nephrectomy and received either spironolactone or water, also for 4 months.

Regarding allograft function, the two experimental groups had significantly greater urine protein and serum creatinine concentration compared to the control groups. The experimental groups also had decreased graft function over time, as the urine protein and serume creatinine concentration steadily increased from baseline over the course of the study. This confirms ongoing renal damage. However, we were not able to demonstrate a difference between the experimental groups regarding renal function, as there were no differences between rats receiving spironolactone or water following transplantation.

Unfortunately, the molecular investigation of pro-inflammatory and profibrotic gene activation, as well as the histopathological analysis, are ongoing and preliminary results are not yet available.

Therefore, initial results indicate, with regard to function of the transplanted kidney 4 months following transplantation, that there is little difference between rats with or without inhibition of aldosterone, however more subtle differences may be illuminated with ongoing molecular and histopathological analysis.

Principal Investigator: Chad W. Schmiedt DVM, DACVS
Co-Investigators: Steven Cogar DVM; Christy Chessman BS, RVT; Cathy A. Brown DVM, PhD; David Hurley PhD.

Vaccination Strategies to Control Runting and Stunting Syndrome in Broilers

Runting and stunting syndrome continues to be a major problem for broiler companies across the United States. RSS is a transmissible disease affecting young broilers between 1-2 weeks of age. Most notably, RSS causes severe weight suppression during the first weeks of age, lack of flock uniformity, diarrhea, and a significant increase in feed conversion. Thus far, numerous infectious agents have been implicated but no etiologic agent has been identified. Studies in our laboratory suggest RSS is likely caused by one or multiple viruses. Early experiments provide strong evidence to suggest that nonenveloped viruses play a role in RSS. In an effort to provide the poultry industry with tools to help mitigate the effects of RSS, we pursued vaccination strategies using tools developed at the Poultry Disease Research Center (PDRC).

Twenty-week-old commercial broiler breeder hens and roosters were divided into treatment groups and placed in isolation houses. Breeder hens in each treatment group were vaccinated three times over the course of 6 weeks with one of four experimental vaccines: 1) recombinant vaccine containing a protein from a novel virus detected in the intestines of RSS affected birds, 2) inactivated RSS Reovirus, 3) inactivated RSS Astrovirus, and 4) inactivated nonenveloped virus. All vaccines were administered with an adjuvant. One group of hens and roosters were not vaccinated and served as negative controls. Once the hens started laying eggs, fertile eggs were collected from each treatment group and set to hatch. Progeny chicks were challenged by oral gavage at a day of age in a previously established RSS challenge model and placed in isolation units. At 12 days of age, all chicks were weighed, euthanized and sections of the small intestine collected for histological evaluation. Parameters used to measure protection were based on body weights and the presence or absence of cystic enteropathy in the intestine (a hallmark lesion of RSS), as well as quantity of lesions in the sections examined.

Results of the studies indicate that the experimental astrovirus and nonenveloped vaccines did not provide adequate protection against body weight suppression, formation of cystic enteropathy, or total number of cysts present in the intestinal sections. The recombinant protein and RSS reovirus vaccine did appear to lessen the severity of RSS challenge based on less severe body weight suppression and fewer cystic lesions in the intestines. While the disease was not prevented by any of the experimental vaccines, the total affects were lessened. Enteric diseases of poultry are often times multifactoral and many factors can contribute to the severity of the disease.

In ongoing studies, we continue to evaluate additional recombinant viral proteins which have been identified by analysis of the viral metagenome of the gut. In addition we are testing one vaccine with a recombinant astrovirus protein which was produced under industrial conditions in collaboration with a vaccine company using their adjuvant. The design of the study is essentially the same as above.

Principal Investigator: Holly S. Sellers
Co-Investigator: Egbert Mundt

Enhancement of Campylobacter Recovery from Carcass Rinses

Initial efforts to culture Campylobacter demonstrated the tendency for Campylobacter to spread and run on the plates making colony counts difficult due to swarming and the inability to form distinct colonies on m-Campylobacter medium without antibiotics. Distinct colony formation was necessary to establish baseline counts without the use of antibiotics. We looked into drying the agar before plating but that proved erratic and unpredictable. Next we tried increasing the concentration of agar above the normal 1.3-1.5% of agar in the media. We found that increasing the agar concentration to 2% provided good consistent colony definition and stopped the swarming seen on agars without antibiotics at standard agar concentrations.

We obtained swine mucin in dry form from Sigma and made up mucin stock, dispensed in 1 ml aliquots frozen at -70C. We also obtained chicken intestines from a processing plant, brought them back to the lab and scraped the intestinal walls to obtain chicken intestinal mucin. We found we had to add about 10% by volume sterile normal saline to allow pipetting of the chicken mucin. We obtained about 200 ml of the mucin, alcohol precipitated the material, and dispensed it in ml aliquots frozen at -70C.

Growth in and on media containing 0.2% chicken or swine mucin appeared to support good growth. Comparisons were made to assess whether concentrations other than 0.2% help or hinder growth of Campylobacter. Early results seemed to indicate a benefit of the addition of swine and to a lesser extent chicken mucin at 0.2% and 0.5% concentration. However, subsequent work has yielded erratic results and this supplement has been abandoned.

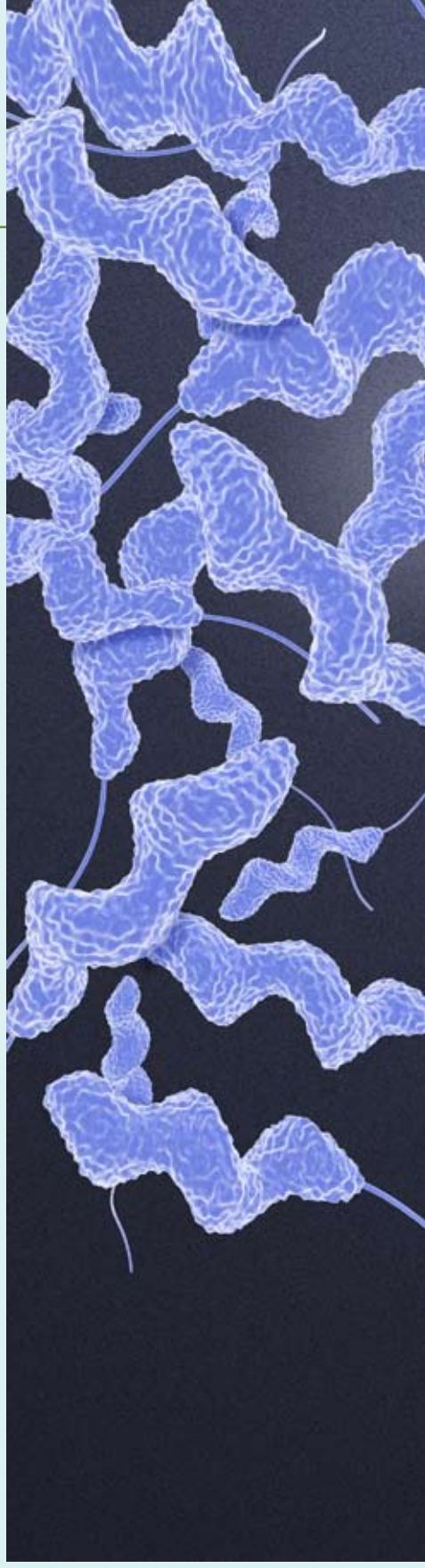
We have had success titrating Campylobacter on solid media and in 96-place deep-well microplates, using the Most Probable Number (MPN) method, and were able to demonstrate correlation of the two procedures.

The initial attempt at producing viable but non-cultivable (VNC) Campylobacter did not work. It appears that 15 minutes at 60C was too harsh but subsequent trials at 5 minutes showed some promise. We will repeat this in future work as time did not permit investigating this phenomenon within the scope of this project. The literature points out that the application of cold temperatures will do the same but time did not permit trial of this approach. For this work 24 hour cultures preliminarily provided more consistent results than 48 hour cultures.

Attempts at using 0.45 and 0.65 micron filters showed that this technique works fine for initial isolation but will not work for enumeration.

Other workers had shown that pre-incubation of MPN cultures at 37C x 4hrs and then continued incubation at 42C after the addition of antibiotics improved recovery and Campylobacter MPN titer endpoints. However, we felt that this was a little cumbersome when testing large numbers of samples and chose to evaluate different media along with pre-incubation of MPN cultures at 37C x 4 hrs with a subsequent incubation at 41C with antibiotics already in the media. Early indications showed significantly improved counts using Tecra broth which was better than Bolton's Broth; both media were better than modified Campylobacter medium. The pre-incubation procedure was preferable to incubation at 41C only in all combinations.

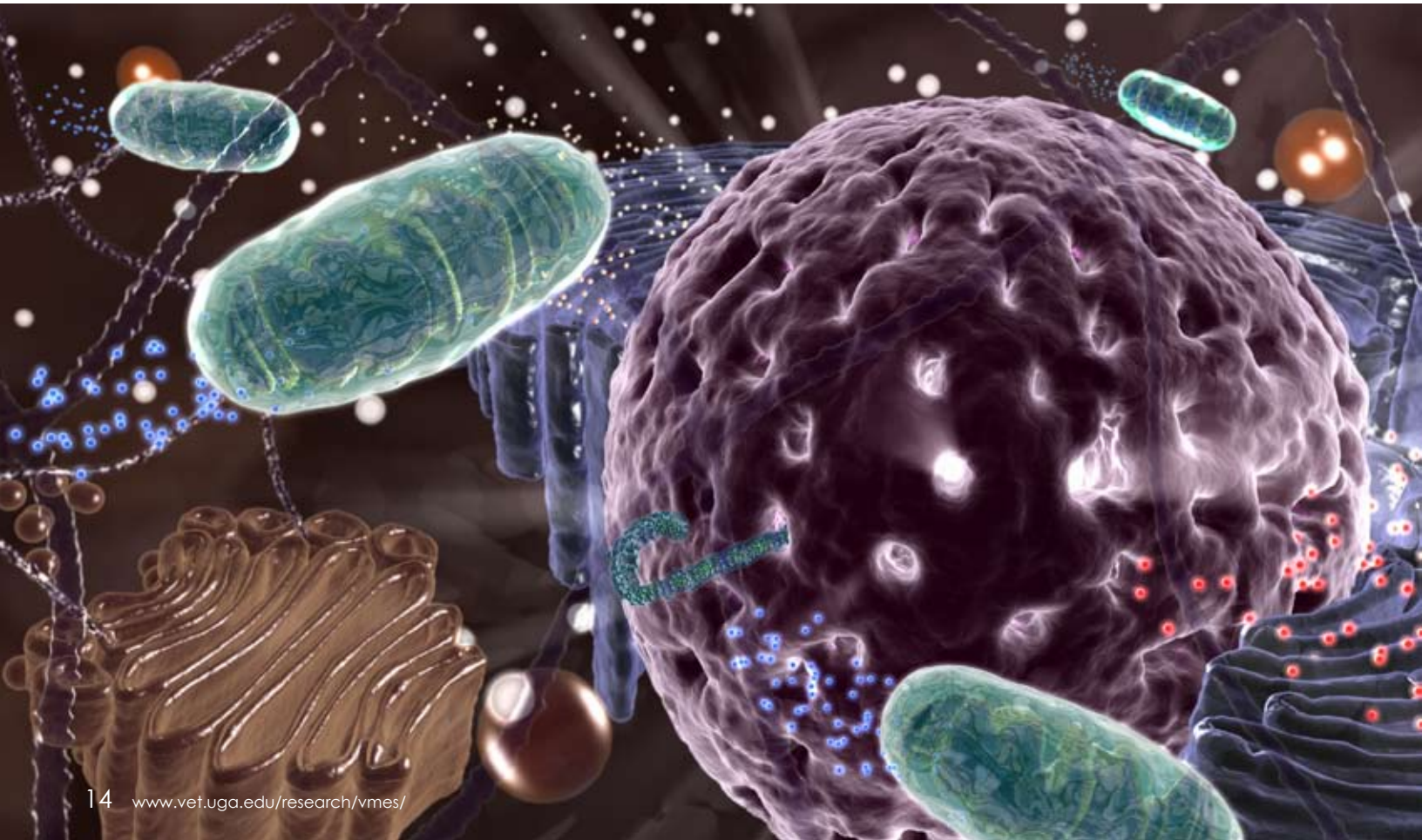
Principal Investigator: Stephan Thayer,
Co-investigators: Lisa Stabler, Roni Swift, and Margie Lee.



Identification of Tumor-Specific miRNA Profiles for Canine Lymphoma

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and have been shown to have critical roles in the development of cancer. Using miRNA microarrays, cancer-specific miRNA expression patterns have been identified in every human cancer analyzed and specific miRNA expression patterns have been associated with disease outcomes. However, the role of miRNA in canine cancer has not been investigated. Lymphoma is one of the most common malignant tumors in dogs, and the incidence is increasing. The objective of this project are to identify miRNAs in normal and neoplastic canine cells and use miRNA expression profiling of a large set of tumor samples to characterize cancer-specific miRNA signatures in dogs. Our long term goal is to use these expression profiles to develop a set of canine cancer-specific miRNA probes and test their predictive, diagnostic and prognostic potential in canine lymphomas. The data generated from this proposal will benefit dogs, their owners and veterinarians by contributing to the development of a state-of-the-art diagnostic tool to more accurately classify, diagnose, and better predict outcomes in canine lymphoma patients. Similar tools are now becoming useful, both diagnostically and prognostically, in a wide variety of human cancers. In addition, the development of canine-specific miRNA microarrays has widespread applications for the assessment of other canine cancers, as well as the potential of providing important insights into cancer pathogenesis.

Principal Investigator: Elizabeth W. Uhl
Co-Investigators: S. Mark Tompkins, Steven E. Suter



Use of Non-oncogenic Retroviral Vectors for gene delivery and in vitro Expression of Immunogenic Proteins of Chicken

The primary goal of this project is to express immunogenic proteins of chicken infectious anemia virus (CIAV) in cell lines of avian origin. The first required and accomplished step was to isolate and clone non-defective endogenous avian leukosis viruses to be used as vectors for gene delivery. Four of such viruses were isolated and three were fully sequenced. Their complete genomic sequences have been deposited in the public database GenBank and a related manuscript has been published. One of such viruses has been cloned into a plasmid vector to be used as an infectious clone for gene delivery. Subclones of this infectious non-defective ALV clone have been produced and are currently being used for gene delivery and expression using reverse genetics techniques. An additional infectious clone of a non-defective ALV is currently being used to deliver foreign genes using the same approach. Initial transfection and co-transfection studies have demonstrated that our approach is successful for gene delivery and expression. Green fluorescent protein (GFP, a reporter protein), was expressed in vitro in the DF-1 avian cell line. In addition, the hemagglutinin gene of AIV (H5), the VP2 gene of IBDV (D78), the VP1 and VP2, and VP1+VP2 protein-coding genes of CIAV (CAG-1), and the HF gene of NDV (B1B1) have been cloned into our endogenous ALV plasmid vector system. Transfection experiments after a modified approach using an ALV cell packaging line have demonstrated successful in vitro expression of IBDV, NDV and AIV proteins. Transfection experiments with the objective of expressing CAIV VP1 and VP2 independently, and VP1+VP2 jointly have been unsuccessful due to cell toxicity interpreted to be caused by the VP2 protein expressed in vitro. We are currently cloning and expressing multiple fragments spanning the capsid gene of CIAV in search of a fragment that can be expressed without toxicity and without losing its immunogenic properties. Our most important long term objective is to develop a system for production of poultry vaccines that do not require the use of chicken embryos, and a system that would be suitable for production of immunogenic proteins from fastidious poultry pathogens.

Principal Investigator: Dr. Guillermo Zavala
Co-Investigator: Dr. Taylor Barbosa

Research Contracts & Grants

Allen, Sheila. National Animal Health Laboratory Network: GA. USDA-CSREES. $298,000
Allen, Sheila. Section 1433 Animal Health and Disease Research Funds - 3rd and 4th Quarter. USDA-CSREES. $65,083
Barton, Michelle. Hydrocortisone replacement therapy in septic foals. Grayson-Jockey Club Research Foundation. $22,250
Barton, Michelle. Hydrocortisone replacement therapy in septic neonatal foals. NIH-National Institutes of Health. $43,451
Berghaus, Roy. Supplement to: Predicting Salmonella loads on broiler carcasses from pre-harvest Salmonella data collected on broiler houses. USDA/NRI (North Carolina State University). $99,369
Brown, Corrie. Characterization of cells in early response to infection with vesicular stomatitis virus. USDA-ARS. $15,000
Brown, Corrie. Delivery of training on specimen collection to Afghan veterinarians. USDA FAS/OCBD/DRDA/RDNR. $13,041
Brown, Corrie. East African Regional Workshop on transboundary diseases and impacts on trade. USDA. $41,066
Brown, Corrie. Project to develop field manual for collection of specimens to enhance diagnosis of animal diseases. USDA-FAS-ICD. $31,295
Budsberg, Steven. Evaluation of analgesic properties of perzinfotel and PLA 695 in a urate crystal model in dogs. Fort Dodge Animal Health. $112,436
Budsberg, Steven. Supplement to: Efficacy and safety of tramadol HCl extended release for the control of pain associated with osteoarthritis in dogs under clinical conditions. Farnam Companies. $1,880
Chen, Shiyou. Activation and functional analysis of response gene to complement 32 in smooth muscle differentiation from neural crest cells. American Heart Association. $58,727
Chen, Shiyou. Smad2 and smooth muscle differentiation from neural crest cells. NIH-National Institutes of Health. $709,817
Corn, Joseph. Exotic arthropod surveillance '09. USDA. $183,750
Corn, Joseph. National feral swine mapping system. USDA/APHIS. $32,788
Dickerson, Harry. Emerging and re-emerging infectious disease residency/PhD program. Merial Limited. $80,000
Dickerson, Harry. T Cells and cutaneous adaptive immunity in channel catfish. USDA CSREES. $375,000
Dickerson, Harry. The University of Georgia 2009 Veterinary Scholars Program: A research training experience for veterinary students. Merck & Company, Inc. $20,000
Dietrich, Ursula. Tolerability and safety of the Aquesys microfistula implant system. Aquesys, Inc. $194,699
Divers, Stephen. Internal sonic/acoustic transmitter implantation in sturgeon (Acipenseridae). US Fish & Wildlife Service. $40,000
Epstein, Kira. Serial Point-of-Care coagulation testing in equine gastrointestinal disease. Morris Animal Foundation. $40,975
Fischer, John. Investigation of and assistance with wildlife disease problems in the SE region of the US. US Dept. of Interior. $238,483
Fischer, John. Relationships that may simultaneously invoice Wildlife Domestic Livestock and Poultry. USDA APHIS. $350,000
Fischer, John. Southeastern Cooperative Wildlife Disease Study State Project. Various States - Tennessee. $272,477
Fischer, John. Wildlife Services. USDA/APHIS/Wildlife Services. $150,000
Fu, Zhen. Developing avirulent rabies virus vaccines. NIH-National Institutes of Health. $289,395
Garcia, Maricarmen. Are the infectious laryngotracheitis virus (ILTV) recombinant viral vector vaccines effective tools for the control of the disease? United States Poultry and Egg Association. $75,000
Garcia, Maricarmen. Validation of Avian Influenza serological tests for differentiating infected from vaccinated animals (DIVA). USDA (University of Maryland). $97,875
Gogal, Robert. Toxicity of bullet fragments and spent lead shot pellets, dosed as grit, to Northern Bobwhite quail. US Department of Defense. $109,000
Hines, Murray E. Classical swine fever surveillance. USDA. $87,252
Hoenig, Margarethe. Mechanisms of insulin resistance in cats. Nestle Purina Co. $80,000
Hogan, Robert J. Determining SARS-CoV host gene requirements by mutagenesis and RNA interference. NIH-National Institutes of Health. $180,872
Hondalus, Mary K. Evaluation of efficacy of a recombinant live Rhodococcus equi vaccine in foals. Ft. Dodge Animal Health. $63,505
Hondalus, Mary K. Nanoparticle delivery of experimental tuberculosis vaccines. Gates Foundation (Harvard University). $99,026
Hondalus, Mary K. Virulence of the opportunistic pathogen Rhodococcis equi. NIH-National Institutes of Health. $283,937
Howerth, Elizabeth. Exploration of the epidemiology and transmission of the newly emerging cattle-epizootic hemorrhagic disease virus in Israel. BARD - Binational Agric. Research and Dev. Fund. $157,000
Jackwood, Mark. Biological testing of low pathogenicity avian influenza virus in domestic avian spp and on farm biosecurity risks assoc with spread of avian influenza. USDA-ARS. $10,000
Kaplan, Ray. Filariasis research reagent resource center. NIH-National Institutes of Health. $52,992
Kaplan, Ray. Phenotypic and genotypic characterisation of moxidectin resistance in cyathostomins. Morendun Research Institute, Scotland. $6,010
Kaplan, Ray. Transfection and analysis of transcription in Brugia malayi. NIH-National Institutes of Health (University of South Florida). $19,529
Keel, Kevin. Disease training of wildlife biologists. USDI. $197,422
Koenig, Amie. Affect of sensor location on performance of an interstitial glucose monitor. ACVIM – American College of Veterinary Internal Medicine. $14,251
Lafontaine, Eric. Development of an adhesin-based vaccine to protect against melioidosis and glanders. US Defense Threat Reduction Agency. $1,119,354
Lafontaine, Eric. Glanders vaccine development. University of Calgary. $51,185
LeRoy, Bruce. Effect of autologous prostate cancer vaccines on the growth and metastasis of a canine prostrate carcinoma xenograft. Cook-Biotech Inc. $24,599
Mead, Daniel. Stable isotope analysis to measure mosquito dispersal in urban environments. Southeastern Center for Emerging Biological Threats. $30,675

Mead, Daniel. Vector-borne disease surveillance and mosquito diagnostic support. Valdosta State University. $16,500
Mead, Daniel. Vector-borne disease surveillance-wild bird and mosquito diagnostic support. Georgia Department of Human Resources. $50,476
Mead, Daniel. Vector-borne disease surveillance-Chatham County. Chatham County. $45,000
Mead, Daniel. Vector-borne disease surveillance-Cobb County. Cobb County. $1,650
Mead, Daniel. Vector-borne disease surveillance-Dekalb County. Dekalb County Board of Health. $8,992
Mead, Daniel. Vector-borne disease surveillance-Dekalb County. Dekalb County Board of Health. $9,900
Mead, Daniel. Vector-borne disease surveillance-Fulton County. Clarke Mosquito Control. $3,300
Mead, Daniel. Vector borne disease surveillance-wild bird and mosquito diagnostic support. Georgia Department of Human Resources. $30,000
Moore, James. Elucidating structure-function relationships of lipid A: A synthetic approach. NIH-National Institutes of Health. $73,749
Moore, James. Gene expression in circulating leukocytes from horses with gastrointestinal diseases. Morris Animal Foundation. $38,664
Moore, James. Learning Biological Processes through Animations and Inquiry: A new Approach. NIH-National Institutes of Health. $102,125
Moore, Julie. Immunopathogenesis of severe malaria during pregnancy. NIH-National Institutes of Health. $297,602
Mundt, Egbert. Characterization and control strategies of low pathogenic Avian Influenza Virus H9N2. BARD - Binational Agricultural Research and Development Fund. $157,000
Mundt, Egbert. Development of a species-independent ELISA for detection of influenza A antibodies directed against H6/H7/H9. USDA (University of Maryland). $83,834
Northrup, Nicole. A multi-center, randomized, observer blind, controlled, Phase III study to determine efficacy and safety of Paclical Vet vs. CeeNu in mast cell tumors grade II and III in dogs. Oasmia Pharmaceutical AB. $31,537
Northrup, Nicole. COTC008: Evaluation of the mTOR inhibitor rapamycin in dogs with osteosarcoma. Morris Animal Foundation/NIH. $4,966
Pellegrini-Masini, Alessandra. Prevalence of Mycoplasma haemolamae infection in South American camelids in the Southeastern United States. Morris Animal Foundation. $25,287
Peroni, John. Use of bioresorbable hydrogels and genetic engineering to accomplish rapid stabilization and healing in segmental long bone effects. Baylor College of Medicine. $602,728
Peterson, David. Cytauxzoon felis: Assessing genetic variability in an emerging feline infectious disease. Morris Animal Foundation. $49,178
Platt, Simon. Pharmacokinetics of a midozalam gel following intranasal delivery in dogs. Morris Animal Foundation. $73,368
Quinn, Fred. Alpha-crystallin as a marker of Mycobacterium tuberculosis latency. Southeastern Center for Emerging Biologic Threats. $10,764
Quinn, Fred. Identification of Francisella tularensis tick-expressed antigents. Southeastern Center for Emerging Biologic Threats. $49,999
Ritchie, Branson. Research Associate in Exotic/Zoo Infectious Disease and Pathology. Riverbank Zoo. $23,000
Robertson, Thomas. Targeting 5-HT in equine laminitis. Grayson Jockey Club. $24,026
Robertson, Thomas. Vagal and glossopharyngeal afferent cell bodies as biological sensors. NIH-National Institutes of Health (University of Virginia). $32,633
Saba, Corey. Randomized trial of 3 dose regimens of GS-9219 in dogs with relapsed B-cell non-Hodgkin's lymphoma. Gilead Sciences, Inc. $112,304
Saliki, Jeremiah T. Avian Influenza, Exotic Newcastle, Scrapie testing and surveillance. USDA APHIS MRPBS. $44,400
Saliki, Jeremiah T. BSE surveillance testing. USDA. $143,406
Saliki, Jeremiah T. Classical swine fever surveillance. USDA. $11,693
Sanchez, Susan. Georgia Veterinary Scholars Summer Research Program. NIH-National Institutes of Health. $41,642
Sanchez, Susan. Georgia Veterinary Scholars Summer Research Program 2009. NIH-National Institutes of Health. $40,779
Schatzberg, Scott. Broadly reactive PCR for detection of viral, bacterial, and rickettsial genera in Pug and Maltese dogs with necrotizing and granulomatous memingoencephalitis. ACVIM. $28,750
Schatzberg, Scott. Degenerate PCR for detection of viral, bacterial, and rickettsial genera in Pug and Maltese dogs with necrotizing and granulomatous meningoencephalitis. AKC-Canine Health Foundation. $67,613
Stallknecht, David. Center of Excellence for Pandemic Flu Research and Surveillance. NIH-National Institutes of Health (University of Minnesota). $1,320,742
Tennent-Brown, Brett S. Real-time continuous interstitial glucose monitoring in neonatal Camelids. ACVIM-American College of Veterinary Internal Medicine. $4,623
Tompkins, Mark. Developing a paramyxovirus-based vaccine for influenza A virus H5N1. NIH-National Institutes of Health/NIAID (Pennsylvania State University). $244,277
Tripp, Ralph. Antibody inhibition of respiratory syncytial virus G protein activity. NIH-National Institutes of Health. $347,815
Tripp, Ralph. Assess the prophylactic efficacy and kinetics in Balb/c mice of each of three micro-powder siRNA formulations that target RSV. AKTIV-DRY LLC. $16,374
Tripp, Ralph. NIAID Centers of excellence for influenza research and surveillance. NIH-National Institutes of Health/NIAID (Emory University). $2,445,235
Uhl, Elizabeth. Protective CMI mechanisms of a dual-subtype FIV vaccine. NIH-National Institutes of Health (University of Florida). $44,250
Vandenplas, Michel. Microarray analysis of gene expression in carbohydrate overload-induced acute laminitis. Morris Animal Foundation. $28,819
Woolums, Amelia. A study to evaluate effect of intranasal & subcutaneous vaccination of calves w/MLV vaccines in face of maternal antibody (IFOMA): a comparison of day 3 & day 60 priming reltv to convtl dlvry@weaning. Intervet-SP. $108,247
Woolums, Amelia. Bovine respiratory disease symposium. USDA NRI. $10,000
Yabsley, Michael. Characterization of Cytauxzoon felis in reservoir and vector populations. Morris Animal Foundation. $15,562
Yabsley, Michael. Effect of host density on parasite transmission among tokay geckos. Brown University. $22,356

Selected Publications

Abell, J. L., Driskell, J. D., Dluhy, R. A., Tripp, R. A., and Y-P. Zhao. Optimization of a multi-well array SERS chip. Proceedings of the SPIE, Volume 7321, pp. 732103-11, 2009.

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The University of Georgia

Veterinary Medical Experiment Station
College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602