VMES 2013
Science in Service to Animals

Tending the Microbial Farm
37th Annual Report
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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.

MISSION

*The VMES mission is to coordinate research on animal disease problems of present and potential concern to Georgia’s livestock and poultry industries.*

OBJECTIVES

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

*The Veterinary Medical Experiment Station is committed to 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.*
Innovation and creativity form the foundation of good science. Researchers with these attributes continually push the boundaries of biomedical knowledge. These same individuals are often the first to translate basic discoveries into practical solutions for enhancing animal and human health. The rapid application of research findings to practical solutions is historically embedded in the missions of land grant universities and experiment stations. As you’ll read in our cover story by Dr. Margie Lee, researchers at the Poultry Diagnostic and Research Center in the UGA College of Veterinary Medicine are investigating and applying basic ecological principles to veterinary medicine in unique ways, which as she states: “may hold the secret to identifying, treating and possibly curing some of our most puzzling medical syndromes.” Dr. Lee describes current thinking and research on the role of microorganisms in maintaining normal homeostasis of the gastrointestinal tract. It is a fascinating and evolving story of immediate relevance to poultry production and veterinary medicine.

This 37th VMES Annual Report gives a synopsis of peer-reviewed, competitive projects and new faculty start-up projects conducted during fiscal year 2013 (July 1, 2012 – June 30, 2013). Projects supported by VMES funding, which is provided by the State of Georgia, and those funded with 1433 Formula funds provided by the United States Department of Agriculture are reviewed by veterinary scientists to ensure quality of the science and to guarantee that they are focused on relevant health issues or disease problems. The research must be innovative and applicable to improving animal health. Additional information on any of these projects can be obtained by contacting the investigators themselves. A list of peer-reviewed publications is provided. These publications represent a selection of VMES-supported work and other scholarly research by faculty in the College of Veterinary Medicine.

It will become evident to the reader perusing the research reports and list of publications in this Annual Report that research in the College of Veterinary Medicine is diverse, but clearly targeted to addressing issues related to animal and human health. As I often emphasize, this diversity is both the strength and challenge of the veterinary profession. Diversity in investigations ranging from the molecular to the whole organism and populations ensures the relevancy of the work to the rapidly changing biomedical and veterinary research environment. The challenge lies in maintaining the focus required for establishing excellence in specific areas. As an example, the college’s investments in infectious disease research have attracted highly collaborative researchers who have focused programs of excellence in cross-cutting fields such as microbial ecology, wildlife disease ecology, and basic vaccinology, to name just a few.

We have listed the names of 25 individuals who received graduate degrees in 2013 after completing a comprehensive training program that included original research conducted under the mentorship of a College researcher. These students are attracted to our programs for the excellent research experiences and mentoring that they find here. The training of future researchers is of utmost importance to fulfillment of the mission of the Veterinary Medical Experiment Station and to meeting the future animal and public health needs of our state, nation and world.

The VMES office by phone, e-mail or website, or directly from the investigators themselves. A list of peer-reviewed publications is provided. These publications represent a selection of VMES-supported work and other scholarly research by faculty in the College of Veterinary Medicine.

The financial tables show that over the past year approximately six research dollars were leveraged for each VMES dollar invested. Expenditures are from all sources including State Appropriations, Extramural Research Funding, and Donations – Includes all expenditures and personnel costs.

Financial Tables

Research Dollars Leveraged

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>VMES Dollars Invested</th>
<th>VMES Dollars Leveraged</th>
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<tr>
<td>2009</td>
<td>$0</td>
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<td>2010</td>
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<tr>
<td>2011</td>
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<td>2012</td>
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</tr>
<tr>
<td>2013</td>
<td>$27,472,528</td>
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</table>

Budget Category | Amount | % of Budget |
<table>
<thead>
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<tbody>
<tr>
<td>Personnel-Researchers/Techs/Research Staff</td>
<td>$1,894,658</td>
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<td>Personnel-Research Administration &amp; Accounting</td>
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<tr>
<td>Travel</td>
<td>$6,888</td>
<td>0.28%</td>
</tr>
</tbody>
</table>

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

Margie D. Lee DVM PhD
Department of Population Health
Poultry Diagnostic and Research Center

Over the years, intestinal excretions have been called many things; none of them complimentary. For the most part, human societies are united in their revulsion of feces, stool, dung, excreta, BM, night soil, and for good reason. In 1849 in an essay titled “On the Mode of Communication of Cholera,” John Snow shared his belief that fecal contamination of well water in London was the cause of the cholera epidemic that sickened hundreds. In the US around the turn of the 20th century, the foundation of the American public health system was based on a typhoid fever investigation in which a young immigrant cook, named Mary Mallon, unintentionally contaminated the food that she served her wards. She was found to be shedding the typhoid bacteria in her feces, which inadvertently contaminated the food that she served her wards. Both of these stories highlight the role of fecal contamination of food and water in causing deadly outbreaks of diseases.

Control of intestinal bacteria has been key in reducing the spread of many infectious diseases, including salmonellosis, polio, E. coli, shigellosis, norovirus, and a multitude of others. Because autointoxication from putrefaction of the colon was once considered to be responsible for a host of maladies, many diseases were treated by removing the offensive matter using colonic or enemas. Despite their historical bad rap, intestinal bacteria have received increased attention in recent years. For example, the Food and Drug Administration is currently faced with regulating the medical use of fecal transplants to treat antibiotic-resistant Clostridium difficile infections; fecal transplants have a high rate of success when antibiotic therapy fails. This finding is evidence that tending the farm of normal flora bacteria can have significant impacts on health. This also provides support for the rising interest in promoting normal flora and for practicing bacteriotherapy through high fiber diets and ingestion of less processed food, yoghurts and other fermented foods, and probiotics to promote better health.

Early humans discovered that fermenting grains, milk and fruit would prolong their storage. Skillful fermentation of milk was accomplished using the stomach of sheep or goats which contained lactic acid-producing bacteria. The normal flora of dairy cows produce milk as an offender; milk and cheese are treated by removing the offensive matter using enemas. Despite their historical bad rap, intestinal bacteria have received increased attention in recent years. For example, the Food and Drug Administration is currently faced with regulating the medical use of fecal transplants to treat antibiotic-resistant Clostridium difficile infections; fecal transplants have a high rate of success when antibiotic therapy fails. This finding is evidence that tending the farm of normal flora bacteria can have significant impacts on health. This also provides support for the rising interest in promoting normal flora and for practicing bacteriotherapy through high fiber diets and ingestion of less processed food, yoghurts and other fermented foods, and probiotics to promote better health.

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An article published a decade ago by Xu and Gordon, entitled “Honor thy Symbionts” challenged the existing human medical dogma regarding the role of normal flora bacteria in intestinal health. Studies with germ-free animals were initially thought to show that animals were better off without their microbial consortium. The animals were raised in sterile conditions and fed sterilized (cooked) food resulting in faster growth rates and less disease. However, researchers subsequently determined that the improvements in health were actually due to the absence of pathogens in the environment. When they removed all microbes, they discovered that the intestinal microbes were essential for normal development of the intestine, digestion, and the immune system. While many pathogens can be members of the intestinal microbial community, this community actually consists primarily of good bacteria that are crucial for development of a normal intestine and its function. Vertebrates, including humans, are hosts for a variety of microorganisms. In fact, there are more bacteria in the intestine than there are host cells in an entire individual, leading to the conclusion that a healthy person is really a consortium of organisms that work together for mutual benefit. Veterinarians have readily accepted this concept because ruminants, such as cattle and goats, rely on microbes to digest their food. The gastrointestinal tracts of ruminants, birds, horses, rabbits, and many other animals have unique properties, such as multi-chambered stomachs (rumen and crop) and oversized large intestines (cecum and colon) that support the healthful benefits of lactic acid-producing bacteria. The concept was first articulated in the early 1900’s by Russian biologist Elie Metchnikoff in a book entitled “The Prolongation of Life” in which he discussed his findings on the healthful benefits of lactic acid-producing bacteria. Metchnikoff’s beliefs regarding beneficial microbes were unique considering the fact that most scientists during this renaissance of microbiology at the turn of the 20th century were describing pathogens and their roles in disease. His work stimulated some doctors to advise their patients to consume more fermented milk products, especially kefir which became very popular in Asia and Eastern Europe. In contrast, bad microbes dominated medical research in the West until recently when broad interest in ecology stimulated interest in how good microbes could help control pathogens.

Fluorescence microscopy reveals communities of live (green) and dead (red) bacteria within the intestine.

Due to the need in improving the efficiency of meat and egg production, many commercial products are available for food animals and poultry. This approach means reducing the amount of feed needed to grow the animals, reducing antibiotic usage, and reducing the presence of human foodborne pathogens within or shed by these animals. The scientific literature is rich with evidence to support the effectiveness of many of these commercial products, although how they work remains somewhat of a mystery. Feeding animals probiotics, which are the live microbes themselves, or prebiotics, which are complex carbohydrates intended to enrich
the growth of beneficial microbes, can have unexpected benefits such as reduced intestinal disease, improved growth rates and feed conversion, and decreased levels of foodborne pathogens such as Salmonella.

Now we know that some allergies, colon cancer, and inflammatory bowel disease may actually be due to an imbalance in the microbial farm. Newborns acquire their first normal flora bacteria from their mothers when they pass through the birth canal. Additional beneficial bacteria are acquired from nursing, sharing food, and physical contact with family members. This is true for many animals, illustrating the importance of family contact in establishing beneficial microflora. In an effort to limit the transfer of infectious diseases and reduce the use of antibiotics in poultry medicine, we have disrupted the transfer of normal flora from mother to offspring, as broiler (meat) chickens are raised distant from the parent flock. As a result, veterinarians are now faced with having to exogenously supply the chicks’ normal flora bacteria in order to reduce intestinal disease. Fortunately, the poultry industry has been proactive in adopting manure practices that enhance transfer of normal flora and seeking commercial products that may be effective. Researchers at UGA’s Poultry Diagnostic and Research Center have been very active in studying the effects of probiotics and prebiotics on poultry health, antibiotic resistance, and Salmonella control by competitive exclusion. Dr. Charles Hofacre organized a group of researchers in this area based on his commercial poultry production experiences tending the microbial farm. The research group includes clinicians (Hofacre and Dr. Steve Davis) and biologists (Drs. Margie Lee and John Maurer) at PDCR who have been successful in acquiring industry, USDA and NIH funding to expand knowledge in poultry microbial ecology. They were among the first researchers to describe the composition of the normal flora of broiler chickens and report that some probiotics were as effective as antibiotics in treating intestinal disease. They also were the first to report the microbial composition of chicken litter, study the microbial ecology of antibiotic resistance in litter, and currently are focusing on the mechanism of competitive exclusion of pathogens such as Salmonella by probiotics.

The advantageous interaction of bacteria and their hosts have been well studied in plants, insects and some vertebrates. The University of Georgia is internationally recognized for its strong core of researchers focusing on the microbial ecology of aquatic and terrestrial environments. While these ecological systems have been viewed by medical researchers as being distinct from the types of interactions that bacteria have with vertebrate hosts, researchers at PDCR have utilized their broad expertise to demonstrate the impact of normal flora in poultry production on bird health and food safety. Their findings have contributed to the efficiency of poultry production in Georgia and impacted the prevalence of Salmonella.

Our new insight is that basic ecological principles are broadly applicable in medicine and may hold the secret to identifying, treating and possibly curing some of our most puzzling medical syndromes.

Cattle production is currently the 2nd largest agricultural commodity in Georgia; there are more than 10,000 cattle operations in Georgia with a total cattle herd of 1.11 million head valued at over $843 million. An integral component in the profitable production of cattle is the control of internal parasites, which can limit cattle health and productivity. This fact is well recognized by cattle producers; in a recent survey, cattle operators “strongly agreed” that parasites “had a significant economic impact on cow-calf operations” three times more often than for any other health condition. Not surprisingly, recent national surveys indicate that 80% of all cattle operations in the United States regularly deworm their cattle.

Macrocyclic lactone dewormers (e.g., ivermectin, moxidectin) are the most commonly used drugs for control of parasites in animals, with annual worldwide sales of approximately $1 billion. These dewormers have revolutionized parasite control, providing an unprecedented level of efficacy, spectrum, safety and convenience. However, in recent years drug resistance has emerged as an important problem. For example, 76% of sheep and goat farms in the southeastern U.S. have resistance to ivermectin and 48% have multiple-resistance to all types of dewormers. Although drug resistance of cattle parasites has received less attention, reports of drug resistance have increased worldwide. Even so, no surveys have been performed in the U.S. Consequently, the extent of the problem and the production costs due to parasite drug resistance in cattle remain unknown, and most cattlemen do not know whether dewormers are working as expected. The major impediment to performing surveys of drug resistance in cattle parasites is the lack of an efficient and cost effective diagnostic test. The only method currently performing surveys of drug resistance in cattle parasites is the lack of an efficient and cost effective diagnostic test. The method currently available is the fecal egg count reduction test, which is time consuming, expensive to perform, and has both logistical and analytical limitations. Consequently, a laboratory-based diagnostic test that is less expensive and more convenient to perform is critically needed.

The primary objective of this study is to optimize and validate laboratory-based biosays for detecting and measuring drug resistance in parasites of cattle. Based on recent studies, the larval migration inhibition assay is the best candidate assay. Therefore, we used this assay to evaluate three populations of the intestinal worm, Cooperia, which is known to be the primary species impacted by drug resistance, two of these parasite populations were resistant and one was reportedly susceptible to macrocyclic lactone dewormers. Third-stage parasite larvae were incubated for 24 hours in 7 different concentrations of the drugs (eprinomectin, ivermectin or moxidectin), after which they were transferred to specialized chambers for 24 hours in which drug resistant larvae migrated and susceptible larvae did not. Migrated and non-migrated larvae were counted and the half maximal effective concentration (EC50) was calculated. Resistant parasites from Herd A had an EC50 of 1.58 μM for eprinomectin, and resistant parasites from Herd B had an EC50 of 0.25, 8.21, and 9.13 for eprinomectin, ivermectin, and moxidectin, respectively. Curiously, the susceptible population yielded very similar results; EC50 were 0.26, 3.79, and 7.58 for eprinomectin, ivermectin, and moxidectin, respectively. Based on these unexpected results, we inquired further about the origin of the “susceptible” worms, and determined that they were in fact highly resistant. Thus, we were unable to compare the results between susceptible and resistant worm populations. Although we were able to optimize the experimental conditions for the assay, the lack of susceptible worms prevented us from validating its diagnostic sensitivity. Fortunately, we now have a confirmed-susceptible population of Cooperia and are establishing an experimental infection in a calf to provide a source of worm larvae for our research. When this is accomplished, we will repeat the study to compare resistant and susceptible worms with the assay. In the interim, we have established a new technology in our laboratory and will test this method to determine if it might be a superior method for laboratory diagnosis of resistance in parasites of cattle.

Validated tests for diagnosing resistance in cattle parasites would address current diagnostic challenges, and the subsequent knowledge gained would lead to improvements in the management and control of parasites. This will ultimately lead to improved health and productivity of cattle in Georgia, the United States and throughout the world.

Co-Investigators: Dr. Bob Storey and Adrian Wolstenholme

Principal Investigator: Dr. Ray Kaplan
Co-Investigators: Drs. Bob Storey and Adrian Wolstenholme

VMES & USDA Formula Grant Funded Projects
CONTROLLING BOVINE PARASITES
Development of Diagnostic Assays for Anthelmintic Resistance in Nematodes of Cattle
Coccidiosis is the most expensive disease that affects the commercial poultry industry, with an estimated annual cost of $2.25 billion (Chapman, 2009). The parasites responsible for this disease are protozoa of the genus *Eimeria*, and their primary effects are reduced growth rate and poor feed conversion. The two main modes of controlling this disease have been incuding anti-coccidial and vaccination. The current expense for preventive medications exceeds $90 million in the United States and more than $300 million worldwide (McDougall, 2008). Even though the costs of preventative medications are high, viable alternatives are diminishing due to the high cost of producing new medicaments, the development of strains of *Eimeria* resistant to anti-coccidial drugs, and the fact that consumers want less medication used in production of food animals.

The results of previous studies indicate that differences in susceptibility to coccidiosis can exist between inbred lines of layers (Bumstead, 1987) but not in meat type or broiler chickens. The objective of this study was to evaluate whether a difference exists in resistance or susceptibility to coccidiosis can exist between inbred lines of layers (Bumstead, 1987) but not in meat type or broiler chickens. The objective of this study was to evaluate whether a difference exists in resistance or susceptibility to coccidiosis and could be a means for reducing intestinal disease.

**Principal Investigator:** Dr. Charles L. Hofacre  
**Co-Investigators:** Drs. Chad M. Malina, Greg Mathis, Roy Berghaus, Elise A. Myers, and Yun-Ting Wang

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**Development of an Equine Adrenocortical Primary Cell Culture System**

Sepsis – a life-threatening complication of bacterial infections – is a leading cause of death in people worldwide. For example, sepsis strikes approximately 1 in 4,000 adults in developed countries, with mortality rates ranging from 30% overall to 40% in elderly patients with severe sepsis. The situation is equally dire in infants and children, as neonatal sepsis is the third leading cause of neonatal death worldwide, behind only premature delivery and birth-related complications. Sepsis is also an important disease in animals, and widely affects dogs, cats, and horses. In newborn horses (foals), neonatal sepsis is particularly important, as it is the primary cause of mortality in both field settings and equine specialty hospitals. My laboratory’s overarching research objective is to characterize and decipher the complex interactions that govern immune and endocrine (hormonal) interactions during bacterial infection in neonatal foals and horses, with the fundamental aim of utilizing this knowledge to decrease sepsis-related mortality in animals and humans.

In septic patients, the immune and endocrine systems are strongly activated and function synergistically to clear the inciting infection and help the patient cope with the stresses that severe illness places on the body. The immune system responds to sepsis by releasing inflammatory substances (cytokines) from immune cells. The endocrine response is coordinated by the hypothalamic-pituitary-adrenal axis, and culminates in the release of the stress hormone cortisol from the adrenal glands. Cortisol helps counteract the effects of severe infection on the body and helps regulate the inflammatory response. This effect of cortisol is critical in sepsis, as an unregulated inflammatory response can be as dangerous as the initial bacterial infection.

Unfortunately, in up to 50% of septic patients, insufficient cortisol is released and the inflammatory response is uncontrolled, a syndrome called Critical Illness-Related Corticosteroid Insufficiency (CIRCI). Septic people and foals with this syndrome have dramatically increased mortality compared to septic patients with adequate cortisol concentrations. At present, we do not understand how CIRCI develops and how best to treat it. Further study in this field requires in vitro laboratory systems to allow us to identify the factors that cause CIRCI in clinical patients. Currently available *in vitro* models involve the culture of adrenal gland cells from rats, mice and cattle, species whose adrenal glands function very differently from those of horses and people, the species in which sepsis and CIRCI naturally occur. Therefore, the purpose of this study was to develop an equine adrenal cell culture system to help our lab and others better understand the causes and treatment of CIRCI in horses and people. This is a difficult task because adrenal cells can change rapidly in culture and lose their ability to make cortisol.

We collected adrenal tissues from several horses, and developed a protocol for successfully isolating individual, live adrenal cells. We optimized culture conditions for these cells, and are able to grow them as culture in the lab for several weeks or longer. Preliminary studies suggest that these cells maintain normal cortisol production during this period, though our work in this area is ongoing. At present, we are also working to develop methods for freezing and thawing these cultured cells while maintaining their cortisol responses. This would greatly increase the number of studies that can be performed using this equine adrenal cell culture system. This system holds promise for use in future studies to investigate many aspects of adrenal gland function in horses and people, and is already in use in our lab in additional studies working to determine the cause of CIRCI in foals and human infants.
Our approach has been to delineate these known in teleost fish regarding the identity and/or functions of alarmins. Alarm signal (alarmins) are surveillance molecules that bind these signal molecules; and determine how the immune system responds. This approach could lead to manipulation of the innate immune system to improve animal and human health.

The objective of the present study was to gain a better understanding of the contribution of a novel mechanism of innate immunity that may provide protection against infectious agents in teleost fish. This mechanism is mediated by a protein of the histone H1 family, referred to as a nonspecific cytotoxic cell cationic antimicrobial protein-1 (NCAMP-1). NCAMP-1, first described in catfish nonspecific cytotoxic cells, functions as a pattern recognition receptor and in soluble form has a broad spectrum of bactericidal activity. Although we have previously identified NCAMP-1 as a pattern recognition receptor in cells of the immune system of mice and fish, studies of its molecular function as a alarm signal and its role in inflammation have not been conducted. We hypothesized that NCAMP-1 is a potential novel alarmin that initiates the acute inflammatory response in teleost fish by activating caspase-1.

The study was designed to provide information about the functional and molecular mechanisms by which NCAMP-1 acts as an alarmin. The rationale was that understanding the signals that regulate expression and release of inflammatory molecules after NCAMP-1 activation of catfish cells should shed light into poorly understood inflammatory mechanisms. Using a combination of molecular and functional approaches in vitro, the cellular responses of immune cells to soluble NCAMP-1 were determined.

The results ex vivo experiments indicate that recombinant γNCAMP-1 binds to catfish white blood cells, which leads to a dose- and time-dependent increase in gene transcription of proinflammatory cytokines (IL-1β and TNF-α). In addition, treatment of cells with rNCAMP-1 produces activation of a specific enzyme, caspase-1, as measured by the opening of its catalytic site. Comparisons with a known teleost alarmin, ATP, demonstrated that similar to ATP, rNCAMP-1 induces non-lytic pore formation in cells as measured by uptake of the dye YO-PRO-1. In vivo experiments further show that NCAMP-1 is constitutively present in catfish serum and the levels of NCAMP-1 increase following microbial infections. Immunohistochemistry analysis of catfish tissues showed staining with anti-NCAMP-1 antibodies in the cytosol of AK and spleen lymphocyte-like cells and in epithelial cells. Catfish T-cell lines G14D and 28.33 expressed NCAMP-1 in the cytosol and in storage granules.

Taken together, the results suggest that NCAMP-1 has a novel role as an alarmin-like protein. These studies have improved our understanding of the mechanisms of activation of inflammatory responses by cells of the innate immune system as well as the contribution of NCAMP-1 to the resolution of bacterial infections. This information provides important clues for future studies aimed at manipulation of innate immune responses in fish to improve protection against infectious agents. This knowledge applies to other food animals as this molecule appears to represent an important innate mechanism of protection that is conserved across species.

Principal Investigator: Dr. Liliana Jaso-Friedmann
A NEW TREATMENT FOR A COMMON BLEEDING DISORDER

Modulation of Immune-Mediated Thrombocytopenia with Polyclonal Equine Immunoglobulin

Because platelets are essential for coagulation, a lack of platelets (termed thrombocytopenia) may predispose to uncontrolled and potentially fatal hemorrhage. Primary immune-mediated thrombocytopenia is an autoimmune disease in which affected animals produce specific antibodies that reduce platelets in the circulation and result in thrombocytopenia. Although conventional treatment includes administration of corticosteroids, these drugs are associated with adverse effects including gastrointestinal bleeding and pre-disposition to infections.

A specific type of human immunoglobulin (Ig) has been used to treat dogs with immune-mediated diseases. However, the limited supply of human Ig makes this form of treatment very expensive for veterinary patients. To address this problem, we concluded that the ability of horses to donate much larger volumes of plasma could ensure a plentiful supply of Ig and reduce the cost of treatment. However, equine Ig has not been evaluated as an adjunctive therapy for canine immune-mediated diseases.

In this study, we hypothesized that equine Ig would be as effective as human Ig in reducing the severity of disease in a mouse model of immune-mediated thrombocytopenia. The first step towards clinical trials with equine Ig in affected dogs was thus to establish the safety and efficacy of equine Ig to treat immune-mediated disease in mice.

We first harvested and purified equine Ig from 12 horses, and then optimized the antibody staining of cell surface markers using a technique called flow cytometry. Whole blood was compared to lysed blood (red blood cells were removed to make other blood elements easier to detect); called flow cytometry. Whole blood was compared to lysed blood; red blood cells were removed to make other blood elements easier to detect. Both techniques gave similar results although the procedure with lysed blood was more consistent. Platelet enumeration using a color bright bead was more consistent. Platelet enumeration using a color bright bead was more consistent. Platelet enumeration using a color bright bead

Principal Investigator: Dr. Jo R. Smith
Co-Investigator: Dr. Robert Gogal

Used an established mouse model of immune-mediated thrombocytopenia, we proposed to compare the efficacy of equine and human Ig to inhibit the decrease in platelet numbers. Initially, we were unable to induce the disease using the published protocol of administering the platelet-depleting antibody intraperitoneally, presumably because the strain of mouse used in our study (C57Bl/6) was immunologically competent whereas the strain used in previous studies was not. By switching to an intravenous route of administration of the antibody, we successfully reproduced transient immune-mediated thrombocytopenia in our strain of mice.

In contrast to the beneficial effects of human Ig documented to occur in people with immune-mediated thrombocytopenia, neither human nor equine Ig prevented the development of thrombocytopenia in the murine model.

To address potential differences between equine and human Ig, we then compared their ability to bind to immune cells from mice. For these studies, cells from mouse spleens were cultured for 24 hours in vitro with either equine or human Ig, and stained with commercially available antibodies. We verified that Ig from both species do indeed bind to these cells. Studies are now being performed to compare binding of human and equine Ig to these cells in vivo to determine if the two Ig behave similarly in vivo and in the animals. A positive result from these studies would provide support for our hypothesis that equine Ig has potential therapeutic value in canine immune-mediated thrombocytopenia.

Principal Investigator: Dr. Jo R. Smith
Co-Investigator: Dr. Robert Gogal

Respiratory Disease in Southern Georgia Cattle

An Immunohistochemical and Epidemiological Survey of Mycoplasma bovis-Associated Bronchopneumonia in Beef and Dairy Cattle

Mycoplasma bovis is an important and emerging respiratory pathogen in beef and dairy cattle. The prevalence, distribution and significance of this bacterial pathogen have not been well defined in Georgia. This retrospective study will utilize the archived tissues at the Tifton Veterinary Diagnostic Laboratory to address these needs. Over 400 cases of bovine bronchopneumonia from a 15-year period (1995-2012) have been identified in the Diagnostic Lab database. Analysis of these data should provide the prevalence of Mycoplasma bovis in beef and dairy cattle, the distribution within the state, and identify any trends involving gender or age of affected animals.

Analysis of these data will also provide information on the incidence of co-infections with other bacterial and viral respiratory pathogens. As an added benefit, analysis of the data should provide important information about the prevalence and distribution of the other respiratory bacterial and viral pathogens (Mannheimia haemolytica, Histophilus somni, Pasteurella multocida, BVD, BRSV, P3, (BRR) in southern Georgia cattle. These other pathogens are important to the cattle industry as they are common components of the bovine respiratory disease complex. Thus far, the immunohistochemistry portion of this study has been completed, and analysis of the data is underway.

Principal Investigator: Dr. Susan Turnquist
Co-Investigator: Dr. Lisa Whittington
Infectious Diseases


Tompkins, S. M. and R. A. Tripp. Subsisting H1N1 Influenza Memory Responses are Insufficient to Protect children suffering from recurrent acute otitis media and chronic otitis media with effusion. Clinical and Vaccine Immunology (19), 1665-71., 2012.


**Pathology**


### Physiology & Pharmacology


Boone, Lindsey.  Doctor of Philosophy – Physiology, Spring 2013
Brown, Kimberly. Master of Science – Veterinary & Biomedical Sciences, Spring 2013
Fine, Kari. Doctor of Philosophy – Infectious Diseases, Summer 2012
Frontera Acevedo, Karelma. Doctor of Philosophy – Veterinary Pathology, Spring 2013
Fung, Hou-Ming. Master of Science – Veterinary & Biomedical Sciences, Summer 2012
Gibizov, Kiersten. Master of Science – Veterinary & Biomedical Sciences, Spring 2013
Heins, Brad. Master of Food Animal Medicine, Fall 2012
Hurley-Bacon, Anne. Master of Avian Medicine, Fall 2012
Kang, Kyung-il. Doctor of Philosophy – Infectious Diseases, Summer 2012
Keeler, Shamus. Doctor of Philosophy – Infectious Diseases, Summer 2012
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Moesta, Alexandra. Master of Science – Veterinary & Biomedical Sciences, Fall 2012
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Neary, Ashley. Doctor of Philosophy – Infectious Diseases, Spring 2013
Oluwadare, Mopelola. Master of Science – Veterinary & Biomedical Sciences, Spring 2013
Owino, Simon. Doctor of Philosophy – Infectious Diseases, Fall 2012
Palomino, Victor. Master of Science – Veterinary & Biomedical Sciences, Spring 2013
Ridenour, Callie. Master of Science – Veterinary & Biomedical Sciences, Summer 2012
Roh, Ha-Jung. Doctor of Philosophy – Infectious Diseases, Spring 2013
Rowley, Sean. Master of Science – Veterinary & Biomedical Sciences, Fall 2012
Rushmore, Julie. DVM-PhD Dual Degree Candidate, Doctor of Philosophy – Ecology, Spring 2013
Silva de Franca, Monique. Doctor of Philosophy – Veterinary Pathology, Spring 2013
Wolff, Bernard. Master of Science – Veterinary & Biomedical Sciences, Summer 2012
Zárate Rendón, Daniel. Master of Science – Veterinary & Biomedical Sciences, Summer 2012
The key to improved animal well-being is animal health.

The key to improved animal health is veterinary research.