Mentor Abstract #12

Spatial distribution of the intestinal microbiota in murine colitis

Principle Investigator:
Albert E. Jergens, DVM, MS, PhD
Iowa State University, College of Veterinary Medicine (ISU CVM)
Veterinary Clinical Sciences Department

Collaborating Investigator(s):
Todd Atherly (USDA-ARS)
Meghan Wymore and Mike Wannemuehler, ISU CVM Veterinary Microbiology and Preventive Medicine Department

Abstract:

BACKGROUND: Studies in humans and animal models of intestinal inflammation strongly implicate intestinal bacteria in the pathogenesis of inflammatory bowel disease (IBD). Routine sampling of the intestinal contents may disrupt the structural organization of the enteric bacteria and may disguise the complex interactions between the intestinal microbiota and the host. Fluorescence in situ hybridization (FISH) combines the molecular identification of bacteria in situ with the direct visualization of the relationships between the bacteria and the mucosa, providing a significant advantage over culture, PCR, and microbial abundance alone.

AIM: To investigate the spatial re-distribution of bacteria in IBD intestinal tissues of mice harboring a defined (8 bacterial species) microbiota.

METHODS: Proximal colonic tissue sections of colitic mice and non-colitis controls will be studied by FISH on a quantifiable basis (figure 1). Temporal changes in mucosal organization will involve FISH labeling of each microorganism with two or more fluorescent probes chosen to maximize discrimination of individual bacterial species. The association between mucosal bacteria and murine histopathologic inflammation will also be investigated.

EXPECTED RESULTS: It is likely that the intestinal microbiota in health is structurally organized, and that the composition of the mucosal bacteria is altered with intestinal inflammation. Our approach using FISH in a unique murine model will allow us to directly compare the locations of different bacterial species in relation to each other and to the mucosa. This is an important research question since IBD is thought to result from aberrant host responses directed against enteric bacteria.
STUDENT ACTIVITIES: FISH techniques, fluorescence microscopy, histopathology, mouse necropsy, data analysis/statistics, and abstract writing. See representative figure below.

FIGURE 1:
Photomicrograph showing 3-color FISH of human pathobiont LF82 (orange) hybridized against all other bacteria (green) in ASF-colonized mice. The colonic mucosa is labeled blue (DAPI).
Mentor Abstract #13

Development of a vaccine delivery device that will maintain life-long high titers of anti-GnRH antibodies.

Principle Investigator:  
Doug Jones, MS, VMD, PhD  
Iowa State University, College of Veterinary Medicine  
Veterinary Pathology Department

Abstract:  
Development of a single-dose non-surgical sterilant which is effective in both male and female cats and dogs would have an unprecedented impact on the global pet overpopulation problem. One approach to this problem is immunocontraception involving vaccination against gonadotropin releasing hormone (GnRH) which is a master reproductive hormone. The primary problem regarding this approach is that antibody levels are not maintained with traditional vaccine strategies. Thus far, research has focused on vaccine formulation (ie, adjuvants) while concepts of antigen delivery and persistence have been neglected. Our central hypothesis is that a vaccine strategy centered on immune-regulated antigen delivery will result in the maintenance of high anti-GnRH antibody titers. The objective of this project is to finalize a vaccine platform consisting of a small implantable device. This implant will be designed to address 3 stages of immunity; 1) priming with a soluble vaccine, 2) boosting with bioerodable polyanhydrides, and 3) long-term immunity with release of vaccine in response to low anti-GnRH antibody levels.
Mentor Abstract #14

Chronic Diseases & Animal Adoptability from Animal Shelters
(Maddie’s Fund project #1)

Principle Investigator(s):
Lin Kauffman, DVM
Iowa State University, College of Veterinary Medicine
Veterinary Clinical Sciences Department

Christine Petersen, DVM, PhD
University of Iowa, College of Public Health
Department of Epidemiology

Abstract:

We have previously published a veterinarian-based identification of chronic diseases or problems that may promote shelter consideration of an animal as “unadoptable/unmanageable” as described in the definitions set forth by the Asilomar Accords. Using this schema, we would like to determine how animals with chronic disease are evaluated by local shelters and further compare the outcomes of these animals in traditional shelters vs. adoption guarantee shelters.

As a second part of this project, we would also like to consider how high adoption rate shelters use special adoption cases to gain PR for shelter. Once this information is collected we would create and share these techniques with smaller shelters to help provide ways that shelters can increase adoption of animals in these more special needs groups. The student would work closely with Dr. Kauffman and Petersen in survey design, IRB and IACUC protocol submission to allow both survey of shelters and possible sample collection from shelter animals. Data collection from the shelters would happen both in person and via email through the summer.
Outcomes of Cats Relinquished to Animal Shelters Due to House Soiling
(Maddie’s Fund project #2)

Principle Investigator(s):
Lin Kauffman, DVM
Iowa State University, College of Veterinary Medicine
Veterinary Clinical Sciences Department

Christine Petersen, DVM, PhD
University of Iowa, College of Public Health
Department of Epidemiology

Abstract:

Previous studies have indicated that behavioral (house-soiling) issues are the number one reason for feline relinquishment to animal shelters. What is not well known is the outcome of these cats once they (re) enter the shelter. Specifically how many of these cats are deemed adoptable vs. unadoptable at intake compared to the overall population of cats in the shelter or cats without identified issues with house soiling. Additionally little is known about the procedure to identify whether the cats have medical disorders that mediate polakiuria or polyuria, etc. The goal of this project is via a survey of 7+ large and small regional animal shelters including “spot checking” feline relinquishment intake and diagnosis of medical vs. behavioral reasons for house-soiling to determine the overall and shelter-sized based outcome of cats identified as house-soilers during intake by their previous owner. The working hypothesis is that the lengths that a shelter will go to diagnose possible reasons for house soiling is directly correlated to the size and resources of the shelter and the current in house cat population. The student would work closely with Dr. Kauffman and Petersen in survey design, IRB and IACUC protocol submission to allow both survey of shelters and obtaining samples from shelter cats. Data collection from the shelters would happen both in person and via email through the summer.
Mentor Abstract #16

Regulation of invasive virulence factors in *Streptococcus pyogenes*

Principle Investigator:
Brian M. Lee, PhD
Iowa State University, College of Veterinary Medicine
Biomedical Sciences Department

Collaborating Investigator:
Gabriela C. Perez-Alvarado
Iowa State University, College of Veterinary Medicine
Veterinary Microbiology and Preventive Medicine Department

Abstract:

Group A Streptococcus (GAS) is associated with a broad range of diseases including scarlet fever, impetigo, pharyngitis, necrotizing fasciitis, streptococcal toxic shock syndrome and the post-streptococcal sequelae of rheumatic fever. Our research is focused on a regulatory endonuclease, conserved virulence factor A (CvfA), which contains a metal-dependent phosphohydrolase domain and regulates the expression of glycolytic enzymes and virulence factors by *Streptococcus pyogenes* in response to low nutrient conditions. The nutrient dependent regulation of protein expression allows for adaptation to various host tissues and represents a potential switching mechanism in the mode of infection from commensal to invasive. Our overall goal is to understand this mechanism of gene regulation by defining the catalytic activity, the RNA binding specificity and the nutrient-dependent signaling response of CvfA. Our approach includes structural studies of CvfA using nuclear magnetic spectroscopy and X-ray crystallography, enzyme catalytic assays to define the mechanism of the phosphohydrolase activity, mRNA decay assays to elucidate the nutrient signaling response, and in vitro selection to define the mRNA sequence specificity of CvfA. The impact of our results may lead to new therapeutic strategies against streptococcal infections following three potential approaches: 1) RNA-based interference with mRNA recognition, 2) down regulation of nutrient signaling pathways through dietary methods, and 3) developing chemical inhibitors of phosphohydrolase activity based on lead compounds identified through catalytic assays. Students may participate in all stages of this project and will receive training in molecular biology, protein chemistry, bioinformatics and structural biology. Preference will be given to students with an academic background that includes introductory microbiology and biochemistry.
Mentor Abstract #17

Is CD8 Imbalance a Risk Factor For Immune Disease in Cocker Spaniels?

Principle Investigator:
Dana LeVine, DVM, DACVIM, PhD
Iowa State University, College of Veterinary Medicine (ISU CVM)
Veterinary Clinical Sciences Department

Collaborating Investigator(s):
Marjory Brooks, DVM, DACVIM (Cornell University)
Emily Phalen (3rd year vet student, ISU CVM)

Abstract:
Cocker Spaniels are predisposed to autoimmune blood disorders including immune-mediated hemolytic anemia and immune thrombocytopenia (ITP). The underlying trigger for these diseases is unknown so current treatments are too general, too often used, and have dangerous side-effects. Understanding the pathogenesis of these diseases could lead to direly needed novel treatment paradigms. Preliminary studies in our laboratory revealed decreased CD8+ T cells in Cocker spaniels compared to other breeds of dogs. Peripheral blood CD8+ deficiency is a feature of many chronic autoimmune diseases in people including systemic lupus erythematosus, Crohn’s disease, and Hashimoto’s thyroiditis (Pender 2012). A similar reduction in CD8+ T cells is observed in healthy relatives of human patients with an autoimmune disease, suggesting that CD8+ abnormalities are genetically determined (Pender 2012). Our pilot study population had an over-representation of intact females so further research is needed to eliminate a confounding factor of sex bias in CD8+ T cell counts.

Study hypothesis: We hypothesize that CD8+ cells are decreased in Cocker Spaniels compared to other breeds of dogs along with CD8+ cytokines and that this T cell imbalance may predispose Cocker Spaniels to autoimmune blood disorders. The discovery of a CD8+ T cell defect will reveal new targets for novel immunomodulatory therapies.

This summer we aim to:

1. Confirm the preliminary data that CD8+ T cells are reduced in Cocker Spaniels by examining larger numbers of Cocker Spaniels and sex-matching them to control dogs of other breeds (equal number of FS, MC, FI, MI in each group).
2. Expand on these preliminary findings by examining B cell counts and cytokine profiles in Cocker Spaniels compared to healthy control dogs of other breeds.
3. Assess cytokine profiles of dogs with ITP (banked samples) to determine if there is a unique cytokine profile in dogs with active immune-mediated disease.

Through this project, you will obtain clinical experience with ITP patients and their typical clinical presentations and will become proficient in preparing samples for flow cytometry, performing flow cytometry, and analyzing flow data. You will also perform Luminex assays to measure cytokines in canine serum.
Mentor Abstract #18

Effects of lysosomal storage diseases on cognitive function of dogs

Principle Investigator:
Suzanne Millman, BSc (Agr), PhD
Iowa State University, College of Veterinary Medicine
Veterinary Diagnostic and Production Animal Medicine Department

Collaborating Investigator:
Matthew Ellinwood
Iowa State University, College of Agriculture and Life Sciences
Animal Science Department

Abstract:

An opportunity exists for a student to develop knowledge and skills in animal behavior and welfare. The primary focus of this summer research position is to explore the progressive effects of lysosomal storage diseases on canine cognitive function, as a component of a multidisciplinary research project. As secondary focus, the student will provide assistance for other ongoing animal behavior and welfare research projects in the Millman lab, typically involving livestock species. Mucopolysaccharidoses (MPS) are fatal neurodegenerative lysosomal storage disorder diseases caused by genetic deficiency of enzymes, which leads to accumulation of the glycosaminoglycan (GAG) heparan sulfate (HS) in the central nervous system (CNS) and other tissues. The resulting progressive neuropathy is typically fatal during the 1st or 2nd decade of life. Canine models of these diseases have been developed, and we are evaluating cognitive function using navigation of two mazes to assess learning and memory. The T-maze test is used to measure simple associative learning via a win-stay paradigm. We also use the T-maze to test reversal learning, an indicator of learning ability and memory. The radial arm maze test uses the dog’s foraging behavior to measure short-term working memory and attention span. Performance in the mazes is video recorded for external validation by an observer blinded to treatment, and to provide opportunity to quantify behavioral indicators of anxiety, pain and physical impairment that explain unexpected changes in performance. The student’s role in this project will be training and testing dogs in the mazes, collecting data from video recordings, and assisting with data analysis and report writing.
Utilization of Neutrophil Extracellular Trap DNA by *Mycoplasma hyopneumoniae*

Principle Investigator:
F. Chris Minion, MS, PhD
Iowa State University, College of Veterinary Medicine
Veterinary Microbiology and Preventive Medicine Department

Collaborating Investigators:
James Roth, DVM, PhD; and Bryan Bellaire, PhD
Iowa State University, College of Veterinary Medicine
Veterinary Microbiology and Preventive Medicine Department

Abstract:

Despite efforts by the swine health industry, *Mycoplasma hyopneumoniae (Mhyo)* continues to have a significant negative economic impact on swine producers. Our lack of knowledge concerning pathogenic mechanisms continues to hinder progress towards resolving this disease in pigs and others like it in various mammalian species including humans. Mycoplasmas have a small genome and are limited in their biosynthetic potential. They lack the biosynthetic pathways for amino acids and purines and pyrimidines. Thus, they must acquire these from their host. This laboratory was the first to identify membrane-associated nucleases in mycoplasmas, presumably to serve as an activity associated with the uptake of nucleic acid precursors. The source of that external DNA in the host has not been defined, however. The recent observation that neutrophils produce an extracellular trap composed of chromatin as a way to control bacterial diseases suggests that this might be a good source of nucleic acid precursors for mycoplasma growth. To support this idea, mycoplasma disease is characterized by a large influx of neutrophils into the area of colonization. To test the hypothesis that mycoplasmas can utilize the extracellular trap DNA as a source of nucleotides, we will establish a precursor uptake assay using fluorescently labeled nucleotides to establish that such molecules can be taken up and incorporated into DNA by *M. hyopneumoniae*, and subsequently be detected by its fluorescence. Second, we will produce PCR fragments with fluorescently labeled nucleotides and show that these fragments can be digested by external nucleases and the precursors taken up and incorporated into mycoplasma DNA. Finally, we will induce extracellular traps in THP-1 cells with fluorescently labeled DNA and interact them with mycoplasmas to show by scanning electron microscopy and confocal microscopy that the mycoplasmas can disrupt the extracellular traps and take up fluorescent nucleotides.
**Mentor abstract #20**

**A randomized controlled trial of IBK vaccine in beef calves and a systematic review of veterinary vaccines or diagnostic tests.**

Principle Investigator:
Annette O’Connor, BVSc, MVSc, DVSc, FACVSc (Epidemiology)
Iowa State University, College of Veterinary Medicine
Veterinary Diagnostic and Production Animal Medicine Department

Abstract:

The aim of this project is to conduct a randomized control trial of Commercial pinkeye vaccine at the ISU McNay farm. The study will be conducted consistent with best practices for randomized trials. Calves will be enrolled in the study in Spring 2015, and outcome data will be collected at weaning. The outcomes of interest are occurrence on pinkeye and weaning weight. Examples of prior trials we have conducted are available (Funk et al., 2009; O’Connor et al., 2011; Gould et al., 2013). The student will also conduct a systematic review of a vaccine or diagnostic assay of importance to veterinary science. It is expected that the review will be published in the peer-reviewed literature. The exact topic for the review will be selected in consultation with the student and other collaborators early in the summer scholars program. Prior students have published reviews on Tritrichomonas vaccines in beef cattle(Baltzell et al., 2013), pain management in livestock(Newton and O’Connor, 2013), and pinkeye vaccine(Burns and O’Connor, 2008) and other topics (O’Connor et al., 2006; O’Connor et al., 2010; da Silva et al., 2014).


Optogenetic manipulation of cortical circuits

Principle Investigator:
Diana Peterson, PhD
Iowa State University, College of Veterinary Medicine
Biomedical Sciences Department

Abstract:

The brain is composed of numerous interconnected circuits which contribute to its ability to pay attention to specific stimuli, learn from the environment, and initiate appropriate behavioral responses. Because the circuits are highly interconnected, the role of each circuit is not understood. New optogenetic techniques enable precise manipulation of an individual brain circuit. The proposed research will use these innovative techniques to isolate and target very specific pathways, alter their function, and examine how these changes influence the animal’s behavior.

The current proposal focuses on the amygdala, a brain region responsible for determining stimulus significance. Although the amygdala has substantial projections to auditory cortex, the function of this pathway has never been examined. Preliminary data indicate that the direct amygdalo-auditory cortex circuit has a transient but substantial influence on auditory processing. Because the amygdala determines stimulus significance, this pathway may be responsible for shifting auditory attention and contributing to acoustic conditioning (learning) within cortex. The planned experiments examine individual circuits for the first time and provide evidence for the hypothesis that auditory conditioning is facilitated by the combined inputs of numerous regions of the brain. To identify the role of the amygdala, the proposed research will deactivate the amygdalo-auditory cortex pathway during behavioral conditioning training. The results will identify how changes in cortical processing influence the animal’s behavioral response. Aberrations in these circuits have been shown in both learning disorders as well as psychiatric illnesses. Therefore, we hypothesize that understanding these circuits will lead to increased insight of how variations in electrical activity result in the varied symptomology these illnesses.
Mentor Abstract #22

Use of a gnotobiotic mouse community for gut microbiome studies

Principle Investigator:
Gregory Phillips, PhD
Iowa State University, College of Veterinary Medicine
Veterinary Microbiology and Preventive Medicine Department

Collaborating Investigator:
Michael J. Wannemuehler, PhD
Iowa State University, College of Veterinary Medicine
Veterinary Microbiology and Preventive Medicine Department

Abstract:

Recent experimental evidence reveals that the bacterial communities (microbiota) that comprise the mammalian gastrointestinal (GI) tract can have a profound influence on the health of the host. Diseases ranging from colorectal malignancies to inflammatory bowel diseases (IBD) have been linked to an abnormal microbiota (dysbiosis) in humans and animal models. Despite the importance of bacteria to the wellbeing of humans and animals alike, how the microbiota influences health and disease is still not well understood. To better understand how specific microorganisms interact with the host, we are using a unique gnotobiotic mouse community, the Altered Schaedler Flora (ASF), which is comprised of animals colonized with only 8 known bacterial species. Despite the low complexity of the microbiota, ASF mouse exhibit normal immune system development and growth. Use of this resource includes monitoring the changes in relative number, spatial distribution and gene expression in response to alterations in diet and following infection with bacterial pathogens. Independent student projects include, but are not limited to, using PCR to measure changes in the abundance of individual ASF community members in response to infection with Escherichia coli, as well as identifying genetic changes in the ASF that occur in response to changes to the gastrointestinal (GI) tract. The ASF model also offers the potential to study exciting new results that indicate that the composition of the GI microbiota may actually influence animal behavior. The overall impact of these studies will lead to a better understanding of how the GI microbiota influences human and animal health and disease.