Project Title: **Food animal antimicrobial susceptibility testing evidence-based medicine guide**

Principle Investigator(s): Adam Krull, DVM, PhD

Collaborating Investigator(s): Amanda Kreuder, DVM, PhD, DACVIM (LAIM)

Abstract: (300 words or less):

With the increase in antimicrobial resistance and a push towards utilizing evidence based medicine in the treatment of bacterial disease in veterinary medicine, readily available pharmacokinetic data for all antibiotic choices at each acceptable dosing strategy needs to be compiled in a handy reference guide for all species. Currently, the only reference guide available is the Target™ Antibiotic Reference Guide to Effective Treatment developed by David Aucoin. While a tremendous reference for canine and feline antimicrobial treatment strategies, no such guide is available for large animal medicine. The summer project would consist of gathering data from published pharmacokinetic studies and beginning to create a reference guide for food animal antimicrobials for one primary species (bovine, swine, sheep, goats or camels). Additionally, the student would recognize deficiencies in the literature and assist in design of a pharmacokinetic study to fill an identified the gap. At the end of the summer, the student will have compiled all relevant data for the species chosen into a convenient guide, identified antimicrobial/dosage/species combinations that still need to be evaluated, and successfully complete a pharmacokinetic trial to address one of those needs.
Project Title: Structure and function of conserved virulence factor A that regulates invasive diseases caused by \textit{Streptococcus pyogenes}

Principle Investigator(s): Brian Lee (BMS)

Collaborating Investigator(s): Gabriela Pérez-Alvarado (VMPM)

Abstract: (300 words or less):
\textit{Streptococcus pyogenes} is associated with superficial infections including scarlet fever, impetigo, and pharyngitis, which may develop into invasive diseases such as necrotizing fasciitis or streptococcal toxic shock syndrome. Sequelae that may develop after streptococcal infection include rheumatic fever or glomerulonephritis. Our research is focused on the regulatory endonuclease, conserved virulence factor A (CvfA), which contains a metal-dependent phosphohydrolase domain and regulates the expression of glycolytic enzymes and virulence factors in response to growth phase and 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 and RNA binding specificity of CvfA. Our approach includes using NMR spectroscopy, X-ray crystallography, enzyme assays, and other biophysical techniques to study the structure, RNA interactions and catalytic activity of CvfA. The impact of our results may lead to new therapeutic strategies against streptococcal infections. Students may participate in all stages of this project and will receive training in molecular biology, protein chemistry, bioinformatics and structural biology.
Project Title: Evaluation of therapies to inhibit Neutrophil Extracellular Trap formation in dogs with immune-mediated hemolytic anemia (IMHA)

Principle Investigator(s): Dana LeVine

Collaborating Investigator(s): Mary Nelson, 3rd year veterinary student (previous summer scholar)

Abstract: (300 words or less):

Immune-mediated hemolytic anemia (IMHA) is a common cause of severe anemia in dogs. IMHA is an autoimmune disease in which autoantibodies develop that target normal red cells for destruction. Unfortunately, IMHA is associated with a tragically high mortality rate (up to 80%) and mortality is mostly attributed to fatal thromboembolic events. The cause of thromboembolism is poorly understood.

Treatment of IMHA involves generalized immunosuppression with various immunotherapies and clot prevention with various antithrombotic medications. Despite these therapies, fatal thrombi still occur. Furthermore, there are no studies comparing the efficacy of different immunosuppressive or antithrombotic therapies in dogs with IMHA, leaving clinicians to select drugs without any evidence.

When activated, neutrophils can extrude neutrophil extracellular traps (NETs), webs of DNA, nucleosomes, histones, and granular proteases. NETs are designed to trap and kill invading microorganisms. However, NETs can also activate clot formation and induce thrombosis. We believe that NETs are important in the pathogenesis of thrombosis in IMHA and we have shown that markers of NETs are increased in the blood of dogs with IMHA (Jeffery et al. 2015). We have preliminary results suggesting that circulating cell-free DNA, a NET marker, is predictive of death in dogs with IMHA. As such, NETs are a logical new therapeutic target in IMHA. We hypothesize that some current IMHA therapies will prevent canine neutrophils’ ability to generate NETs, i.e. NETosis. We will assess current immunosuppressive and antithrombotic therapies for their ability to prevent canine neutrophils from undergoing NETosis ex vivo. In so doing we will determine which therapies can prevent NETosis and thus which would be better frontline IMHA treatments.

Through this project you will learn to isolate canine neutrophils and stimulate NETosis (https://www.jove.com/video/54726/a-simple-fluorescence-assay-for-quantification-canine-neutrophil). You will also have the opportunity to practice venipuncture skills and you will become an expert on IMHA.
Project Title: Population Ecology and Control of Avian Pathogenic *E. coli* (APEC) in Poultry

Principle Investigator(s): Catherine M. Logue

Collaborating Investigator(s): Lisa K. Nolan, and Nicolle Lima Barbieri

Abstract: (300 words or less): Poultry production and its sustainability are threatened by acute fatal septicemia or sub-acute pericarditis, airsacculitis, salpingitis, and peritonitis (together termed as colibacillosis) caused by Avian Pathogenic *Escherichia coli* (APEC). Control of APEC is elusive, as its virulence and risk factors are poorly understood. Previous studies have identified virulence and antimicrobial resistance plasmids as contributors to disease. Current evidence shows that plasmid-bearing APEC are emergent that are more virulent and resistant and have greater potential to cause human disease with antimicrobial interventions favoring their selection in the production environment. It is imperative we understand what is causing these plasmid-bearing APEC to emerge.

Our academic and industrial partnership seeks to understand the biology and ecology of APEC and risk factors for colibacillosis. **Our primary hypotheses are:** (i) Comprehensive sampling for *E. coli* and metagenomics analysis from particular niches in the production environment will provide insight into the succession of strains in a poultry house where APEC is harbored and the selective pressures favoring their selection. (ii) Pressure from continued use of antibiotics and some disinfectants predispose birds to APEC infection by selecting more virulent and multi-drug resistant (MDR) strains. **Our secondary hypotheses are:** (i) APEC plasmids confer a selective advantage for *E. coli* survival because these plasmid-bearing strains have not diminished. (ii) Current ‘on-farm’ production practices favor survival/selection of plasmid-bearing *E. coli*.

We will test the **hypothesis** that antimicrobial interventions such as use of antibiotics, disinfectants and heavy-metal containing feed supplements in poultry production, select for higher proportions of APEC among the total *E. coli* population and that repeated use of interventions prevents reversion to non-pathogenic populations. Our **approach** to testing this hypothesis will be to collect empirical data from the poultry production system on-farm and at the processing plant. To do so, we will map the poultry-associated metagenome over time, and ascertain its composition, resistome and virulome, before and after antimicrobial interventions.

Insights will be generated to provide the poultry industry with tools to reduce the impact of APEC and occurrence of colibacillosis thereby improving the health and welfare of billions of birds but also improving the potential impact of APEC on human health.
Mycoplasma hyopneumoniae continues to cause economic losses in the swine industry despite the availability of commercial vaccines and significant advances in our understanding of the disease process. The hypothesis of this proposal is that Mycoplasma hyosynoviae can serve as a unique vaccine delivery vehicle for M. hyopneumoniae antigens in swine thereby providing a stronger mucosal immune response. M. hyosynoviae is known to reside in the tonsillar tissues, an immunological tissue capable of seeding immune reactive cells to organ systems throughout the body, particularly to mucosal tissues. Whether stimulation of this tissue can provide protection against lung infections in the pig has not been clearly delineated. To test this hypothesis, we will construct a modified M. hyosynoviae strain expressing one or more of the members of the P97-P102 gene family members from M. hyopneumoniae and test it in swine for its ability to generate immune responses against those antigens. To accomplish this long-term goal, we must first demonstrate an ability to genetically manipulate M. hyosynoviae by transformation. We will then construct transposon vectors expressing M. hyopneumoniae antigens, transform them into M. hyosynoviae, and demonstrate expression of those antigens in M. hyosynoviae. Finally, we will infect pigs with the modified M. hyosynoviae strain and monitor immune responses against the cloned antigens. Our initial target(s) will be members of the P97/P102 gene families of M. hyopneumoniae, notably P97, which we have studied for years and have various immunological reagents available to study its expression. These studies will reveal the potential for using M. hyosynoviae as a vaccine delivery vector and provide basic information about promoter sequence recognition and protein secretion in M. hyosynoviae. Depending on the project’s progress in the next several months, the Summer Scholar will be involved in the construction of antigen expressing vectors, transforming M. hyosynoviae and monitoring expression of the cloned antigens.
Project Title: The molecular basis for biofilm formation ability in Avian Pathogenic *Escherichia coli* (APEC).

Principle Investigator(s): Lisa K. Nolan

Collaborating Investigator(s): Nicolle Lima Barbieri; Catherine M. Logue

Abstract: (300 words or less):
Avian pathogenic *Escherichia coli* (APEC) is the etiologic agent of colibacillosis, an important cause of morbidity and mortality affecting all facets of the poultry industries worldwide. Though APEC’s abilities to persist in the poultry environment, resist antimicrobial efforts and acquire genetic information from other microbes are well known, the role of biofilms, known to contribute to these traits in related bacteria, is poorly understood for APEC. Here, we propose to fill this gap in order to understand the role of biofilms in the pathogenesis of colibacillosis and to better advise producers on how to prevent this disease in their flocks.

Biofilms are microbial communities that adhere to one another, ensuring their survival and providing an environment for genetic exchange. Resistance of biofilm-forming bacteria to antimicrobial drugs and detergents complicates biofilm elimination during cleaning and disinfection on poultry farms and in industrial settings. To date, little information has been available on the genetic determinants of biofilm production by APEC. With annual multi-million dollar losses and animal health at risk, elucidating the mechanisms of biofilm formation is an urgent matter. Therefore, the aim of this study is to evaluate the molecular basis for biofilm formation in APEC. To do so, we will create a random mutant library using Signature Tagged Mutagenesis (STM), and from this library, identify mutants impaired in biofilm formation as compared to the Wildtype (WT). Finally, we will select putative biofilm formation genes for further phenotypic characterization.

The primary outcome of this research will be the elucidation of the genetic basis of biofilm formation in APEC. From this, new targets for colibacillosis control will also be identified. This novel approach will serve to improve the quality of poultry production and lessen dependence on classical strategies using antibiotics and other means.
Project Title: A randomized controlled trial to assess differences in weight gain associated with treatment with Tulathromycin (Draxxin) to Oxytetracycline for the treatment of infectious bovine kerato-conjunctivitis.

Principle Investigator(s): Annette O'Connor

Abstract: (300 words or less):
The aim of this trial is to assess weight gain associated with treatment with either oxytetracycline or tulathromycin in calves with pinkeye (IBK). Infectious bovine keratoconjunctivitis (IBK) is one of the most common and important production-limiting disease of pre-weaned beef calves. IBK or pinkeye produces a range of clinical signs and decreased weaning weights in affected calves. In the absence of additional vaccine evidence, antibiotic therapy is the likely best approach in minimizing the impact of IBK. In a recent review of antibiotic treatment options for IBK, we attempted to use a mixed treatment comparison meta-analysis to determine which of the various drugs used to treat IBK was superior. However, sparse data meant that it was not possible to make such inference. This review highlighted the need for a well-conducted independent assessment of treatment options for IBK and provided the motivation for the trial. Oxytetracycline and tulathromycin are registered for use for IBK in the US, therefore producers need information about the comparative efficacy of these products. We will randomly assign calves with IBK to these treatment options and follow them over the summer to determine weight gain. The weight gain will then be compared.

The student will work on two projects over the summer. First, the student will work on the 2nd year of a two-year randomized controlled trial. The student will also work on updating systematic reviews of vaccination and treatment options for various reviews including pinkeye, PCV-II, BRD that we have conducted. This includes data collection, data analysis and working with web interfaces for end-users. The student suited to this position should have willing to work with cattle, collecting data and be interested (and preferably have experience) with coding and web applications (R, R-Shiny, python, etc) and be comfortable working independently.
Innovative tools for effective control and eradication of porcine reproductive and respiratory syndrome

Fernando Osorio, Hiep Vu, Eric Weaver & Asit Pattnaik; Nebraska Center for Virology & School of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln,

Veterinary vaccinology (the study of strategies for designing and developing immunogens for use in animals) has always steered the progress on the science of vaccines, with advantage over similar applications in humans. The possibility of testing the efficacy of the final outcomes in the target host species plus the great economic significance of several of the major viral diseases affecting swine sustain the significance that swine vaccinology has for the development of novel, next generation vaccines.

Our research has always centered on the pathogenesis of and the immune response to swine viral infections, studying mainly porcine reproductive and respiratory syndrome (PRRS) which is the most important viral disease of swine. The disease is prevalent worldwide; causing tremendous loss to swine producers. Vaccine is the most cost-effective tool to control PRRS. Unfortunately, commercially available PRRS vaccines are not effective. Overall, using PRRSV as center model, our comprehensive research goal is to develop an improved, rationally designed broadly protective vaccine against PRRSV. Along this line we are conducting at least 3 main lines that may be of interest to veterinary students in their summer research projects:

1) We have developed a revolutionary concept that may have profound implications for successfully vaccinating swine herds against PRRSV: using bioinformatics and molecular biology we developed a synthetic virus (i.e. a PRRSV consensus strain non-existing in nature), with the broadest possible coverage against all the strains circulating in the field. We have now advanced to an attenuated form of this consensus virus, which could be used to clinically evaluate multiple parameters of vaccine efficacy in vaccinated pigs.

2) We have now extended the same concept to an experimental design of an universal swine influenza virus consensus sequence vaccine, whose clinical and in vitro efficacy may have great significance not only for the US farm economy but also great implications in public health.

3) We have developed an experimental line of chimeric PRRSV viruses (genetically obtained versions of mixed types of PRRSv strains) that would serve as powerful tools to find subunit vaccine candidates (non-replicating viral protein-based immunogens) which could be used to discover and dissect the parts of key PRRSV proteins that could induce powerful broadly neutralizing antibodies in vivo when inoculated in pigs.
Project title
Investigation of the prevalence of vestibulovaginal septa in dogs with recurrent urinary tract infections

Principle investigator
Jean-Sébastien Palerme, DVM, MSc, DACVIM

Abstract
The importance of normal anatomy in the prevention of urinary tract infections is well recognized. Internal (such as ectopic ureters) as well as external (such as recessed vulvas) anatomical abnormalities have been identified as risk factors for recurrent urinary tract infections in dogs. Surgical or endoscopic correction of these abnormalities has been associated with decreased frequency or complete resolution of infections.\(^1\)\(^2\) Vestibulovaginal septa (VVS) are frequently identified during routine cystoscopic procedures to evaluate dogs for a variety of lower urinary tract signs such as incontinence, hematuria or pollakiuria and the common recommendation is to section the septa when they are found.\(^3\) However, their association, if any, with urinary tract infections remains unclear. Indeed, these structures are commonly seen in dogs without lower urinary tract signs.

In this study, we aim to assess if VVS (as well as other abnormalities) are more commonly found in dogs with recurrent urinary tract infections than in a population of dogs without any urinary tract signs. This shall be done by (1) prospectively recruiting a population of dogs with no urinary disease that are undergoing anesthesia for reasons other than cystoscopy. In addition, (2) we will retrospectively recruit cases that underwent cystoscopies for investigation of recurrent urinary tract infections from the medical records of ISU as well as other veterinary institutions. Results of this study could offer insight into the cause of recurrent urinary tract infections as well as help determine if ablation of VVS is medically justified.
Project Title: Effect of fat loading on intestinal lacteals in healthy dogs

Principle Investigator: Jean-Sébastien Palerme, DVM, MSc, DACVIM

Abstract:
Lymphangectasia is a common cause of protein-losing enteropathy in dogs. An integral part of the diagnostic work up of these cases involves the use of gastroduodenoscopy and coloileoscopy to evaluate the mucosa as well as obtain endoscopic biopsies of the small intestines. A classic endoscopic finding in these dogs is the presence of dilated lacteals, which demarcate themselves from the rest of the mucosa by their white appearance. Some gastroenterologist recommend the administration of a lipid-rich meal prior to endoscopy in order to enhance the appearance of these lacteals and facilitate diagnosis. However, no studies have been performed to evaluate the efficacy of these meals nor has the exact timing or dosage been evaluated. Using a newly available non-invasive technology in veterinary medicine, capsule endoscopy, we propose to evaluate the effect of fat-loading on the small intestines of a population of healthy dogs.