Project Title: CRISPR-based diagnostic assay for Cache Valley virus

Principal Investigator(s): Brad Blitvich

Veterinary Scholar Focused Abstract: (300 words or less):

Cache Valley virus (CVV) is a mosquito-transmitted virus that is distributed across most of the United States. CVV infections in sheep are common and often result in pregnancy loss and severe congenital defects such as arthrogryposis and hydranencephaly. Diagnosis of CVV is based on three main laboratory methods: virus isolation, reverse transcription-polymerase chain reaction (RT-PCR) and plaque reduction neutralization test (PRNT). One disadvantage of the virus isolation procedure is the need to maintain a continuous cold-chain during transportation of the sample from the field. RT-PCRs require sample manipulation (e.g. RNA extraction) and expensive machinery (e.g. a thermocycler). PRNTs are labor-intensive and take over a week to perform. Recent advancements in the field of molecular diagnostics have been made possible due to the groundbreaking discovery that bacteria have heritable adaptive immunity mediated by the CRISPR-Cas system. In this grant application, the power of CRISPR-Cas will be harnessed to develop a portable diagnostic assay for the rapid, inexpensive and specific diagnosis of CVV. The assay will detect viral RNA without the need for technical expertise, user-training or specialized equipment, allowing it to be used by veterinarians and producers in the field. The timely diagnosis of affected sheep is critical for disease prevention and control and the mitigation of economic losses.
Project Title: Parasites in Calves

Principal Investigator(s): Matt Brewer

Collaborating Investigator(s): Katy Martin, Jeba Jesudoss

Veterinary Scholar Focused Abstract: (300 words or less):

*Tritrichomonas foetus* is a sexually-transmitted protozoan parasite of cattle. Our laboratory is interested in the diagnosis and treatment of this organism. In our current project, we are attempting to create a bovine calf model for the disease. The summer scholar will be responsible for laboratory work, including maintaining parasite cultures and processing samples for ELISA and PCR. The student will also assist with other ongoing projects in the parasitology laboratory, as needed. Students are encouraged to talk to Dr. Brewer if they are interested in this project.
Project Title: Identification of risk factors associated with increased respiratory disease in finishing beef cattle

Principal Investigator(s): Grant Dewell

Collaborating Investigator(s): Vickie Cooper, Drew Magstadt, Renee Dewell, Annette O’Connor

Veterinary Scholar Focused Abstract: (300 words or less):

Despite advancements in vaccines and antibiotics, Bovine Respiratory Disease (BRD) continues to be the primary cause of disease among feedlot cattle. In fact, recent trends have indicated that feedyard morbidity and mortality associated with BRD have been steadily increasing in recent years. Although many research projects have focused on individual BRD pathogens or prevention strategies, there has not been a thorough investigation of why BRD is increasing among feedyard cattle.

The specific objectives of this project are: 1) Determine the incidence of BRD in typical Iowa feedyards. 2) Identify risk factors associated with increased morbidity and mortality associated with BRD among different groups of cattle.

Specifically, for objective one 20 feedlots will be solicited to provide morbidity and mortality information for a six month time period. For objective two, two feedlots will be recruited to provide complete history, management and disease information.

We expect to determine the current baseline of BRD in Iowa feedyards. This information will provide an important benchmark that future researchers can use when addressing BRD issues. Additionally, it will provide needed background for extension and outreach efforts geared at addressing BRD issues. Identification of risk factors associated with increased BRD morbidity and mortality in feedyards can be used by producers and veterinarians to establish potential mitigation strategies for control of BRD. Additionally, researchers can leverage outputs from this project for funding to conduct nationwide studies on BRD risk factors and to develop novel intervention strategies for risk factors that do not currently have effective control measures.
Project Title: Development of a clinically relevant lameness model for evaluation of analgesic strategies in dairy cattle

Principal Investigator(s): Patrick Gorden

Collaborating Investigator(s): Rochelle Warner, Jonathan Mochel, & Locke Karriker

Veterinary Scholar Focused Abstract: (300 words or less):

Pain is a physiological response that cattle often experience as a result of pathological conditions, including lameness. Pain associated with lameness continues to be a substantial welfare concern in the dairy industry. With the exception of transdermal flunixin for pain control associated with interdigital pododermatitis (foot rot), extra label therapies are required to provide analgesia for other painful conditions. One of the major reasons for a lack of approved analgesics for lameness pain in cattle is a lack of a clinically relevant lameness model which could be used to conduct analgesic studies. Additionally, the underlying mechanisms for pain (chronic nociceptive vs. neuropathic pain) require different therapeutic approaches. In a recent project, our research team demonstrated effectiveness of analgesics in cows experiencing mild lameness. In this project, we propose to first develop a clinically relevant, chronic lameness model similar to what has been done in swine. After development of the lameness model, we will use computer simulation to compare results of biomarkers and lameness detection modalities from lameness induction to cattle that experience naturally occurring lameness. We then propose to utilize the lameness model to design a year-2 project which evaluate effective analgesic strategies towards lameness.
Project Title: Vitamin A and Zinc co-supplementation to support mucosal immunity and promote enhanced resistance to bovine respiratory infection

Principal Investigator(s): Jodi McGill

Collaborating Investigator(s): Stephanie Hansen

Veterinary Scholar Focused Abstract: (300 words or less):

Bovine respiratory disease is the leading cause of morbidity and mortality among feedlot cattle in the Midwest and worldwide. Our research is directly focused on identifying strategies to reduce the impact of respiratory disease on feedlot cattle, and thus, speaks directly to IHLAC’s major research priorities. Vitamin A (VA) is a fat-soluble vitamin that is essential for optimal function of the immune system in the respiratory tract. Zinc (Zn) is an essential trace mineral that is important for the immune response to intracellular pathogens, such as viruses. Importantly, VA and Zn regulation are intricately linked. Zinc is essential for transporting VA from the liver and into the blood. VA is important for Zn absorption and utilization by the liver. Studies in humans have shown that both Zn and VA statuses directly impact morbidity associated with respiratory infections; however, little is known about the impact of acute illness on VA or Zn utilization by ruminants. There is currently a worldwide shortage of synthetic VA, causing many to consider the most appropriate, and cost effective, strategies for supplementing cattle with VA during times of need. In other species, acute respiratory disease is linked to VA deficiency, and a subsequent increase in susceptibility to secondary infection. In agreement, our preliminary studies in preweaned calves show that acute respiratory infection has a significant negative impact on circulating and stored VA levels, causing vitamin- and mineral-replete calves to become VA deficient. Currently, the effects of this deficiency on the animal’s susceptibility to subsequent secondary infections are not known. It is also unclear if acute infection will have a similar impact on weaned calves. Here we propose to explore the dynamics of Zn and VA concentrations in the weaned calf during acute viral infection. Our objectives are to: 1) determine the effect of acute respiratory viral infection on VA and Zn status in weaned calves; and 2) determine the impact of VA and Zn co-supplementation on the outcome of acute respiratory infection in vitamin- and mineral-replete calves. We hypothesize that acute respiratory viral infection will negatively impact the micronutrient status of weaned calves, and that VA/Zn co-supplementation will counteract the micronutrient-depleting effect of infection, and positively impact disease outcome. Our results will significantly enhance our understanding of the impact of nutrition on respiratory health, and will identify supplementation strategies which will provide optimal benefit to the animal and subsequently, the producer.
Project Title: Generation of Site-Specific Mutants of *Mycoplasma hyopneumoniae*

Principal Investigator(s): Chris Minion

Collaborating Investigator(s):

Veterinary Scholar Focused Abstract: (300 words or less):

*Mycoplasma hyopneumoniae* (*Mhyo*) is a significant pathogen of swine. A major component of the Porcine Respiratory Disease Complex, *Mhyo* continues to cause significant economic problems world-wide for the swine industry. In terms of colonization, its pathogenesis has been documented, but *Mhyo*’s effect on the immune surveillance of the host is not well understood nor are many of the features of adherence and colonization in the host. The surface of *Mhyo* is complex with approximately 100 of the 650 proteins already identified as surface proteins. Major contributors to the overall pathogenesis of the organism are the gene family members of P97 and P102, components of an adherence operon. P97 is the main ciliary adhesin and P102 has unknown functions but is thought to function in adherence as well. All paralogous members of the two families undergo proteolytic processing as part of their maturation process; this processing occurs either as they translocate through the membrane or after arrival on the membrane surface. The goal of this project is to determine which of the 5-6 possible protease genes are responsible for this processing, and if processing is a prerequisite for virulence in swine. *Our hypothesis is that loss of proteolytic processing of P97 results in attenuation of Mhyo.* We will construct mutations in the protease genes by genetic recombination and test them for their ability to process the P97/P102 gene family members by immunoblot. Identification of the protease gene products responsible for P97/P102 processing could potentially provide new targets for treatment of *M. hyopneumoniae* infections or vaccine development.
Project Title: Fecal microbiome transplantation as an antibiotic-alternative against gut dysbiosis and porcine postweaning diarrhea

Principal Investigator(s): Shankumar Mooyottu

Collaborating Investigator(s): Alex Ramirez

Veterinary Scholar Focused Abstract: (300 words or less):

Post-weaning diarrhea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) is an economically important disease in weaned piglets. Currently, antibiotics are used in the swine industry to control enteric infections including PWD in pigs. The overuse of antibiotics is directly linked to the emergence of antibiotic-resistant bacteria which poses a threat to human and animal health. In this context, identifying a safe antibiotic-alternative to prevent PWD in piglets has become a priority.

Recent studies have confirmed that disruption of gut microbiome (gut-dysbiosis) induced during weaning transition is a critical factor involved in PWD pathogenesis. Therefore, stabilizing the gut microbiome during weaning transition could be an effective strategy for controlling PWD. Recently, fecal microbiome transplantation (FMT) has been established as a radical and effective therapy to prevent gut-dysbiosis and control important enteric disease in humans. FMT is highly effective against gut-dysbiosis, pathogen colonization, and gut inflammation, both prophylactically and therapeutically. However, no studies have been conducted to test the efficacy of FMT in preventing gut-dysbiosis and PWD in pigs. This proposal investigates FMT as an effective, inexpensive and environmentally-friendly alternative to antibiotics for preventing gut-dysbiosis and ETEC infection in weaning piglets.

For testing this hypothesis, 3-week-old piglets will be provided with fecal microbiome mix orally during weaning transition. On day 10, piglets will be challenged with ETEC, and the diarrhea score, bacterial load, and histologic scores will be assessed post infection for 7 days in treatment and respective control groups. Additionally, piglets will be sacrificed on 4th and 7th-day post-infection, and intestinal samples will be collected. The fecal microbiome of different treatment groups will be analyzed using high-throughput sequencing technology.

The results from this research will provide the swine industry with a readily executable, non-antibiotic strategy to control PWD in piglets, and provide insights on ‘microbiome modulation by FMT’ as a potential strategy to promote gut-health in swine operations.
Project Title: Comparison of Intranasal and intramuscular administration routes of a universal pig M2e flu vaccine in pig model

Principal Investigator(s): Tanja Opriessnig, Dr med vet, PhD

Collaborating Investigator(s): Phillip Gauger, DVM, MS, PhD

Veterinary Scholar Focused Abstract: (300 words or less):

1. Statement of the problem: Influenza A virus (IAV) infection of pigs continues to remain a threat to the global pig population and is considered one of the most important diseases in US swine herds. The M2e protein, a small highly conserved protein expressed on the IAV surface, has been proposed as an ideal target for a universal flu vaccine. Recently, differences in the M2e genetic structure among IAV strains have been identified which appear to be related to the most common host species (i.e. human, pig, or avian hosts). The objective of this study is to determine if vaccination of M2e administered via the intranasal route, is successful in protecting pigs from challenge with IAV strains.

2. Hypothesis to be tested: Our hypothesis is that vaccination of pigs by the intranasal route with a pig-specific M2e epitope matched to other pig isolates available in GenBank will result in a stronger mucosal immune response and protect pigs against challenge better than compared to intramuscular administration and will protect pigs against challenge with contemporary IAV isolates.

3. Experimental plan and expected results:
An M2e vaccine candidate has been selected and will be tested in February 2019 using two vaccine administration routes: Intranasal and intramuscular. The humoral immune responses against the peptide (IgG and IgA against M2e) will be assessed by M2e-specific in house ELISAs and the more effective vaccination route will be used in the main study. We expect that intranasal vaccination with the M2e-based vaccine will result in result in a higher local immune response. Serum from vaccinated pigs will be tested against various IAV strains in vitro using serum neutralization assays. In the main study pigs will be challenged with one of two contemporary IAV strains to assess vaccine efficacy. A total of 36 pigs from an IAV free source will be randomly assigned to one of five groups. At 3 and 6 weeks of age, VAC-IAV1 and VAC-IAV2 pigs will be vaccinated while the other three groups remain non-vaccinated. At 8 weeks of age, pigs in groups NV-IAV1 and VAC-IAV1 will be challenged with IAV1 isolate and pigs in NV-IAV2 and VAC-IAV2 will be challenged with IAV2 isolate. All pigs will be necropsied 5 days later. Serum will be collected at regular intervals and tested for IgG and IgA antibodies. Nasal swabs will be collected at challenge and daily afterwards and tested by IAV real-time PCR for IAV RNA shedding. Macroscopic and microscopic lung lesions will be scored and the scores will be compared among groups. This study is expected to serve as a proof-of-principle. We expect that vaccinated pigs will have reduced clinical signs, reduced IAV shedding and reduced lesions compared to unvaccinated pigs. This work will not only broaden our understanding of M2e and its ability to protect pigs against heterologous IAV strains but it could also result in a universal pig flu vaccine capable of protecting pigs against diverse flu strains of human or pig origin. This is an important next step into accumulating information on the efficacy of a novel M2e-based vaccine in the pig model. The results of this ILHAC study will lead to submission of external competitive grant proposal(s) to USDA, NIH and private biopharmaceutical industry partners in the near future.
Group C
2019 ISU CVM SSRP Mentor Abstract #9

Project Title: Recombinant interferon to control viral pathogens of swine

Principal Investigator(s): Chandra Tangudu

Collaborating Investigator(s): Bradly Blitvich

Veterinary Scholar Focused Abstract: (300 words or less):

Interferons (IFNs), particularly IFN-α and IFN-β provide one of the first lines of defense against viral infections. Many viruses that affect swine, i.e. porcine reproductive and respiratory syndrome virus (PRRSV), Seneca Valley virus (SVV), porcine epidemic diarrhea virus (PEDV) and swine influenza virus (SIV), encode proteins that block IFN activity. These viruses have a devastating economic impact on the swine industry. Restoring the anti-viral state in pigs by IFN administration could significantly reduce the economic losses associated with viral disease outbreaks. IFN treatment has been successfully used for human healthcare; for example, IFN therapy is the most effective treatment against hepatitis C virus. We have recognized the lack of cost-effective IFN for use in the swine industry. Our goal is to produce inexpensive, industrially scalable and functional porcine IFN using recombinant technology. To achieve this, we will engineer Bacillus megaterium bacterium to produce porcine IFN-α and IFN-β then purify the recombinant proteins and assess their abilities to suppress PRRSV, SVV, PEDV and SIV in porcine cells in vitro. Our studies will directly test the hypothesis that recombinant porcine IFN significantly reduces the ability of the aforementioned viruses to replicate in porcine cells. If our hypothesis is proven correct, recombinant IFN will be tested using an in vivo model in future experiments.
Project Title: Functional genomics of Campylobacter hepaticus associated with chicken spotty liver disease

Principal Investigator(s): Zuowei Wu

Collaborating Investigator(s): Orhan Sahin

Veterinary Scholar Focused Abstract: (300 words or less):
Spotty Liver Disease (SLD) is an acute infectious disease of layer poultry, occurs mostly in barn housed or free-range layer flocks, and causes an acute drop in egg production of up to 35% with a mortality rate as high as 15%, incurring great economic losses to poultry producers. SLD has become an important concern for the poultry egg and meat industries. Although the disease was originally reported in the USA and Canada in the 1950s and 1960s, respectively, the aetiological agent was not identified until 2015 and 2016 when a new Campylobacter species, C. hepaticus, was determined to be the causative agent of SLD in the United Kingdom and Australia, respectively. In the United States, C. hepaticus was identified from livers of laying hens with SLD in 2017. Despite the recent advancement in identifying C. hepaticus as the etiological agent of SLD, little is known about the epidemiology and pathogenesis of C. hepaticus. To begin to close this critical knowledge gap, we propose in this project to study the genomic features and virulence factors of C. hepaticus from the United States by pursuing the following two specific aims. In Aim 1, we will characterize the genomes of C. hepaticus isolated from the U.S., and in Aim 2 we will identify the genetic determinants associated with the virulence of C. hepaticus. We anticipate that the obtained results will reveal the genomic properties of the C. hepaticus strains present on the U.S. farms and identify the virulence factors associated with SLD. These findings will significantly advance our understanding of molecular epidemiology and pathogenesis of SLD and will also be useful for the development of rapid diagnosis and effective vaccines against Campylobacter-induced SLD.