Group C
2018 ISU CVM SSRP Mentor Abstract #1

Project Title: Calf infection Model for *Tritrichomonas foetus*

Principle Investigator(s): Matt Brewer

Collaborating Investigator(s): Jeba Jesudoss Chelladurai

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

Tritrichomonas is a sexually transmitted protozoan parasite that infects cattle. The disease causes early embryonic death and severe economic losses in the cattle industry. There are no treatments for trichomoniasis. Control measures typically involve testing and culling infected bulls. However, it is difficult to procure cohorts of infected cows or bulls for scientific studies. In this project, we will aim to develop a calf model of infection where the organism is cultivated in the laboratory and inoculated into heifer and bull calves. The student will grow the organism, monitor infection status by PCR and culture, and measure the immune response to the parasite by ELISA and western blot.

Group C
2018 ISU CVM SSRP Mentor Abstract #2

Project Title: Development and field validation of a tracheal-bronchial sampling device for detection of *M. hyopneumonaiae* in naturally infected pigs.

Principal Investigator(s): Maria Clavijo

Collaborating Investigator(s): John K Jackman, Bailey Arruda

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

Mycoplasma hyopneumoniae (Mhp) continues to be a significant cause of respiratory disease in grow-finish swine populations, with reported annual industry losses of $400 million. Effective prevention and control of Mhp requires the implementation of accurate and comprehensive diagnostic protocols. Given that Mhp establishes infection in the lower airway, tracheo-bronchial mucus appears to be the most sensitive sample, compared to oral fluids and upper respiratory swabs. However, there are no commercially available tracheo-bronchial sampling devices. Thus, veterinarians have relied on the use of modified tracheal catheters for detection in live pigs. These “quick and dirty” tools have not been properly validated and do not have the needed features to collect adequate amounts of mucus. In this study, we propose to develop and validate a practical, sensitive, safe and low-cost disposable tracheo-bronchial sampling device that can be combined with commercially available swabs; thus, absorbing higher amounts of mucus and increasing the sensitivity of the protocol. Our hypothesis is that the novel tracheo-bronchial device will be more sensitive for Mhp detection compared to the traditional catheter, thus improving significantly the diagnosis of Mhp in swine farms. In this study, we will leverage resources and animals from on-going studies to validate the novel tracheo-bronchial device. We will collect a robust set of samples (n= 406) from experimentally and naturally infected pig populations of different ages, sources and with varying levels of prevalence for estimation of the sensitivity and specificity of the novel device. We expect to develop a sensitive tool that can be easily implemented at the farm level and used to assess infection dynamics in pig herds. More importantly, it is expected that this novel tool will help producers develop improved control and elimination strategies, resulting in enhanced production efficiency, animal health and well-being.

Group C
2018 ISU CVM SSRP Mentor Abstract #3

Project Title: Understanding the epidemiology of Ornithobacterium rhinotracheale in commercial turkeys in Iowa

Principal Investigator(s): Mohamed El-Gazzar

Collaborating Investigator(s): Yuko Sato

Veterinary Scholar Focused Abstract: (300 words or less):
Statement of the problem: Isolation of a microbe from diseased individuals does not mean it is the cause of the disease. To identify the causative agent, we have to tease it out of all normal flora and opportunistic pathogens carried within animal bodies. Ornithobacterium rhinotracheale (ORT) was first identified in 1993 as a microbe often isolated from poultry with respiratory disease. Since then, ORT has been implicated in poultry respiratory disease throughout the world without sufficient evidence to support its role as a primary pathogen. Control and treatment schemes have been devised, including common use of autogenous vaccines. In spite of that, ORT is often listed as one of the top diseases in turkeys. While it is true that ORT can be isolated from birds with respiratory disease, the majority of challenge studies aiming to reproduce the disease produced minimal to no disease. Clinical signs in those attempts were reproduced only when ORT is combined with other respiratory pathogens. Additionally, ORT can be isolated from up to 100% of apparently healthy poultry flocks. Moreover, we don't know the source of infection or the major transmission routes.

Hypothesis to be tested: We hypothesize that ORT can exist as part of the normal flora of poultry respiratory system without producing disease. In other words, ORT is not a primary pathogen, but rather an opportunistic pathogen.

Experimental plan and expected results: We plan to conduct surveillance to estimate prevalence of ORT in turkey flocks with and without respiratory disease. The expected results is that the prevalence of ORT in turkeys with respiratory disease vs turkeys without respiratory disease will not be significantly different. This would improve our understanding of the disease and help guide our approach to its control and prevention.

Group C
2018 ISU CVM SSRP Mentor Abstract #4

Project Title: Precision Animal Health – Identifying biomarkers that provide rapid, accurate prediction of bovine respiratory disease.

Principal Investigator(s): Terry J. Engelken

Collaborating Investigator(s): Jacek Koziel, Vicki Cooper, Annette O'Connor

Veterinary Scholar Focused Abstract: (300 words or less):
Traditional methods of identifying cattle affected with bovine respiratory disease (BRD) have relied upon a combination of a subjective evaluation of clinical signs and change in body temperature. These methods have been shown to have low accuracy in actually identifying animals with BRD. Furthermore, when making decisions concerning the injection of an antibiotic in groups of calves at arrival (metaphylaxis), animal caregivers typically rely on incomplete information and historical assumptions to justify this practice. This leads to an overreliance on antibiotic therapy at a time when consumer groups are calling for decreased use and veterinary associations are emphasizing the need for the judicious use of antimicrobials. Therefore, diagnostics need to be developed that will enable us to accurately identify calves with BRD and precisely target antibiotic use.

Metabolomics utilize analytical methods to identify differences in small molecules or proteins between animals in different physiological states. This type of analysis has been used to identify disease states in different species using various body fluids (serum, plasma, saliva, nasal secretions). It has the advantage of being noninvasive and able to evaluate multiple samples in a relatively short period of time. This technology can be utilized to enhance the accuracy of BRD diagnosis and laboratory diagnostics. The main objective of this study is to utilize metabolomic techniques to profile beef calves of known BRD status and compare those results with normal control animals. The second objective is to identify the molecules or metabolites that are different between BRD calves and controls so that they can be evaluated as potential disease biomarkers.

Group C
2018 ISU CVM SSRP Mentor Abstract #5

Project Title: Comparative pharmacokinetics of meloxicam between healthy post-partum versus mid-lactation dairy cows

Principal Investigator(s): Patrick Gorden

Collaborating Investigator(s): Rochelle Warner

Abstract: Pain is a physiological response that cattle often experience as a result of pathological conditions or through implementation of common management procedures. Meloxicam is a non-steroidal anti-inflammatory drug that is often utilized by bovine veterinarians to control pain in their patients. Pain control measures in bovines involves extra label therapies, as there are no labeled products currently available for pain control. In a recent project (Summer Scholar 2016), our research team discovered significant differences between plasma and milk drug concentrations between post-partum and mid-lactation cows following oral meloxicam therapy. This would necessitate longer withdrawal periods for meat and milk in the post-partum cow following meloxicam therapy, as compared to our normal recommendations. In the summer of 2017, we compared drug depletion in plasma following IV and oral therapy between immediate post-partum and mid-lactation cows. That work was consistent with previous work, in that it took longer for the drug to deplete in plasma in post-partum cows compared to the mid-lactation cohorts following meloxicam administration via both routes.

In the upcoming summer, our research group will conduct a parallel design trial with six groups of eight post-partum cows to determine the length into lactation that this phenomenon persists. Each group of post-partum cows will receive oral therapy at different days following calving. Milk samples will be collected and analyzed using liquid chromatography-mass spectrometry (LC-MS) to determine milk meloxicam concentrations. As a result of this approach, we hope to define the establishment of withdrawal periods in the post-partum cow and also determine how long into lactation veterinarians would need to prescribe longer withdrawal periods.

Group C
2018 ISU CVM SSRP Mentor Abstract #6

Project Title: Genomic epidemiological analysis of mastitis *Klebsiella pneumoniae*

Principal Investigator(s): Ganwu Li

Collaborating Investigator(s): Patrick Gorden

Veterinary Scholar Focused Abstract: (300 words or less):
Mastitis caused by gram-negative bacteria is a major concern on many well-managed US dairy farms. The main bacterial pathogens that cause mastitis in the coliform family are Escherichia coli and Klebsiella pneumonia (K. pneumonia). Mastitis due to Klebsiella infection is more severe on average and results in higher milk loss and mortality of the affected cows compared with mastitis due to E. coli infection. Furthermore, antimicrobial therapy and other ancillary therapies have limited effects against Klebsiella. The dairy farm environment is thought of as the major source of mastitis K. pneumoniae. However, it seems that environmental Klebsiella strains are greatly heterogeneous with different virulence; some strains are virulent and most are non-virulent. Accurate identification of potential sources of pathogenic Klebsiella isolates is important for implementation of preventative measures, but requires thorough understanding of molecular epidemiology and pathogenesis. This new understanding could serve as a new tool for Klebsiella detection in the mammary gland. Here we will test our hypothesis that specific genetic traits enable some K. pneumoniae strains to colonize mammary gland and cause mastitis more efficiently than other strains. We will sequence 100 K. pneumoniae strains isolated from mastitis (60 strains) and healthy cows and environment (40 strains). Genomic epidemiological analysis will be performed in K. pneumoniae strains currently circulating in the USA. The core genome, pan-genome, antibiotic resistome, and virulome (a set of all genes that contribute to virulence) of K. pneumoniae from mastitis and from healthy cows and the environment will also be identified and compared. In addition, identified putative virulence genes will be experimentally verified by using targeted gene deletion combining with in vitro and in vivo models. Additionally, virulence genes-based multiplex PCR will be developed for the detection of mastitis K. pneumoniae. Once completed, this project will lead to a better understanding of molecular epidemiology and virulence mechanisms of Klebsiella sp. mastitis and result in the development of new molecular tool for the separation of virulent Klebsiella strains from non-virulent ones. The results of this project will also provide basis for future development of additional vaccines or vaccine antigens based on virulence factors against Klebsiella mastitis.

Group C
2018 ISU CVM SSRP Mentor Abstract #7

Project Title: Family oral fluids-based PRRSv monitoring in due-to-wean piglets

Principal Investigator(s): Daniel Linhares

Collaborating Investigator(s): Derald Holtkamp, Jeff Zimmerman

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

1. Statement of the problem
Current PRRS monitoring schemes are based on individual pig sampling, and are laborious, logistically complicated, and have poor sensitivity to detect PRRS virus at low prevalence. This study will investigate the use of family oral fluids (FOF) sampling (a population-based sampling method) to surveil PRRS virus in breeding herds undergoing PRRS virus elimination (i.e. at low prevalence of infection).

2. Hypothesis to be tested
We hypothesize that FOF-based monitoring of due-to-wean piglets is a diagnostically sensitive and specific method for detecting PRRSv infection in due-to-wean piglet litters. Proving this hypothesis will represent a major improvement in PRRSv monitoring by enabling more frequent sampling of more pigs, thereby increasing the probability of detecting PRRSv in very low prevalence herds.

3. Experimental plan and expected results
In herds undergoing PRRSV elimination (PRRSV prevalence < 10%) we will compare PRRSV detection in serum versus family oral fluids collected from the same population of due-to-wean piglets. Blood samples will be collected from all due-to-wean piglets in the farrowing room with the oldest piglets. Likewise, family oral fluids will be collected from all due-to-wean litters. All samples will be individually tested by rRT-PCR in the Iowa State University Molecular Diagnostic Research and Development Laboratory.
The data generated by this study will allow generating sample size guidelines to assist veterinarians and producers to surveil breeding herds for PRRSV in herds expected to be at low infection prevalence.

Group C
2018 ISU CVM SSRP Mentor Abstract #8

Project Title: Monitoring ISU VDL data for signs of emerging diseases

Principal Investigator(s): Rodger Main

Collaborating Investigator(s): Daniel Linhares

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

1. Statement of the problem
The swine industry has been recently bombarded with emergence / re-emergence of pathogens, including new variants of Influenza A virus, novel enteric coronaviruses (Porcine epidemic diarrhea virus, Deltacoronavirus), vesicular disease (Senecavirus A), and clinical nervous system-associated viruses. The economic impact of emerging pathogens in livestock operations is correlated with the time-to-detect-disease, and time-to-respond to those challenges. However, there is currently very limited efforts in place to early detect emerging pathogens in the swine industry. Thus, there is an urgent need to establish practical and effective monitoring and surveillance systems to early detect emerging and re-emerging pathogen activity in the swine and other livestock industries.

2. Hypothesis to be tested
The Iowa State University has the world's largest Veterinary Diagnostic Laboratory (VDL) in number of cases, processing >1.2 million samples per year. We propose that ongoing strategic mining of VDL data results in early detection of signals associated with emergence of pathogens. This, in turn, allows the early characterization of novel or modified pathogens, and the implementation of appropriate rapid response measures, minimizing the economic impact of pathogens in the swine industry in Iowa and the US.

3. Experimental plan and expected results
Our group has recently developed a swine disease reporting system (SDRS), which consists of consolidating VDL data (test results) of major pathogens affecting swine in the US. The SDRS reports trends of pathogen test results over time, geographical area, age group, and specimen. However, the program does not have analytical capabilities. This study will develop and incorporate analytical tools for the automated detection of significant changes on test results of the major pathogens affecting swine. This will allow early identification of disease threats affecting swine. The project has the ability to be expanded to other livestock and poultry disease syndromes.

Group C
2018 ISU CVM SSRP Mentor Abstract #9

Project Title: Evaluation of animal welfare and efficacy associated with a novel technology for rapid humane depopulation of laying hen flocks

Principal Investigator(s): Suzanne T. Millman

Collaborating Investigator(s):

Veterinary Scholar Focused Abstract: (300 words or less):
Depopulation of laying hen facilities is a critical aspect of managing animal disease outbreaks, and is also a routine practice at the end of the laying production cycle in regions where end-of-lay hens have no market value. Depopulation is a point of public scrutiny due to concerns of animal welfare, which can be as risk since the hens have no or low economic value. One method of depopulation involves exposure to carbon dioxide gas (CO2), which the AVMA considers to be a conditionally acceptable method of euthanasia of poultry. However, there are practical constraints associated with CO2 because of handling required and potential for suffocation at thigh loading rates when live hens may be piled on top of still conscious hens. In addition, to address animal welfare concerns the AVMA recommends procedures in which hens are first exposed to low concentrations of CO2 gas (<30%) to induce insensibility before higher noxious concentrations are experienced. Recent research in the Millman lab demonstrated that laying hens will willingly enter a chamber containing 25% CO2 gas to access mealworms, and hens remain in the chamber until loss of consciousness occurs. Conversely, hens avoid 50% CO2 concentrations and above.

A new technology, the Hen Sleeper, has the potential to mitigate these animal welfare concerns associated with CO2 depopulation. The device consists of a series of conveyor belts that move hens through increasing concentrations of CO2 gas. Hens are placed in an entry chute, from which they slide through a curtain and into the first conveyor which is maintained at 25-30% CO2, then progress into lower conveyors at 50% and 70% CO2, before exiting into a dumpster 2-minutes after entry. Preliminary trials with the Hen Sleeper on commercial laying hen facilities provide promising results, suggesting the device can be used to depopulate 10,000 hens per hour at a maximum loading rate. There is a need for independent validation of Hen Sleeper in terms of efficacy and animal welfare.

Group C
2018 ISU CVM SSRP Mentor Abstract #10

Summer Scholar's Research Program
Chris Minion
Professor, VMPM

Mycoplasma hyopneumoniae continues to cause economic losses in the swine industry despite the availability of commercial vaccines and advances in our understanding of the disease process. This project involves the development and testing of a new vaccine approach against *M. hyopneumoniae*, one which uses a second mycoplasma to deliver antigens to a major mucosa-associated lymphoid tissue (the tonsils). This approach has two major advantages: mycoplasmas have an alternate codon usage and are difficult to express in non-mycoplasma hosts; second, *Mycoplasma 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 accomplish this goal, we will construct transposon vectors expressing *M. hyopneumoniae* antigens since the simplest way to introduce recombinant DNA into mycoplasmas. Our initial target will be P97, the ciliary adhesin of *M. hyopneumoniae*, which we have studied for many years and have various immunological reagents available to study its expression. We are in our first year of a two-year project and have accomplished construction of several vectors for expressing P97 in mycoplasmas. Our next step is to transform these constructs into *M. hyosynoviae,* and demonstrate expression of those antigens in *M. hyosynoviae*. Finally, we will infect pigs with the modified *M. hyosynoviae* vaccine strain and monitor immune responses against the cloned antigens.

A Summer Scholar student will learn how to grow and modify mycoplasmas, learn some gene cloning techniques, gain hands-on experience with PCR, and potentially be involved in the swine studies including the analysis of the immunological response to the vaccine strain.

Group C
2018 ISU CVM SSRP Mentor Abstract #11

Project Title: Potential of carvacrol as an antibiotic alternative for promoting gut health in piglets

Principal Investigator(s): Shankurmar Mooyottu

Collaborating Investigator(s): Alejandro Ramirez

Veterinary Scholar Focused Abstract: (300 words or less):
Currently, antibiotics are widely in use to control enteric diseases in piglet. The overuse of antibiotics is directly linked to the emergence of antibiotic-resistant bacteria and poses a threat to both human and animal health. Thus, the use of antibiotics in the industry is being tightly re-examined by regulatory agencies. In this scenario, alternate, environmentally friendly and economically viable approaches are required to control enteric disease in commercial swine operations.
Due to inherent antimicrobial and anti-virulence properties, and their comparatively selective inhibitory effect on enteric pathogens, phytophenolics are considered to be a potential alternative to antibiotics in swine operations. Moreover, the gut microbiome has been established to have a critical role in the pathogenesis of swine enteric diseases. Thus, stabilizing gut microbiome during suckling and weaning period could be a potential strategy to prevent enteric diseases in piglets. Carvacrol (CR) is a natural food-grade phytophenol that has potent anti-virulence properties against major pathogens such as E. coli and C. difficile. Furthermore, CR is found to have a protective effect on gut microbiome.
Since the development of healthy gut microbiome and attenuation of virulence of enteric pathogens are important in piglet life as early as in first ten days of birth, this proposal focuses on the efficacy of CR supplementation in suckling piglets. We propose teat spraying (in sow) as a strategy to supplement CR in suckling piglets. The effect of CR as a teat spray will be assessed as follows: 1) Gut microbiome analysis using 16s rRNA sequencing; 2) Fecal CR concentration using GC-MS; and 3) Fecal pathogen carriage by enumerating C. difficile and E. coli.
The results from this study will help the industry to utilize a readily available, food grade and safe antibiotic-alternative to circumvent the impact caused by current and future restrictions on antibiotic use in swine operations.

Group C
2018 ISU CVM SSRP Mentor Abstract #12

Project Title: Comparison of intranasal and intramuscular administration routes of novel monovalent and bivalent universal pig M2e flu vaccines in the pig model

Principal Investigator(s): Tanja Opriessnig

Collaborating Investigator(s): Anbu Karuppannan

Veterinary Scholar Focused Abstract: (300 words or less):
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 influenza A virus (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 determine if vaccination with a novel complex norovirus vaccine platform administered via the intranasal route, using a monovalent vaccinate containing a pig M2e (pM2e) is successful in protecting pigs from challenge with IAV strains containing pM2e. Furthermore, we will also assess if a bivalent vaccine containing both pM2e and human M2e (hM2e) may even have a broader cross protection by neutralizing IAV strains containing pM2e or hM2e in vitro. The complex platform that will be used in this study has been previously utilized in monovalent but also in polyvalent form. Antigens for three pathogens were successfully expressed resulting in detectable immunity against all three pathogens in the mouse model. Co-expression of two or three antigens has been shown to result in higher neutralizing antibody levels against each of the antigens than singular expression.

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 compared to intramuscular administration and will protect pigs against challenge with a contemporary swine IAV isolate.

Group C
2018 ISU CVM SSRP Mentor Abstract #13

Project Title: Mitigation of castration pain in neonatal piglets using aspirin and/or needleless injection of local anesthetic into the teste or spermatic cord.

Principle Investigator(s): Alex Ramirez, Jesse Goff

Collaborating Investigator(s):

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

Project Summary
One important animal welfare consideration involves painful procedures in piglets foremost of which is castration – which are generally performed without anesthesia or analgesia. Castration is done to prevent boar taint of the meat of male pigs. Concerns over failure to provide analgesia during castration have caused the EU countries to enact legislation barring the practice in the near future. Solving this problem is our objective. There are currently no approved analgesics for use in piglets. Flunixin meglumine and meloxicam are injectable non-steroidal anti-inflammatory drugs (NSAID) used for specific disorders such as pneumonia in pigs, but their use for castration pain is considered off-label at present. An analgesic that can be used legally is aspirin (acetylsalicylic acid), an old but effective NSAID which can be given orally (avoiding the pain of an injection). Aspirin at two doses will be tested in piglets at processing to determine if it provides analgesia to the piglets without adversely affecting the piglets. Local anesthesia can also be provided by lidocaine administered into the teste and spermatic cord. Unfortunately several needle sticks in and around each testicle is needed to provide satisfactory anesthesia. It also takes a couple of minutes to work. To work around this problem we propose to test needle free injectors to deliver lidocaine into the teste and the deeper region of the spermatic cord. Needle free injectors have been used to precisely deliver vaccines to piglets and other species such as baby chicks with good success. Pig weight and health will be monitored from castration until they are weaned. Effective analgesia /anesthesia should include a reduction in vocalization during castration, reduced cortisol response and reduced time spent lying down away from the sow and littermates (touch avoidance).

Group C
2018 ISU CVM SSRP Mentor Abstract #14

Project Title: Xenotransfusion and diphenhydramine as a proposed therapy for the treatment of anemia due to *Haemonchus contortus* parasitism in goats

Principal Investigator(s): Joe Smith

Collaborating Investigator(s): Amanda Kreuder, Austin Viall, David Borts, Jon Mochel, David Wong

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

Complications from parasitic infections, such as anemia, are significant causes of decreased production and mortality for goat producers. Treatment of severe anemia due to parasitism often requires emergency blood transfusion, however, field transfusion of anemic goats is often complicated due to a lack of availability of suitable herdmate donors as production status, pregnancy status, and body weight all limit the amount of blood available for transfusion. On farms with significant parasitism problems requiring blood transfusions, the other animals available for on farm donation are frequently anemic themselves. In addition, the cost of maintaining blood donors for all necessary species is cost prohibitive for most veterinary practices. In small animal medicine, the practice of xenotransfusion (transfusing one species with the blood of another) is commonly performed in emergency situations with the transfusion of anemic cats with blood from dogs1. Xenotransfusion is theoretically possible in other species as well; therefore, identifying one ruminant species that could be used to xenotransfuse multiple others would have profound animal welfare implications. The biological similarities between goats and cattle would make cattle ideal blood donors, particularly as being a larger animal, more blood can be collected from a cow than from a goat. Preliminary work performed by our research group transfusing bovine blood into two goats demonstrated that while xenotransfusion is indeed possible, appropriate antihistamine pre-medication for prevention of transfusion reactions will be necessary. At this time, however, practitioners are limited to extrapolating antihistamine therapy from similar species as there are no pharmacokinetic studies of these drugs in goats. To further assess the suitability of xenotransfusion as a therapy for anemia in goats we propose to test xenotransfusion in goats, and study the effects of an antihistamine (diphenhydramine) on the goats undergoing xenotransfusion.