B1 – Dr. Brewer

Project Title: Enhancing control of *Trichomonas foetus* through improved diagnostic testing and control

Principle Investigator(s): Matt Brewer

Collaborating Investigator(s): Katy Martin

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

*Trichomonas foetus* is a protozoan parasite causing early embryonic death and economic losses in the cattle industry. Our lab is interested in developing a chute side diagnostic test that could be used for detection of the parasite in the field. In past studies, we characterized the “immunoproteome”, or the repertoire of antigenic epitopes from the parasite. In this study, we will screen serum from vaccinated and infected animals against these epitopes using microarrays. The student will be responsible for investigating ‘hits’ from the microarrays via ELISA. In addition, the student may have the opportunity to investigate the usefulness of these epitopes for a vaccine in a calf model of disease. If you are interested in this project you are encouraged to discuss with Dr. Brewer prior to ranking this project.
**Project Title:** Evaluation of protective efficacy of a universal flu vaccine against avian influenza in poultry.

**Principal Investigator(s):** Michael Cho

**Collaborating Investigator(s):** Yuko Sato, Mohamed El-Gazzar

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

Influenza virus is a major public health concern as it causes significant morbidity and mortality. The CDC estimates that the virus infected 9.2-35.6 million people, of which 12,000-56,000 died annually since 2010. The total economic burden related to influenza infections in humans is estimated at more than $86 billion/year. Influenza virus can also cause acute, severe respiratory diseases in many animal species, such as swine, horses, cattle, dogs, cats and poultry. In 2014-2015, the H5 highly pathogenic avian influenza (HPAI) epizootic outbreak devastated the poultry industry in North America as the largest recorded animal disease outbreak resulting in the death of over 48 million poultry. The USDA has been actively involved in looking at development, evaluation, and acquisition of efficacious vaccines for future control strategies. Currently, there are no licensed vaccines against avian influenza virus (AIV) that are broadly protective. The major roadblock in developing protective vaccines against influenza virus is antigenic variation. The overall protection of current vaccines is conferred by antibodies against the globular head domain of hemagglutinin (HA1), which is highly variable. Consequently, the vaccine efficacy is largely dependent on how closely the vaccine strains match viruses that will be circulating. In this regard, a universal influenza vaccine that can provide protection against all or most influenza strains is highly desirable. We have recently generated a universal swine flu vaccine using the “stem” domain (HA2) of hemagglutinin from H1 and H3 swine influenza isolates. In this project we will develop a vaccine that can provide broad protection specifically for poultry using a consensus sequence of H5 AIV isolates. Through participating in this research project, students will gain better understanding of virology, molecular biology, vaccinology and immunology. Students can apply learned knowledge to develop vaccines against any veterinary pathogens.
Project Title: A time space investigation for better understanding of the epidemiology of Ornithobacterium rhinotracheale in commercial turkeys in Iowa

Principal Investigator: Mohamed El-Gazzar

Co-Investigators: Amro Hashish, Yuko Sato
Veterinary Diagnostic and Production Animal Medicine

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

Ornithobacterium rhinotracheale (ORT) is a gram negative bacteria implicated in respiratory diseases in poultry. First identification of ORT was in 1993 from multiple cases of poultry flocks suffering from respiratory disease. Since then, ORT has constantly been listed as one of the top diseases affecting the turkey industry. Antimicrobials, vaccines and other control measures are often used to reduce losses caused by this pathogen making ORT one of the costliest diseases facing the turkey industry. However, there is no sufficient evidence to confirm ORT is a primary respiratory pathogen. 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 clinical signs. Disease was reproduced in poultry only when ORT was combined with other respiratory pathogens or other stress factors. Additionally, ORT can be isolated from up to 100% of apparently healthy poultry flocks. Moreover, our understanding of the epidemiology of the infection, transmission dynamics and source of infections of ORT is rudimentary. We hypothesize that ORT is not a primary respiratory pathogen, but rather an opportunistic pathogen, and present and exist as part of the normal flora of poultry respiratory system without producing disease. In order to test our hypothesis, we plan to conduct surveillance to estimate prevalence of ORT in turkey flocks with and without respiratory disease. This will allow us to study the prevalence of ORT in flocks showing respiratory signs vs flocks with no respiratory signs (apparently healthy). Additionally, we will genetically investigate multiple isolates from the two groups to determine the relatedness between them. This project aims to improve our understanding of ORT as a respiratory pathogen, source of the infection, transmission and eventually help in formulating more effective prevention and control strategies.
Project Title: Development of Core Genome Multilocus Sequence Typing (cgMLST) Scheme for genotyping of avian isolates of *Pasteurella multocida*

Principal Investigator: Mohamed El-Gazzar

Co-Investigators: Amro Hashish, Yuko Sato
Veterinary Diagnostic and Production Animal Medicine

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

Fowl Cholera (FC) caused by *Pasteurella multocida* (PM) is of major economic significance as one of the most important respiratory bacterial diseases. Among poultry, turkeys are the most susceptible to the disease. FC has been ranked within the top ten in a list of the most threatening health issues for the American turkey industry in 2018. Additionally, FC has a very long history in USA and has been described in Iowa State as early as 1867 where losses had occurred in turkeys and other poultry species.

Development of a core genome MLST scheme would allow the classification of avian *P. multocida* into epidemiologically reliant types and would represent a sensitive (i.e. able to separate unrelated strains,) specific (i.e. able to link related strains,) and stable typing method. Furthermore, it would correlate with serotyping classification but could provide deeper understanding of *P. multocida* transmission dynamics and global epidemiology.

We plan to collect diverse reference strains and samples of *P. multocida* in order to develop a cgMLST scheme as a standardized portable sequence typing assay. This cgMLST would allow better understanding of the epidemiology of FC including the source of infection, transmission, route of infection, and the pathogenesis of the disease. This new assay would replace the serotyping or current genotyping assays with a less expensive and less time consuming assay, yet provide more relevant and more detailed information about FC outbreaks in the poultry industry.
Title: Suitability of an Air-liquid interface porcine respiratory epithelial cell cultures to investigate the early immune responses in Mycoplasma hyopneumoniae infection in pigs.

Principal investigator: Luis G. Gimenez-Lirola, Associated professor VDL-VDPAM

Mycoplasma hyopneumoniae (MHP) is a swine respiratory pathogen that causes enzootic pneumonia. MHP establishes infection in the mucosal epithelia of the lower respiratory tract of pigs. MHP attaches to the ciliated respiratory epithelium, causing clumping and loss of cilia leading to epithelial cell death and altering the function of the mucociliary apparatus. Overall, the destruction of the mucociliary apparatus and the ‘ability’ of MHP of modulating the immune response, enhances the susceptibility of infected pigs to secondary pathogens. However, the precise mechanisms of how MHP avoids the host immune response and establishes a persistent infection are still unclear. Therefore, more research toward a better understanding of the mechanisms of innate and mucosal immune interactions between MHP and respiratory tract of pigs are needed. In recent years, stem cell research has made significant progress towards establishing ex vivo tissue models that mimic biologically and physiologically to the original tissue/organ of the animal. This group have already established an air-liquid interface porcine respiratory epithelial cell cultures (ALI-PRECs) that resembles the pseudostratified epithelial lining of the tracheobronchial region of the swine respiratory tract. The air-liquid interface grown porcine respiratory epithelial cells were able to secrete mucins and expressed active ciliary movement. This proposal aims to use ALI-PRECs model as in vitro infection model to characterize MHP infection, its interaction with the respiratory epithelia, and to expand our knowledge on the early innate immune response to the infection. We aim to complete the remaining cellular characterization (immunocytochemistry) of this model and further evaluate specific molecular markers (e.g., mucins, glycan’s, MHCs, TLRs) in ALI-PRECs in comparison to tracheal sections obtained from the experimental pigs. To achieve this, we will perform a transcriptional analysis of various genes in ALI-PRECs and compare their expression in tracheal tissue sections.
Project Title: Virulence assessment of six genomic islands in Streptococcus equi subspecies zooepidemicus associated with high swine mortality

Principal Investigator: Ganwu Li

Co-PI: Eric Burrough, Orhan Sahin, Nubia Macedo, Panchan Sitthicharoenchai

High mortality events due to Streptococcus equi subspecies zooepidemicus (S. zooepidemicus) in swine have not previously been reported in the United States. In late September and early October 2019, escalating swine mortality ranging from 10-50% was reported from a buying station in Ohio and from an abattoir in Tennessee. To determine the genetic relatedness, 24 S. zooepidemicus isolates including isolates from swine and other animal species were sequenced using the Illumina MiSeq and Nanopore sequencing platforms. MLST and phylogenetic analysis, based on core single-nucleotide polymorphisms, revealed clustering of the isolates from Ohio and Tennessee with the Chinese strain (ATCC 36246) associated with outbreaks of high mortality in pigs in 1975. In contrast, these were highly different from another swine isolate, which was not associated with high mortality, and from most isolates from other animal species. Comparative genomic analysis identified several genomic islands that are present in strains from cases of high mortality, but absent from those strains that did not cause high mortality. We hypothesized that these genomic islands contribute to the virulence of S. zooepidemicus and enable them to cause high mortality. Here, we propose to study the genetic basis of bacteremia induced by S. zooepidemicus by pursuing the following two specific aims: 1) To develop a mouse model to study bacteremia properties of S. zooepidemicus, and then use this model 2) to determine the roles of identified genomic islands in S. zooepidemicus in producing bacteremia using gene-specific mutagenesis. We anticipate the results will advance the understanding of pathogenesis of bacteremia due to S. zooepidemicus and will aid in the development of means to control this emerging disease in pigs.
Project Title: Effect of pooling of family oral fluids (FOF) on the probability of detection of PRRSV RNA by PCR

Principal Investigator: Daniel C L Linhares

Co-PI: Marcelo Nunes de Almeida, Giovani Trevisan, Cesar A. A. Moura, Edison Magalhaes, Will A. Lopez-Lopez

Porcine reproductive and respiratory syndrome virus (PRRSV) has the ability to sustain infection in the swine breeding herd (and suckling pig population) at very low prevalence (<5%). Therefore, monitoring protocols require a large number of pigs to be sampled to provide a great probability of virus detection. For example, to detect PRRSV at 3% or 2% there is the need to sample 90 or 149 pigs respectively to provide a 95% probability of detection of at least one positive sample when testing by RT-PCR. Alternatively, there has been a recent development with the use of family oral fluids (FOF)-based sampling. FOF consist of a liquid sample obtained from a cotton rope exposed to the sow and respective piglets at the farrowing room, after allowing them to chew on it for about 30 minutes. FOF are collected around weaning age (21 days of age). Results from our recent work have demonstrated that collecting 20 FOF (i.e. sampling 20 farrowing crates with sows and respective litters) provide an equivalent probability of detecting PRRSV than sampling 150 pigs (i.e. detecting virus infection at ~ 2% prevalence). This has significantly improved monitoring & surveillance protocols, but it is still cost-prohibitive for most producers, as a typical herd weans 2+ farrowing rooms per week. In other words, submitting 20 samples per room for 2 rooms per week for PCR testing ($25/sample) would cost $1,000/week for PRRSV monitoring. Therefore, this study will investigate the effect of pooling of family oral fluid samples on the probability of detection and potential cost reduction for monitoring programs.