

C1 – Derscheid

Project Title: Development of live/dead qPCR assay for *Mycoplasma hyopneumoniae* to improve gilt acclimation and elimination programs

Principal Investigator(s): Rachel Derscheid

Collaborating Investigator(s): Pablo Pineyro, Calvin Ko

Would the proposed project be in-person or virtual? In person

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

Mycoplasma hyopneumoniae (MHP) is the cause of enzootic pneumonia in swine and a contributor to Porcine Respiratory Disease Complex. It continues to be an economic burden as well as a diagnostic challenge, due to its inherent growth properties and pathogenesis within individual pigs as well as within swine herds. Although culture using specialized mycoplasma media is considered the gold standard, Mhp is notoriously fastidious to grow in vitro. Therefore, real-time polymerase chain reaction (qPCR) is the most widely used method for routine Mhp detection because of its high accuracy, fast turnaround, high throughput, and utility for use with tissue samples collected postmortem or clinical samples collected from live pigs. The biggest disadvantage for qPCR is that live and dead cells will be equally detected (Pieters and Maes, 2019), which poses a problem in evaluation of disease status and controlling enzootic pneumonia from swine herds. Viability qPCR (v-qPCR) detection system is a qPCR assay coupled with propidium monoazide (PMA). They are positively charged molecules thus they are excluded by intact, negatively charged, bacterial cell walls/membranes but can enter bacteria with damaged cell-wall/membranes. In these cells with damaged membranes, PMA intercalates DNA every 4–5 nucleotides. Therefore, only the DNA from bacteria with compromised cell walls/membranes is bound by PMA and is thus not amplified by qPCR. We hypothesize that the v-qPCR method can be adapted and optimized in Mhp to enhance the quality analysis of lung tissue homogenates and thus improve acclimatization protocols, leading to better control and elimination of enzootic pneumonia in the swine industry. Results could potentially be presented at the annual meeting of the American Association of Veterinary Laboratory Diagnosticians and/or the American College of Veterinary Pathologists. The impact of the project would be immediate, with the laboratory using the developed assay as standard operating procedure.

C2 – Almeida

Project Title: E. coli genotyping using SmartChip Real-time PCR System- an affordable alternative to gel-based PCR

Principal Investigator(s): Marcelo Almeida

Collaborating Investigator(s): Rahul Nelli and Pablo Pineyro

Would the proposed project be in-person or virtual? In person

Veterinary Scholar Focused Abstract: (300 words or less): Post-weaning colibacillosis remains one of the major enteric diseases challenging swine production in the US, causing mortality and poor growth performance, leading to increased production costs. In a recent case series investigation, E. coli was detected in high-impact cases (90-100% and 15-20% mortality). Whole-genome sequencing (WGS) identified an adhesin gene (tia) not previously reported in association with E. coli strains in cases of post-weaning diarrhea (PWD); however, associated with cases of human diarrhea. The clinical significance of the tia gene in association with ETEC and PWD remains unknown. However, changes in the genetic profile of E.coli in the US swine population leading to unexpected high virulence need to be investigated. To this end, diagnostic laboratories must be adequately prepared to detect any changes in genotype and incorporate newly identified genes in high throughput direct diagnostic assays. To accomplish this high throughput testing needs, we propose designing, developing, and validating a real-time PCR/quantitative PCR (qPCR) for E. coli genotyping to distinguish even the isolates with the tia gene. To perform E. coli genotyping, the ISU-VDL currently uses a well-established gel-based PCR assay. Although gel-based genotyping is widely accepted, it has limitations, such as labor-intensive, low-throughput, and low-resolution primer amplification of target genes. Meanwhile, qPCR is a probe-based high-resolution amplification and quantitation assay with high-throughput capabilities. This study will use SmartChip Real-Time PCR System for genotyping E.coli. To this end, we have already designed primers and probes for E.coli toxin, pilus, and adhesin genes, including a recently detected tia adhesin gene. Using this newly developed qPCR assay, we will analyze 200 known positive samples for hemolytic E. coli isolated from cases of post-weaning colibacillosis. In addition, WGS will be performed in 40 E. coli isolates containing the tia and 20 without tia to confirm the specificity of the newly developed qPCR assay.

C3 – Pineyro

Project Title: Development of a Senecavirus A mRNA vaccine prove of concept for the development of FMD vaccine

Principal Investigator(s): Pablo Pineyro

Collaborating Investigator(s):

Would the proposed project be in-person or virtual? In person

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

Project Summary Senecavirus A (SVA) causes sporadic outbreaks of vesicular disease (VD) and epidemic transient neonatal losses (ETNL) in a large percentage of sows and grower-finisher pigs. These vesicular lesions are indistinguishable from other foreign vesicular diseases with large economic implications including foot and mouth disease (FMD), vesicular exanthema of swine (VES), swine vesicular disease (SVD), and vesicular stomatitis. Following SVA infection, a robust neutralizing antibody and T cell response correlates to short term viremia and the resolution of clinical disease. Currently, there are no licensed SVA vaccines available to producers. Meanwhile, epidemiological data supports that SVA outbreaks occur, resulting in a multifactorial impact to the swine industry. Impact of SVA and importance of the problem: • SVA persistent infection in sow and grower-finisher farms are prompt to VD outbreaks • The majority of foreign animal disease (FAD) investigations are due to swine VD outbreaks • Direct and indirect costs are associated with VD outbreaks at various levels including: o Federal resources- FAD investigation and laboratory costs o Animal care- increase number of days to slaughter, increased feed conversion or reduced feed intake, treatment costs o Reallocation of farm resources, labor costs o Packing plants- closures and delays in harvest schedule o Cost and risk associated with live pig exports Since an mRNA vaccine platform provides the ability to deliver specific SVA viral protein (VP) mRNAs, we hypothesize that translated viral proteins will elicit a strong neutralizing antibody and T cell response resulting in safe and effective option to control and prevent SVA infection. Also, the mRNA platform provides additional flexibility to create an mRNA vaccine for FMD. To test our hypothesis, we will clone the SVA VP sequences into a plasmid and utilize in vitro transcription to generate mRNA. These transcripts will be encapsulated into a lipid nanoparticle delivery system, where protein expression will be evaluated in multiple cells lines (IFA and western blot). Next, pigs will receive two immunization two weeks apart to assess neutralizing antibody response (FFN) and T cell response (recall stimulation). Lastly, the two most promising vaccine candidates will be selected for an animal challenge study. Pigs will receive two immunizations two weeks apart and will be challenged two weeks after the second vaccine dose. Samples will be selected periodically to assess viral shedding (RT-qPCR) on oral fluids and feces, antibodies neutralizing activity (FFN) and T cell response (recall stimulation) on serum. The information gained in this project will help in identifying a potential vaccine for the control of SVA outbreaks in addition to an mRNA vaccine platform that can be applied to FMD vaccine design.

C4 – Almeida/Linhares

Project Title: Pathologic and clinical effects of an F18 enterotoxigenic *Escherichia coli* containing a *tia* adhesin gene

Principal Investigator(s): Marcelo Almeida, Daniel Linhares

Collaborating Investigator(s): Pablo Pineyro, Nubia Macedo, Ganwu Li

Would the proposed project be in-person or virtual? In person

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

Post-weaning colibacillosis (PWC) remains one of the main enteric challenges in U.S swine production, causing mortality, poor performance, and increasing treatment costs. In a recent case-series investigation, F18+ *E. coli* was isolated from cases of high economic impact where an F18-specific autogenous vaccine that historically prevented clinical PWC failed to protect the pigs. This might suggest that the F18+ *E. coli* strains have acquired a new pathogenic trait. The whole-genome sequencing of these isolates identified the adhesin gene *tia* not previously reported associated with enterotoxigenic *E. coli* (ETEC) strains in pigs. This study aims to investigate 1) if the *tia* gene plays a role in the adherence and pathogenicity of an ETEC-F18+/*tia*+ strain and 2) if a commercial competitive product confers protection against this ETEC-F18+/*tia*+ isolate. A wild-type ETEC F18+/*tia*+ isolated from a PWC outbreak with high mortality (20%) will be used to generate three mutants with specific deletions (F18+/*tia*-, F18-/*tia*+, and F18-/*tia*-) by Lambda Red recombination system. A challenge study will be conducted in 3-week-old pigs (n = 72), which will be randomly allocated into 9 groups (negative control, vaccinated and non-vaccinated and challenged with F18+/*tia*+, vaccinated and non-vaccinated and challenged with F18+/*tia*-, vaccinated and non-vaccinated and challenged with F18-/*tia*+, vaccinated and challenged and challenged with F18-/*tia*). Mortality, weight gain, fecal score, *E. coli* fecal shedding, rectal temperature, and coccobacilli attachment to the intestinal mucosa will be compared amongst different treatments. This study will reveal whether ETEC strains containing the *tia* gene combined with F18 are more pathogenic than strains lacking the *tia* gene. Additionally, this study will assess the efficacy of the current commercial competitive exclusion products against the new ETEC F18+/*tia*+ strains. Direct and readily applicable information will be generated to update current therapeutic methods or develop new methods against this new ETEC strain.

C5 – Linhares

Project Title: Expanding and validating the PRRSV surveillance “toolbox” for weaning age pigs

Principal Investigator(s): Daniel Linhares

Collaborating Investigator(s): Gustavo Silva, Giovani Trevisan, Onyekachukwu Osemeke

Would the proposed project be in-person or virtual? In person

Veterinary Scholar Focused Abstract: (300 words or less): Ascertaining the Porcine reproductive and respiratory syndrome virus (PRRSV) status of pigs around the time of weaning (18 to 21 days of age) is crucial as these pigs, in most cases, leave the breeding herds to other sites; a false negative test has grave consequences especially in the wake of recent outbreaks with reemerging PRRSV strains. Serum sampling is the most reliable sampling approach for PRRSV investigation, but when prevalence is low ($\leq 3\%$) it requires a large number of samples to detect the virus ($n \geq 100$). In comparison to other (alternative) sampling options, serum sampling requires skill, more manpower, is less animal welfare friendly, and is often impractical for frequent PRRSV surveillance in large herds. For these reasons, since 2018 alternative samples are the most frequently submitted samples for PRRSV surveillance in the US, according to the swine disease reporting system (SDRS) database. Processing fluids, an alternative sample type, are obtained in the first week of life of the piglets and do not represent the status of the pigs at the point of weaning. Family oral fluids are obtained around weaning and require that pigs interact with sampling ropes.

Other alternative samples such as nasal swabs, buccal swabs, and blood swabs are also used for PRRSV surveillance; but have no guidelines for an appropriate sample size. The focus of this study is to test the hypothesis that; there is no statistical difference in the PRRSV detection rates between each of the listed alternative sample types compared to serum. The listed alternative samples and serum will be matched for about 330 pigs and diagnostic performances evaluated. Results will be used to build a sampling guideline.