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Anaplasmosis in Illinois: A Survey of Economic Impact and Prevalence

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Anaplasmosis, caused by the rickettsial hemoparasite *Anaplasma spp*. (*A. marginale* in the U.S.), is the most prevalent tickborne disease of cattle worldwide. Clinical case cost of anaplasmosis in the U.S. is conservatively estimated at over $400 per animal with a total estimated cost to the beef industry greater than $300 million per year. The most recent anaplasmosis serological survey of Illinois (published in 1997) revealed a statewide prevalence between 7.1 and 10.7%. Anecdotal information from Illinois cattlemen and veterinarians suggests the incidence of anaplasmosis is far greater today. In the absence of effective vaccines, control of anaplasmosis in the U.S. is predicated on implementing biosecurity practices and administering low doses of tetracycline during months with the highest infection risk. Successful implementation of these control strategies requires knowledge of the regional anaplasmosis prevalence. In 2017, we initiated a pilot survey of Southern Illinois cattle from 27 herds in 14 counties, funded by the Illinois Beef Association. Based on limited sampling, the prevalence in the counties surveyed has increased from 19.6% in 1997 to 60% in 2017. The goal of this proposal is to accurately determine the current statewide level of *Anaplasma marginale* infection and, from Illinois beef cattlemen survey and performance data, the current control method(s) and economic impact of bovine anaplasmosis. Based on our preliminary data, we hypothesize that the prevalence is markedly increasing within Illinois and is having a measurable economic impact on Illinois producers. A stratified random sampling procedure will be used to collect representative samples from beef herds. The sample will be stratified on location (county) and herd size. Results from this project will be disseminated to cattle producers, veterinarians, and other industry stakeholders throughout Illinois. This information will provide Illinois beef industry stakeholders with the information to conduct herd specific cost-benefit analysis of prevention programs and determine the impact of the disease on animal health and welfare. Results from this study will be used as the foundation for future proposals aimed at mitigating the impact of *A. marginale*.

Pharmacokinetics and Pharmacodynamics of Oral and Intravenous Apixaban in Horses

Ryan Fries

Coagulation disorders are common in equine medicine and can become an important complication in hospitalized horses. Activation of a hypercoagulable state in horses is associated with ischemic and inflammatory disorders, infectious agents, and iatrogenic causes. Coagulopathies in these patients can contribute to prolonged hospitalization times and increased mortality. Prophylactic administration of antithrombotic drugs has been recommended in order to reduce complications of thrombosis and improve outcomes. The most effective drugs used for these conditions in equine species are aspirin and heparins. These drugs are known to have side effects and clinically efficacy is not well established. Given the potential for severe complications associated with vessel thrombosis and a general lack of effective therapy, an alternative to aspirin and heparin is needed. Apixaban is a novel, commercially available, orally administered factor Xa inhibitor. Apixaban treatment in humans can be safely used by means of standard administration with no need for continuous monitoring of coagulation. Therefore, apixaban holds great potential for a variety of thrombotic conditions. On the basis of existing data from human studies showing excellent efficacy of apixaban in patients with thromboembolism and deep vein thrombosis, apixaban would appear to be a strong candidate to fulfill this need in horses at risk for thrombosis. The objective of this study is to determine the

pharmacokinetic and pharmacodynamic properties of apixaban after oral and intravenous administration in healthy adult horses so that future studies may evaluate the clinical efficacy of apixaban at reducing thrombotic complications in horses.

Effect of Nerve Growth Factor-β in Pregnancy Outcomes in Dairy Cows
Fabio Lima

Nerve Growth Factor-β (NGF) is a seminal plasma protein that improves corpus luteum (CL) development and progesterone production when administered systemically to heifers. Recent studies in our laboratory revealed that Angus cows treated with purified bull NGF not only had increased progesterone production, but also had enhanced early embryonic development, as determined by an upregulation of interferon-stimulated genes (ISG) and increased pregnancy-specific protein B (PSPB) concentrations. In camelids, the luteotrophic effect of NGF is attributed to increased production and an extended duration of pre-ovulatory luteinizing hormone (LH) secretion from the anterior pituitary. The expression of the NGF receptor (TrkA) is upregulated in response to LH treatment in bovine theca cells, suggesting a potential positive feedback mechanism within the NGF signaling pathway. This same study also demonstrated that NGF directly stimulates the production of androgens, progesterone, and prostaglandin E2 (PGE) and proliferation of thecal cells in bovine ovarian tissues. After ovulation, theca cells differentiate into small luteal cells that, in response to LH binding, produce an early rise in progesterone that is essential for supporting initial embryonic growth. Increased PGE production in the pre-ovulatory follicle (POF) also provides another possible rationale for improved steroidogenesis within the CL. This pro-angiogenic molecule increases vascularity and improves the ability for signaling molecules (such as LH) to reach ovarian tissues. Indeed, enhanced PGE production may explain why llamas treated with NGF experienced increased CL vascularity and progesterone concentrations when compared to gonadotropin releasing hormone (GnRH)-treated controls. While this particular mechanism has yet to be studied in cattle, it is known that CL blood flow is significantly correlated with progesterone production in the early luteal phase9. The current literature, supported by our ongoing studies, suggest that NGF may be used to alter ovarian function and improve cattle fertility. There is, therefore, a critical need to assess if NGF can be used to improve pregnancy outcomes in dairy cows. Additionally, validating a recombinant protein will provide a more economically feasible and consistent way to expand NGF use in the cattle industry. Our long-term goal is to establish a role for NGF as a tool for improving pregnancy rates in cattle artificial insemination (AI) programs. The overall objective of this proposal is to determine how administration of NGF affects CL development and subsequent pregnancy per AI for the first service and pregnancy loss. Our central hypothesis is that treatment with both recombinant bull NGF (R-NGF) and purified bull NGF (P-NGF) will improve CL function, maintenance of pregnancy, and overall pregnancy outcomes. The rationale for the proposed research is that the results will provide us with evidence if seminal plasma-derived NGF benefits of luteal function can be translated into higher fertility. We foresee that after completing the proposed studies, NGF will become a useful tool to decrease early embryonic losses due to luteal insufficiency in dairy cattle.

Prioritization of Candidate Risk Genes for Equine Osteochondrosis
Annette McCoy

Osteochondrosis (OC) is a widely recognized form of developmental orthopedic disease that affects up to 40-60% of young foals across breeds. There is a clear genetic component for OC risk, with heritability estimates of 15-52% depending on disease definition (Philipsson et al, 1993; van Grevenhof et al, 2009). Reduction of incidence, early intervention, and preventative management require early identification of horses with genetic risk; however, the specific

genetic risk alleles underlying OC are unknown. Our global hypothesis is that OC is caused by novel genetic variants affecting pathways related to normal skeletogenesis. Our preliminary work identified several genomic regions of interest (ROIs) for OC, each containing many potential candidate genes. However, once genomic loci for disease association have been identified by GWAS, prioritization of candidate genes located within ROIs is hampered due to an incomplete understanding of normal developmental/regulatory pathways and a lack of knowledge regarding tissue-specific and condition-specific gene expression for key tissues. In this proposal, we address this by generating and utilizing transcriptomics data and network analysis to functionally prioritize candidate risk genes, and identify novel putative risk variants, within each ROI. Future studies will focus on validation of these putative risk variants in an independently sampled population. Our integrated GWAS/transcriptomics approach will directly benefit Standardbred horses and become a resource for future OC research, while also significantly contributing to our understanding of equine musculoskeletal biology.

Osteogenic Shift of Synoviocytes in Equine Osteoarthritis
Matthew Stewart

Osteoarthritis is the major cause of wastage and lost performance in equine athletes and also negatively impacts many people and pets. Although articular cartilage degeneration is the pathological hallmark of osteoarthritis, the disease involves pathological changes in several intra- and peri-articular tissues, including the synovial membrane that lines the inner surface of joint cavities. Synovium generates a plasma ultra-filtrate that nourishes articular chondrocytes and minimizes intra-articular friction during joint motion. Under normal conditions, the cells within the synovial membrane, synoviocytes, have a very limited capacity to undergo osteogenic differentiation and produce bone. However, excessive intra- and peri-articular bone formation is a major feature of osteoarthritic joints and our preliminary data support the idea that the environment within osteoarthritic joints shifts resident synoviocytes to a ‘bone cell’-like phenotype. The proposed experiments will address this idea by analyzing synoviocyte characteristics from normal and matched arthritic joints, by determining whether the inflammatory cytokines that drive osteoarthritis also induce this phenotypic transition in synoviocytes, and by investigating a potential bone morphogenetic protein-mediated mechanism for this phenotypic shift. These experiments will provide insights into the mechanisms by which joint pathology progresses in osteoarthritis and may identify target pathways for new therapies to mitigate osteophyte/enthesophyte development.

Genetic Dissection of Rhoptry Neck Proteins in *Cryptosporidium parvum*
Sumiti Vinayak

Cryptosporidium parvum is a protozoan parasite that causes diarrheal disease (Cryptosporidiosis) in a wide range of agricultural and farm animals. It primarily infects calves, lambs, goat kids, and causes huge economic losses to the agricultural industry due to mortality, stunted growth of animals, increased labor costs and veterinary assistance. The *C. parvum* infectious stage (the spore-like oocysts) are highly resistant to environmental stress and standard disinfection procedures, thus making them difficult to eliminate from animal housing facilities. There are no effective drugs or vaccines to treat or prevent the disease. Therefore, there is an urgent need to devise new treatment and prevention strategies to control *C. parvum* in agricultural animals. To develop novel therapeutics, it is critical to understand the biology of the parasite. However, the field is lagging behind due to absence of a continuous culture system to grow *C. parvum*, poor animal models, and lack of genetic tools. To overcome these hurdles, we have recently established a powerful technology to genetically manipulate this agriculturally important pathogen and a robust animal model system to propagate the parasites. There is a

huge gap in our understanding of the machinery used by *C. parvum* to invade the host cell. In closely related parasites such as *Plasmodium falciparum* and *Toxoplasma gondii*, proteins released from specialized rhoptry organelles located at its anterior end are known to play a key role in invasion and host-pathogen interactions. We mined the *C. parvum* genome and identified two genes that encode for rhoptry neck proteins (RONs) that are orthologs of RON6 and RON9 found in *T. gondii* and *P. falciparum*. Integrating our expertise in functional genetics and cell biology, we will uncover the function of these novel rhoptry neck proteins (named *Cp*RON6 and *Cp*RON9) in *C. parvum* invasion, host-parasite interactions and disease pathogenesis. The results obtained from this project will provide novel insights into the parasite biology that can be exploited for the development of novel drugs and vaccines for disease intervention in agricultural animals.

Deciphering Innate Protective Mechanisms against *Toxoplasma gondii* in a Pig-Derived Macrophage Cell Line
William Witola
Toxoplasma gondii is a parasite that causes economic losses in livestock through abortions world-wide, including in the USA, but for which no effective treatment, nor vaccine exist. Development of control strategies for *T. gondii* will require identification and validation of host molecules that confer resistance to *T. gondii* infection. In our preliminary studies, we compared a *T. gondii*-susceptible Brown Norway rat versus a *T. gondii*-resistant Lewis rat and found that, in response to *T. gondii* infection, the Lewis rat upregulates genes for GTPase Immunity Associated Proteins (GIMAPs), namely, GIMAP4, 5, and 6, that engage early innate mechanisms to kill *T. gondii* in host cells. GIMAPs are recently reported small GTPases that have conserved orthologs in livestock, but their functions in host defense against intracellular pathogens are completely unknown. Thus, our focus in this proposal is to test the functionality of GIMAP4, 5 and 6 in mediating resistance to *T. gondii* infection in a pig-derived macrophage cell line, and decipher their molecular networks and modes of action. These studies will provide important new knowledge in that we will for the first time characterize and validate mechanisms involving a novel class of immunity-associated GTPases and their partners in mediating resistance to *T. gondii* in an agricultural animal cell line. Our studies will potentially unveil strategies for designing interventions against *T. gondii* infection in livestock.

A Promising Intranasal Inactivated PRRS Whole Virus Vaccine
Federico Zuckermann
Since the early 1990s, when porcine reproductive and respiratory syndrome (PRRS) virus was first identified as a major pathogen of swine, researchers have been attempting to develop effective vaccines against this troublesome virus. Although PRRS modified live virus vaccines are somewhat effective, their demonstrated ability to revert to virulence is a major detriment to their use for effective control of this pathogen. The existence of an efficacious inactivated vaccine against PRRS virus would be a powerful tool to empower efforts to control and eventually eradicate this virus. We have preliminary data indicating that the intranasal (IN) immunization of swine with a vaccine consisting of inactivated PRRS whole virus is able to stimulate virus-specific IgA in nasal secretions in PRRS virus-naïve animals, as well as measurable levels protective immunity against a homologous virus challenge. As comparison, we will also measure the same parameters with the same dose of the immunogen but formulated properly for intramuscular administration. Here we are advancing the hypothesis that a properly formulated PRRS virus vaccine, consisting of inactivated whole virions, in combination with a suitable adjuvant, delivered intranasally constitutes a safe an PRRS virus vaccine capable of eliciting acceptable levels of protective immunity. Objective: This project

aims to ascertain the immunogenicity and efficacy of a properly formulated inactivated PRRS whole virus vaccine administered intranasally. We fully expect that the mucosal PRRS vaccines being examined will result in a vaccine capable of reducing the economic losses due to PRRS. An effective inactivated vaccine against PRRS virus is likely to have a major impact on the control of this virus and have a major economic benefit to the pork industry.