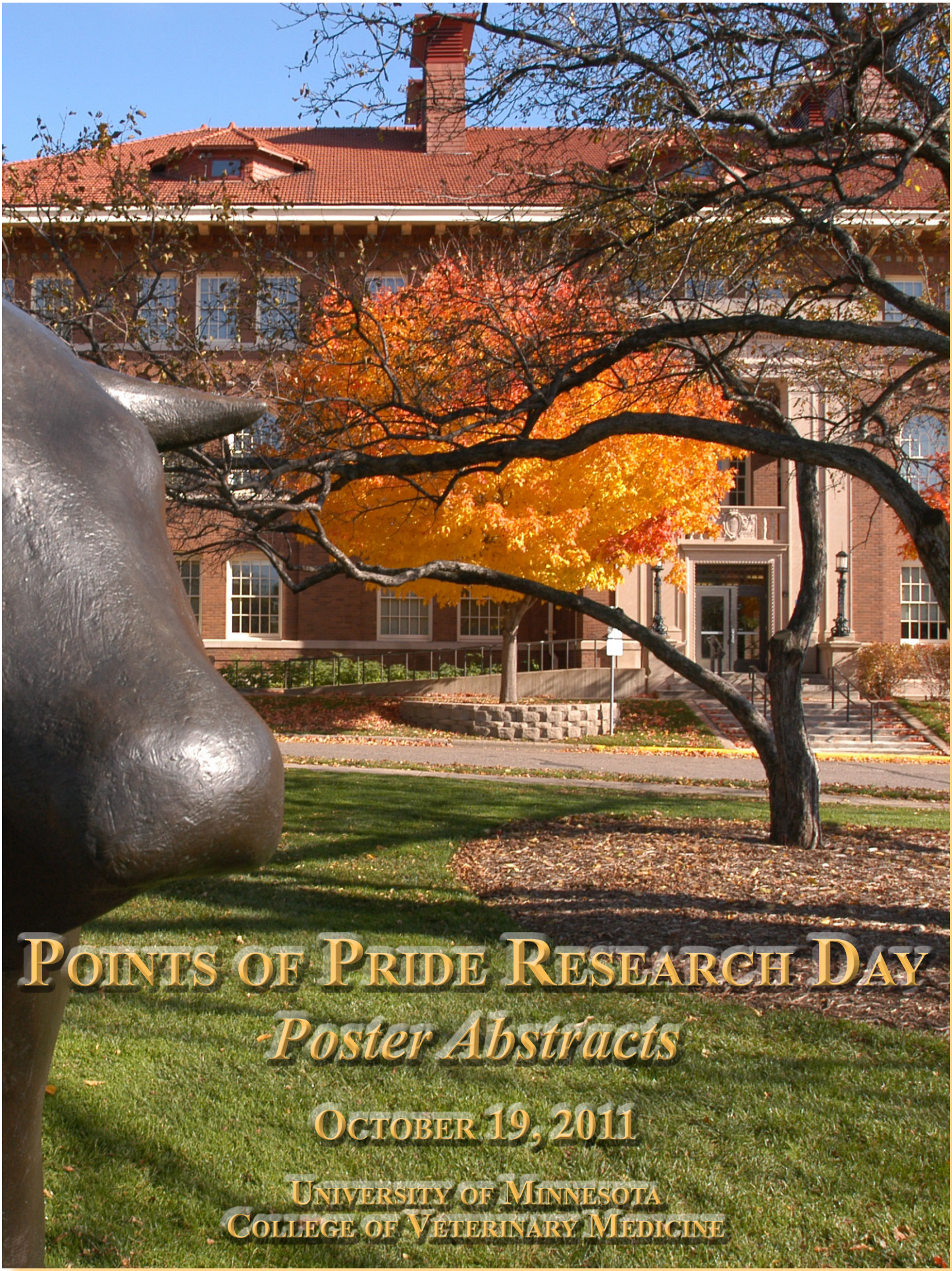

Points of Pride Research Day

Poster Abstracts

October 19, 2011

University of Minnesota
College of Veterinary Medicine

SS-1

Candidate Genes Underlying Locomotion in the Thoroughbred and Standardbred

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Members of the equine species are unique, in that artificial selection pressures have resulted in breeds with different gait phenotypes. Our recent work has demonstrated that gaited horses from multiple breeds show evidence of selection in a single region of the genome, despite differences in each breed's gait phenotype. We hypothesize that this shared region across breeds may be permissive for gait, and further that this locus works with other gene(s) specific to each breed that modify the gait, resulting in specific gait phenotypes. Using a population differentiation statistic (di), regions under putative differential selection in Standardbreds exhibiting two different gait phenotypes were identified. Genes in these regions were chosen as candidates based upon conservation of haplotypes in the region as well as our hypothesis that locomotion is influenced by brain development and neural pathways.

Two genes on chromosome 15 were investigated further. EMX1 is thought to play a role in the development of the corpus callosum. LRRTM1/CNNA2 is associated with handedness in humans. Primers specific for these genes were designed and DNA from Standardbred trotters, Standardbred pacers and Thoroughbreds was amplified using PCR and then sequenced.

EMX1 contains two exons that are annotated in the horse. Approximately 700 base pairs were sequenced, covering most of the gene. In Exon 2 a SNP was found where trotters were heterozygotes. All annotated exonic regions were successfully sequenced in LRRTM1/CNNA2. In Exon 2, two SNPS were found in Standardbred pacers. Neither resulted in an amino acid change.

Future research should focus on the identification of the brain and neural mechanisms involved in locomotion and in turn the genes controlling those pathways.

SS-2

Keratinocyte-microbial interactions in an *in vitro* canine skin model.

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Atopic dermatitis is a chronic inflammatory condition that affects dogs and humans at an estimated frequency of 10-20% of the general population (Marsella & Girolomoni, 2009). Patients with atopic dermatitis are very prone to develop bacterial skin infections. Knowing how the epidermal cells respond to bacterial exposure will offer more options to better manage these recurrent infections that significantly aggravate the primary disease. The goal of this study is to establish canine skin equivalents for analysis of bacteria and keratinocyte interactions. These cultured keratinocytes will be exposed to the commensal skin bacterium *Staphylococcus epidermidis* and the pathogenic *Staphylococcus pseudintermedius*, each separately and then combined, to determine the innate immune response mounted by the cells by measuring the levels of a panel of cytokines. My hypothesis is that co-inoculation of the two bacteria will result in attenuation of the pro-inflammatory effects of the pathogen.

To date, I have conducted several experiments inoculating rat skin equivalents with the aforementioned bacterial species, and have been collecting and culturing skin samples from dogs undergoing elective ovariohysterectomies. This study is the first collaborative effort focused on epidermal cells. It is important to provide valuable insight into the pro- and anti-inflammatory effects of common bacteria colonizing the epidermis and form a baseline for future studies unlocking the pathophysiology of atopic dermatitis.

SS-3

The Role of Alpha-Dystrobrevin in Cardiac Muscle Costameres

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Duchenne muscular dystrophy (DMD) is a uniformly fatal degenerative muscle disease resulting from mutations in the cytoskeletal protein dystrophin. Dystrophin connects cytoskeletal components and the membrane, through interactions with a complex of proteins and glycoproteins. This interaction is thought to provide mechanical stability to the membrane during muscle contraction.

One of the proteins in this complex is dystrobrevin. Dystrobrevin binds to dystrophin's C-terminal and to the sarcoglycans, a group of glycoproteins also associated with dystrophin. Our preliminary data indicated that loss of dystrobrevin led to weakened interaction between dystrophin and the membrane.

In spite of this weakened interaction, no significant difference in resting hemodynamic function was observed between dystrobrevin knockout (DBko) mice and wild type mice. We hypothesized that the low level of interaction observed in DBko mice might be sufficient to maintain hemodynamic function at rest, but insufficient under stress. To examine this, we applied pharmacologic stress using isoproterenol to DBko, mdx (dystrophin deficient) and wildtype mice over four days and performed immunohistochemistry to assess the degree of cardiac muscle injury. We also performed a treadmill study in which DBko, mdx and wildtype mice were run downhill on a treadmill and their hearts, tibialis anterior, gastrocnemius, triceps and diaphragm were assessed using IHC for damage.

We found increased cardiac muscle damage in DBko and mdx mice after low dose isoproterenol exposure compared to wildtype. Furthermore, high dose isoproterenol resulted in significant mortality in DBko mice, while wildtype mice survived to the end of the protocol. We also observed decreased treadmill running performance in DBko mice relative to that of mdx and wildtype mice. These findings suggest that the weakening of the dystrophin-membrane interaction with loss of dystrobrevin produces a subclinical phenotype at rest, but prone to muscle damage under stress.

SS-4

Defining Normal Acylcarnitine Profiles in Horses

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Lipid storage myopathies (LSM), due to defects in lipid metabolism, are characterized by an abnormal accumulation of fat within muscle fibers leading to muscle degeneration. Screening for LSM in humans usually involves analysis of concentrations of specific chain lengths of acylcarnitine in serum or blood collected on dried blood spot (DBS) cards. Specific defects in lipid metabolism produce characteristic elevated levels of certain chain lengths of the mitochondrial free fatty acid transporter acylcarnitine within the blood. LSM have recently been identified in horses with severe rhabdomyolysis based on excessive lipid accumulation in muscle fibers. Conventional diagnostic methods rely on muscle biopsy samples but an easier approach would involve analysis of whole blood or serum samples.

The objective of this project was to establish normal values for blood acylcarnitines in horses stored as either serum or on DBS cards. The effect of breed and collection method on acylcarnitine profiles in 11 Quarter Horses, 10 Thoroughbreds and eight warmbloods was evaluated in blood samples collected as either serum or whole blood spotted on DBS. Free carnitine as well as C2 up to C18 acylcarnitines were analyzed by GC mass spectrometry. A two way ANOVA was used to compared the effect of breed and collection method. The results of this study identified normal ranges for free carnitine ,C2 through C18:2 acylcarnitine concentrations in serum and DBS cards for each breed. Concentrations across breeds showed small statistically significant differences in certain acylcarnitines, however, the differences were small enough that one set of normal ranges can be used when assessing potential LSM cases. DBS cards resulted in significantly lower free carnitine and short-chain acylcarnitines and higher long-chain acylcarnitine concentrations.

In conclusion, either DBS or serum can be utilized for the analysis of acylcarnitines in horses, however, the corresponding normal ranges determined in this project for each collection method should be utilized when evaluating horses for defects in lipid metabolism.

SS-5

A Pilot Study to Evaluate the PRRSV Carrier Status of Vasectomized Boars in Herds Attempting PRRSV Elimination

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Porcine reproductive and respiratory syndrome (PRRS) continues to be the most economically important disease affecting the swine industry. It has become standard practice for infected sow farms to attempt virus elimination using a "load, close, and homogenize" protocol. Recently, certain herds have failed to eliminate resident virus. Due to the propensity for viral persistence in lymphoid tissue and the reproductive tract, a proposed reservoir has been vasectomized boars (V-boars). Therefore, the following objectives were proposed: 1) Determine whether V-boars can harbor PRRSV. 2) Determine the site of viral persistence 3) Determine if carrier boars can be detected using ante mortem sampling techniques.

Samples were collected from V-boars across 8 sow farms averaging 3000 sows in size with 5 to 15 weeks remaining in their elimination protocols. Serum and oropharyngeal samples were collected. When possible, boars were euthanized and tonsil, lymph nodes, penis, testis, epididymis, ductus deferens, vesicular gland, prostate, and bulbourethral gland were collected. Samples were tested for PRRSV RNA by PCR and the ORF 5 region of positive samples was nucleic acid sequenced. A total of 174 V-boars were tested. All sera were negative while 13% (23/174) of oropharyngeal scrapings were PCR positive. Select boars were necropsied and 100% (8/8) were tonsil positive and 63% (5/8) were lymph node positive; however, no virus was detected in the reproductive tract. Regarding ante-mortem samples, oropharyngeal scraping samples from 3/8 boars were PCR positive. All sequences indicated the presence of resident PRRSV (>99% homologous to previous isolates).

Results indicate that V-boars can harbor resident PRRSV, that the tonsil is the predominate site of persistence and that carriers can be detected using ante-mortem diagnostic methods.

SS-6

Putative DNA-binding Genes and their effects on the Persistence and Dissemination of *bla*$_{\text{CMY-2}}$ positive IncA/C plasmids

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Multidrug resistance (MDR) is a condition enabling a microorganism to resist multiple antimicrobial agents. Multidrug resistant bacteria are emergent in many ecological niches, particularly among enteric bacteria of production animals. The ability to rapidly change a bacterium to a MDR phenotype is most often conferred via the acquisition of conjugative plasmids. The most concerning plasmids in this regard are those that have broad host range and an innate ability to acquire MDR-encoding elements. The IncA/C plasmids are an example of such a plasmid type, and they have recently emerged worldwide among *E. coli* and *Salmonell*a spp. in production animals (4). This plasmid group was previously identified in a wide variety of *Proteobacteria* in the environment, but their recent introduction into production animal microbial populations poses a great threat to animal and human health.

Recent genome sequencing efforts have revealed that these plasmids contain a highly conserved core backbone, with three primary accessory regions that contain genes implicated in a host bacterium's MDR phenotype. While the genetic structure of the IncA/C plasmid group is well defined, little is known about the basic biology of these plasmids. Allelic exchange was used to generate three gene knockouts including a putative Hu-IHF gene, a putative plasmid stability regulator gene, and an H-NS-like gene.

The objective of this study was to characterize how these plasmid encoded genes affected the conjugation efficiency and fitness cost of the plasmid under laboratory conditions. It was found that Hu-IHF deletion shows the least conjugations across the *Salmonella* serovars (Dublin, Kentucky, and Newport). It was found in all salmonella strains, that H-NS-like gene deletions had a decrease in fitness. In the environmental strains (*Vibrio* and *Paracoccus*) the plasmids conferred a fitness increase. Deletion of the HNS-like gene resulted in the greatest cost among all the bacterial strains. The results from this study give us a better understanding of the biology of the Inc A/C plasmid and this gives us insight into how to possibly mitigate the persistence of this plasmid.

SS-7

Determining the Role of Cytochrome P450 Family 1, Subfamily A, Polypeptide 2 (CYP1A2) in Achilles Tendinopathies

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The objective of this study was to investigate a potential relationship between cytochrome P450 mutations and fluoroquinolones in the pathophysiology of spontaneous Achilles tendon rupture in the dog. Specifically, the gene cytochrome P450 family 1, subfamily A, polypeptide 2 (CYP1A2) was investigated, since it is the gene responsible for metabolizing fluoroquinolones. Exonic sequences within the CYP1A2 gene from 13 dogs diagnosed with spontaneous Achilles tendon rupture were compared to their specific sex, reproduction status, age, and breed matched controls. Six single nucleotide polymorphisms (SNPs) were identified within the sequences. Results indicated that these single nucleotide polymorphisms are not statistically significant when analyzed both by allele and by genotype frequencies.

Our final conclusion is that these six single nucleotide polymorphisms found within the CYP1A2 gene are most likely not associated with Achilles tendon rupture and concurrent use of fluoroquinolones or other potentially related types of medication.

SS-8

Characterization of canine ADAM17 and its role in inflammation

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A Disintegrin and A Metalloproteinase 17 (ADAM17) is a membrane-associated surface protein widely expressed in leukocytes, and is responsible for processing, cleaving, and activating other cell surface proteins, such as Tumor Necrosis Factor-α (TNF- α) , L-selectin adhesion molecule, et al. It does this by cleaving these membrane proteins, regulating their function through a process known as ectodomain shedding, leading to a multitude of effects, such as modulating the activity of cytokines, cytokine receptors, and adhesion molecules which are involved in leukocyte recruitment, activation, migration, and apoptosis signaling. The proteolytic actions of ADAM17 plays important roles in numerous bodily functions, including acting as a TNF- α-converting enzyme (TACE) to generate mature TNF- α, a cytokine important in many inflammatory disease states. To date, there have only been studies looking at ADAM17 in the human and the mouse, which are not necessarily the best models for studying the role of ADAM17 in cases of trauma, and trauma-induced inflammation, or sepsis.

In this study, we do a novel characterization of ADAM17 in the canine through the use of Flow Cytometry and Western Immunoblotting. We also had the opportunity to compare the expression of ADAM17, L-selectin, and Mac-1 in normal, healthy dogs against dogs coming into the ER for trauma/infectious/septic reasons. Our results demonstrated that canine ADAM17 is very similar to ADAM17 in humans and mice, both in structure and activity, making it a suitable model for study in the progression of certain inflammatory and disease states. These results will allow future studies to be done on canine models that can represent real cases of trauma/inflammation/sepsis, since human studies can't be done, and studies with mice may not be accurate since the trauma/inflammation/sepsis must be induced. Future studies of canine ADAM17 will hopefully lead to a better understanding of the variety of ADAM17 functions, which will lead to useful therapies targeting ADAM17 in such disease states, for humans and animals alike.

SS-9

Inhibition of receptor tyrosine kinases in canine hemangiosarcoma

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Canine hemangiosarcoma (HSA) is a highly malignant neoplasia of blood vessel forming cells in dogs. Consisting of up to 7% of all canine cancers, current therapies do not substantially extend survival times beyond diagnosis. Poor treatment responses indicate that we may not be targeting effectively the cells responsible for tumor survival. We hypothesized that targeting the cancer stem cell (CSC) population with small molecule tyrosine kinase inhibitors (TKIs) combined with current treatment protocols may effectively eliminate the CSC population and lead to improved outcomes. We have established a culture method that supports the formation of tumor cell spheres from canine HSA and enriches for CSCs. Sphere cells showed increased chemoresistance, exclusion of vital dyes indicating increased ABC drug transporter activity, and higher invasive capacity. Sphere cells also showed increased expression of CD117 (c-Kit) and CD115 (CSF-1R), two receptor tyrosine kinases (RTKs) expressed by lineage progenitor cells and more highly expressed by sphere cells than their monolayer counterparts. We examined the effect of the TKIs, nilotinib (CD117) and cFMS inhibitor IV (CD115), on inhibition of both HSA monolayer and sphere cells. Nilotinib inhibited growth of monolayer cells and decreased the concentration of paclitaxel needed to effectively kill tumor cells in culture. Nilotinib also inhibited the expression of two ABC transporters, ABCB1 and ABCG2, commonly involved in drug resistance. Cell proliferation studies performed on sphere cells using nilotinib showed little or no decrease in cell viability. Short-term viability assays using cFMS inhibitor IV showed no effect on either monolayer or sphere cells indicating that long-term assays may be needed. Our results suggest that nilotinib may be an effective treatment for HSA either alone or in combination with chemotherapy agents. Ongoing work will examine the effect of other TKIs on cell viability and ABC transporter expression in HSA CSCs.

SS-10

Reduction of Osteoarthritis Severity in MIF Knock Out Mice

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Osteoarthritis (OA) is the most common form of arthritis affecting upwards of 27 million Americans; however, there are no treatments currently available that reduce the severity of the disease. Degradation of articular cartilage matrix, a key feature of OA, is through the action of matrix metalloproteinases (MMP). It has been demonstrated that macrophage migration inhibitory factor (MIF) is produced by chondrocytes and can up-regulate synovial cell production of MMPs. Because MIF-knock out (KO) mice live longer than control mice housed under similar conditions, we hypothesized that these animals also may have a reduced severity of OA. A histological grading scheme was used to evaluate lesions of osteoarthritis in the stifle joints of one-year-old MIF-KO mice from a stock with equal contributions from C57BL/6J and 129/SvJ strains (n=13) and age-matched wild type (n=13) mice to determine if the MIF KO mice had a reduced prevalence and severity of osteoarthritis. Mid-coronal histological sections of one stifle joint/mouse were stained with hematoxylin & eosin and safranin O. Evaluations were confined to the medial tibial plateau and meniscus and included measurements of thickness and area of articular cartilage, calcified cartilage, and subchondral bone; the number of chondrocytes and area occupied by necrotic chondrocytes in articular cartilage; and area of meniscus. In addition, subjective scores were assigned for articular cartilage structure (extent of fibrillation and clefting) and extent of loss of safranin O staining. Findings indicate that the MIF-KO mice have fewer and less severe lesions of osteoarthritis compared with the controls, thus suggesting that the absence of macrophage migration inhibitory factor in these mice may reduce the development of osteoarthritis.

SS-11

An Evaluation of Stalosan® F Powder for Deactivation of PRRSv

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Many hog operations utilize liquid disinfectant boot baths as a part of biosecurity protocols aimed at minimizing the spread of PRRSv. The objective of this study was to test Stalosan® F, a powder disinfectant, as an alternative to a liquid disinfectant boot bath, for the deactivation of PRRSv under various temperatures and in the presence or absence of fecal matter. Clean, dry rubber boots (n=24) were randomly assigned to one of two treatment groups (12/trt) which consisted of boot baths with either Stalosan® F power or Synergize liquid disinfectant. Ingelvac® PRRS MLV vaccine was applied (2 mL of a 1:100 dilution of the re-constituted vaccine) with a syringe to the bottom of each boot. The inoculated boots were consecutively placed in the boot bath per treatment group and boots were sampled and tested by PCR 1,3, and 5 min post-disinfection, at both 8°F and 85°F, and both in the presence and absence of fecal matter. Neither of the products tested were 100% effective at deactivating PRRSv. Stalosan F was significantly more effective at deactivating PRRSv than Synergize with the presence of fecal matter at both temperatures tested ($p < .0001$), while Synergize was more effective without fecal matter present. Overall, Stalosan F produced significantly less PRRSv positive samples than Synergize in the conditions tested and would be a beneficial alternative to current liquid boot baths.

SS-12

Analysis of PRRSv outbreaks using mapping of farms and risk-based surveillance scoring

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Currently, there are approximately 20 PRRSv regional control projects throughout the United States collecting farm information to promote a collaborative effort towards limiting the transmission of this virus. Challenges to these regional projects include consent of participation, collection of adequate information, and communicating presence of outbreaks in a non-threatening atmosphere. To address these obstacles, I have developed a testing program based on estimated risk of each site. To establish a standardized method of site testing priority, this scoring system incorporates inventory, PRRS status, testing dates, farm location, sources and destinations of pigs. This scoring system can be used to optimize allocation of testing funds to higher-scoring areas and sites. Geostatistical interpolation of site data allows participants in regional control projects to share progress while maintaining confidentiality. This risk-based surveillance testing system and mapping require more use and in-field validation to be evaluated for risk estimation.

GS-1

A Transposon-Based Genetic Screen in Mice to Identify Drivers of Histiocytic Sarcomagenesis

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Histiocytic Sarcomas (HS) are rare neoplasms derived from hematopoietic or mesenchymal stem cells known to have a poor prognosis. Primary lesions of HS appear in the spleen, lymph nodes, lung, bone marrow, skin and subcutaneous tissue. The genetic etiology of HS is largely unknown and HS is difficult to manage clinically. We have developed a forward genetic screening method in mice that can identify mutations capable of causing cancer initiation and progression. The forward genetic screens use the *Sleeping Beauty* (SB) DNA transposon as a random somatic mutagen, capable of both activating proto-oncogenes and inactivating tumor suppressor genes. The system relies on the use of a Cre/Loxp-regulated SB transposase transgene and mutagenic transposon vector transgenes that are mobilized by the transposase. Using Cre recombinase under the control of a tissue specific promoter allows for selective, tissue specific mutagenesis in particular cells of interest. Tumors generated in these mice are analyzed for recurrent transposon insertion sites (called common insertion sites or CIS) using ligation-mediated PCR in combination with high-throughput sequencing. CIS have been found to harbor known and novel cancer genes. Thus, this approach provides us with candidate cancer driver genes. As in human cancer, our results often reveal a small number of frequently mutated genes that cooperate with a greater number of rare mutant genes that drive formation of tumors. We hypothesized that SB-mediated transposition of an oncogenic transposon in myeloid lineage cells in mice could cause mutations in oncogenes and tumor suppressor genes leading to the initiation and progression of HS. We generated and aged 190 experimental and control transgenic mice. The triple transgenic, experimental mice express SB transposase in myeloid lineage cells containing an oncogenic transposon by virtue of a Cre recombinase transgene under the control of the *Lysozyme M* promoter. The triple transgenic mice became moribund, much faster than control double transgenics, beginning around one year of age. Necropsies were preformed on all moribund animals and animals that reach 18 months of age. Tumors were collected and analyzed by HE staining. Initial pathology suggests that 30% of the experimental animals developed HS. We are currently performing immunohistochemistry to confirm the tumor type. Tumors will be further analyzed for transposon insertion sites to identify candidate cancer genes by mapping transposon insertions to the mouse genome from many independently generated tumors. We expect that these experiments should identify candidate cancer genes that will help greatly our understanding of the etiology of histiocytic sarcomas and lead to targeted therapies.

GS-2

Non-terminal animal model of acute joint injury causes early osteoarthritis

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There is a well-recognized need for a translational model of acute joint injury and early osteoarthritis (OA). The purpose of this study was to develop a non-terminal equine model that demonstrated clinical and morphological evidence of the onset and progression of OA. We hypothesized that creation of an osteochondral (OC) injury in the metacarpophalangeal (MCP) joint of the horse would result in clinical, radiographic, arthroscopic, and histologic changes characteristic of early OA.

Twenty-two clinically and radiographically normal age- and sex-matched Quarter Horses were randomly divided into 1 of 2 groups: (1) horses (n=11) that had an OC fragment created arthroscopically on the proximal dorsomedial aspect of the first phalanx in one MCP joint and a sham operation in the contralateral joint at week 0; and (2) unoperated exercise control horses (n=11). At week 16, OC fragments were arthroscopically removed. Every 2 weeks throughout the study, force plate analysis, joint range of motion, and effusion scores were recorded on all horses and joint fluid, serum and urine were collected for future biomarker analyses. A repeated measure ANOVA with Tukey's for multiple comparison was used for analysis of clinical data. Week 0 and 16 radiographs and arthroscopic videos were blinded and graded for OA changes. Synovial membrane biopsies obtained from all OC injured joints at weeks 0 and 16 and from sham joints at weeks 0 (n=5) and 16 (n=11) were blinded and graded histologically, as were articular cartilage samples obtained from the third metacarpal bone at week 16 in OC injured (n=11) and sham (n=6) joints. All scoring systems except that used for cartilage were analyzed using a Kruskal-Wallis test with a Dunn's multiple comparison test; a Mann Whitney t test was used for cartilage. $P<0.05$ was considered significant. All procedures were approved by institutional animal care and use committees.

Osteochondral fragments were successfully created and multiple clinical and morphologic changes consistent with early OA were demonstrated. OC injured horses exhibited a decrease in forelimb symmetry ($P<0.001$) at week 2 on the force plate, indicating pain. Subjective joint effusion scores increased in OC injured limbs compared to baseline throughout the entire study period ($P<0.001$). Radiographic scores showed subtle but significant change in the OC injured limbs. Arthroscopically, synovial membrane from OC injured joints exhibited mild changes (hyperemia, villus thickening, and proliferation). However, cartilage damage within the joint was more pronounced. Histologically, fibrillation/fissuring, chondrocyte cell death and proliferation (repair) were significantly greater in OC injured cartilage compared to sham cartilage, with the majority of changes restricted to the superficial zone. A non-inflammatory fibrotic reaction was seen in OC injured synovium.

Creation of an OC fragment in the equine MCP joint results in acute traumatic joint injury that has mild but consistent clinical and morphological features of early OA. This non-terminal injury model will be useful for defining biomarker changes of early OA and for monitoring response to therapy of this disease.

GS-3

Fecal Bacterial Diversity of the Wild Mantled Howling Monkey (*Alouatta palliata*)

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Mantled howling monkeys (*Alouatta Palliata*) are New World Monkeys living in the Neotropics whose diet is primarily composed of foliage. In howlers, the primary sites of microbial fermentation are in the cecum and colon where a diverse group of microbes with cellulose-digesting abilities dwell. This digestive specialization is known as hindgut fermentation.

Commensal microbial communities play a key role in animal and human health, and the characterization of these populations is essential to understand divergent adaptations in closely related species. The emerging field of metagenomics combined with high throughput sequencing allows direct, unbiased interrogation of microbial populations, thus enabling the investigation of unique dietary differences in human and non-human primate species that may reveal the role of microbial communities in primate speciation. Here we present the first study of the structure of the intestinal bacterial community of the mantled howling monkey, using high-throughput sequencing and metagenomic analysis.

Our study showed that the fecal microbiome of the mantled howler was dominated by phyla Firmicutes (59.01%) with Bacteroidetes (13.81%), Unclassified (17.49%) and TM7 (3.61%) in lower concentrations. Compared to other non-human primate species examined in previous studies, the phylum TM7 comprised a much higher percentage of the fecal microbiome in mantled howling monkeys. This factor makes mantled howlers unique among other nonhuman primates previously studied in regards to their gut microbial community structure.

GS-4

MicroRNA Regulation of CD38 Expression in Human Airway Smooth Muscle (HASM) Cells

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Background: CD38 is a trans-membrane protein with multiple enzymatic functions. Cyclic ADP-ribose, the product of ADP-ribosyl cyclase activity of CD38, mobilizes intracellular Ca2+ and contributes to myocyte contractility. *In-vivo* studies in mouse models of asthma showed that CD38 null mice develop attenuated airway hyperresponsiveness (AHR). Inflammatory cytokine TNF-α induces CD38 expression in HASM cells through both transcriptional and post-transcriptional mechanisms. The 3' UTR region of CD38 mRNA carries targets for several microRNAs including miR-140-3p. MiR140-3p expression was down regulated in HASM cells exposed to TNF-α. **Hypothesis:** We hypothesized that miR-140-3p regulates TNF-α-induced CD38 expression in HASM cells by interacting with 3'UTR of CD38 mRNA. **Methods:** HASM cells were transiently transfected with miR-140-3p mimic oligonucleotides and CD38 mRNA expression was determined. HEK 293 cells or NIH3T3 cells were co-transfected with luciferase-CD38-3'UTR reporter plasmid and control oligonucleotide or miR-140-3p mimic oligonucleotide and luciferase activity was determined. The luciferase reporter assays were also performed using a mutant luciferase-CD38-3'UTR construct, which has the miR-140-3p target mutated. **Results:** MiR-140-3p mimic inhibited TNF-α-induced CD38 mRNA expression in HASM cells. MiR-140-3p mimic transfected HEK-293 or NIH-3T3 cells showed marginally reduced luciferase activity compared to the control oligo-transfected cells. In cells co-transfected with miR-140-3p target-mutated reporter construct, luciferase activity remained unaltered by miR-140-3p mimics. **Conclusion:** In HASM cells, miR140-3p regulates TNF-α-induced CD38 expression at least in part through binding to 3'UTR of CD38 mRNA. This novel regulatory mechanism involved in CD38 expression presents potential therapeutic targets to down regulate CD38 and thus attenuate AHR in airway disorders such as asthma.

GS-5

Adeno-associated virus mediated gene transfer to sensory neurons innervating the murine colon

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The colon is innervated by extrinsic primary afferent fibers whose cell bodies reside in thoraco-lumbar and lumbo-sacral dorsal root ganglia (DRG). These fibers transmit sensory inputs from the colon to the spinal cord under normal conditions and under conditions of visceral hypersensitivity. In histological preparations, the extrinsic afferent fibers cannot be distinguished from intrinsic fibers of the enteric nervous system because all known neurochemical markers of DRG neurons are also present in enteric neurons. In the present study, the gene for green fluorescent protein (GFP) was delivered to mouse DRG neurons using adeno-associated virus serotype 8 (AAV8)-mediated gene transfer via direct lumbar puncture. Histological analysis of colon from AAV8-GFP treated mice demonstrated GFP expression within colonic nerve fibers. To determine the relative contribution of thoraco-lumbar and lumbo-sacral dorsal root ganglia to GFP-positive nerve fibers in colon, we evaluated the expression of GFP in L6, L5, L1, and T13 DRG of rAAV-GFP treated mice. The number of GFP-positive neurons increased rostrally in lumbar DRG, peaking in L1 DRG. Remarkably, the level of transduction dropped dramatically in the adjacent T13 DRG. The transduction gradient varied considerably in lumbar DRG among AAV8-GFP treated mice. Substantial variability was also seen in the density of colon GFP-ir fibers. Interestingly, higher density of labeled fibers correlated with higher number of GFP-positive neurons in L6 DRG. Retrograde labeling with Cholera Toxin B (CTB) was used to confirm GFP expression in sensory neurons innervating colon. These results demonstrate the utility of intrathecally delivered AAV8 vectors for gene transfer to sensory neurons innervating the colon.

GS-6

Ontogenetically distinct cancers of the dog form tumor spheres in a shared culture condition

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Cancer stem cells (CSC), have been demonstrated in a variety of cancers; however, shared properties have not been reported as a recurrent characteristic of CSC from different tumors. We hypothesized that CSC from three ontogenetically distinct cancers share molecularly defined properties. To test the hypothesis, we enriched CSC by formation of multicellular three-dimensional tumor spheroids in serum-free culture conditions. First, we established an invariant culture method that supported formation of tumor spheroids from canine hemangiosarcoma, osteosarcoma, and glioblastoma cells. The cells from these tumor spheroids showed increased chemoresistance, excluded vital dyes (an indicator of elevated ABC drug efflux transporter activity), and differentially expressed a complement of genes that included drug efflux transporters. Cells from the tumor spheroids also show increased capacity to engraft and survive as in vivo xenografts under conditions of limiting dilution. Genome-wide gene expression profiling is complete for seven samples from two tumor types. We observed uniform changes in the molecular profile of cells from the tumor spheroids along one component when data were analyzed using principle component analysis. This is one of several parameters that define the molecular separation between cells derived from tumor spheroids and cells cultured under conventional monolayer conditions. Taken together, our results suggest that there are shared molecular properties among our CSC from ontogenetically homologous and ontogenetically distinct tumors. However, unique drivers of gene expression associated with CSC from each cancer also are apparent. Ongoing work will use additional bioinformatic algorithms to define both shared and unique molecular properties of CSC from these different cancer types.

GS-7

Development of a Novel Antiviral Therapy for Feline Leukemia Virus Infection

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Retroviruses are RNA viruses that replicate through a DNA intermediate and are associated with many important diseases. Human immunodeficiency virus (HIV) causes AIDS and human T-cell leukemia virus (HTLV) causes an adult T-cell leukemia/lymphoma. Avian and mammalian retroviruses have been used as models for the study of retroviral replication and disease pathogenesis. These model systems remain important for better understanding retrovirus pathology and drug therapy. The development and use of antiretroviral drugs for the treatment of HIV infection has saved millions of lives. The rapid emergence of drug resistance and off-target effects provide a continual need for identifying novel drug targets and developing new antiretroviral drugs. Recent studies have indicated that decitabine and gemcitabine, two anti-cancer drugs, have anti-HIV activities and can inhibit HIV infectivity by elevating the viral mutation rate by a process called lethal mutagenesis. In this study we investigated the breadth of efficacy and antiretroviral mechanism of action for these drugs with feline leukemia virus (FeLV), a model gammaretrovirus, in order to identify new antiretroviral drugs that can be used for the treatment of FeLV in companion felines. Current treatment options for FeLV infection include the antiretroviral drug AZT and immunomodulators, but limitations such as drug toxicity and variable efficacy creates an opportunity for the identification of additional drugs to treat viral infection. We demonstrate a dose-dependent inhibition for FeLV infectivity with decitabine and gemcitabine, as well as 2 anti-HIV drugs - tenofovir and raltegravir – in the absence of cellular toxicity. Such studies may lead to the development of these drugs for use in the treatment of FeLV infection.

GS-8

Validation of a Radioimmunossay for the Measurement of Porcine C-Peptide Concentration in Non-Human Primate Serum.

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The xenotransplantation of porcine pancreatic islets is a promising approach to reversing type 1 diabetes and is under investigation in several animal models. Non-human primate (NHP) models are of interest because of the genetic and anatomical similarity between NHPs and humans. Porcine islets transplanted into NHPs continue to produce porcine proinsulin (and thus porcine insulin and c-peptide). The objective of this work was to determine if an assay produced for the quantification of porcine c-peptide (PCP) concentration in porcine serum could reliably be used to measure the concentration of PCP in NHP serum. A commercially available PCP kit (Millipore, Billerica, MA, cat no. PCP-22K) was investigated. The assay characteristics of sensitivity, precision, linearity and recovery were tested using NHP serum collected after porcine islet transplantation to determine if pre-determined assay validation criteria would be met using this method. Calculated assay sensitivity matched the manufacturer reported sensitivity of 0.01 ng/mL. Intra-assay precision, using repeated measurement (n=5) of PCP concentration in NHP serum containing 2.20 ng/ml PCP, was 2.6%. Inter-assay precision, using repeated measurement (n=3) of PCP concentration in the same serum sample was 5.9%. Linearity was demonstrated in NHP serum containing PCP at dilution factors of 1.0, 1.33, 2.0 and 4.0; mean percent-of-expected concentration was 100%, 99%, 99% and 102% respectively. Recovery was demonstrated by adding varying concentrations (0, 0.5, 1.0 and 2.5 ng/ml) of PCP standard to NHP serum in duplicate, four times in one assay. Mean percent recovery (observed concentration/expected concentration) was calculated as 100%, 101%, 101% and 103% respectively. These data show that PCP concentration can be reliably (precise, sensitive, linear, accurate) measured in NHP serum and that the test method meets established validation acceptance criteria providing a useful tool for xenotransplantation models using pigs and NHPs.

GS-9

Dissociation of Porcine Reproductive and Respiratory Syndrome Virus Neutralization from Antibody Production to Major Envelope Protein

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Porcine reproductive and respiratory syndrome (PRRS) is the most severe infectious disease facing the swine industry worldwide. The etiologic agent is PRRSV, an enveloped arterivirus. GP5 and M, the major envelope proteins, form disulfide-bonded heterodimeric complexes that are involved in PRRSV entry into porcine alveolar macrophages (PAM). Previous studies identified several neutralization epitopes in the GP5 polypeptide, containing amino acid residues (AA) 29-52, and potential unknown neutralization epitopes in M protein. To determine whether PRRSV-neutralizing antibodies (NA) are directed to GP5 and M ectodomain polypeptides, we constructed three recombinant single-chain proteins: the two predicted ectodomains of GP5 (GP5-5'), the two ectodomains of M (M-5'), and all four ectodomains (GP5-M). In particular, GP5-5' contains the reported neutralization epitopes. We found that the convalescent pig sera after PRRSV infection contained various levels of antibodies (Abs) to those proteins, but the Ab titers did not correlate with the NA response. Immunization of pigs with these recombinant proteins did not induce a detectable NA response. Finally, protein-specific Abs that were purified from PRRSV-neutralizing serum by affinity chromatography showed no neutralizing activity, whereas the protein-specific Ab-depleted sera retained similar neutralizing activity as the original sera. The results, therefore, do not support that PRRSV-NA are directed to the ectodomain polypeptides of GP5/M.

GS-10

Role of leukocyte ADAM17 in regulating inflammation during Sepsis

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Inflammation is the body's initial response to infection, which can be harmful when excessive, as exemplified in sepsis inflammatory syndromes. Ectodomain shedding is a proteolytic process that directs both instantaneous and prolonged alterations in the activity of various cytokines, cytokine receptors, and adhesion molecules, and ADAM17 is a key membrane metalloprotease involved in this process. At this time, very little is currently known about the *in vivo* function of ADAM17 in regulating inflammation during infection. In this study, we generated Adam17 gene targeted mice (ADAM17-null mice) in which only the leukocytes lacked functional ADAM17, and then examined its role in the inflammatory and host responses during peritoneal sepsis. ADAM17-null mice showed significantly increased survival and bacterial clearance during sepsis, which was associated with a reduction in systemic proinflammatory cytokine levels and bacterial burden. An underlying mechanism accounting for the enhanced host response in ADAM17-null mice is a very rapid yet transitory infiltration of neutrophils into the peritoneal cavity of ADAM17-null mice when compared with control mice. We are currently examining the molecular processes that underpin the accelerated recruitment of neutrophils in ADAM17 null mice. Overall, our study provides the first direct evidence of the instrumental *in vivo* role of leukocyte ADAM17 in modulating inflammation and host resistance during sepsis.

GS-11

Effects of surgery and post-operative intra-articular corticosteroids on synovial fluid collagen biomarkers in an equine model of osteoarthritis

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PURPOSE: The purpose of this study was to investigate the effects of arthroscopic surgery and post-surgical intra-articular administration of triamcinolone acetonide (TA) on collagen synovial fluid biomarkers using an animal model of osteoarthritis. We hypothesized that collagen synthesis and degradation would increase in synovial fluid after removal of osteochondral fragments, and that there would be greater collagen degradation in the TA treated group than in saline controls.
METHODS: In 7 normal adult Quarter Horses an osteochondral fragment was arthroscopically created on the dorsal medial aspect of the first phalanx in one metacarpophalangeal joint (MCPJ). MCPJ synovial fluid was collected at five time points. After the third time point, horses were divided into 2 treatment groups: horses that received TA and horses that received saline injected into the injured MCPJ. MCPJ synovial fluid CPII, C12C, C2C, and CTXII concentrations were evaluated at all time points using ELISAs and concentrations were compared using unpaired t-tests (significant at $P<0.05$).
RESULTS: Sixteen weeks after creation of an osteochondral fragment, concentrations of CPII, C2C, C12C, and CTX II all significantly increased. Surgery to remove osteochondral fragments did not result in any biomarker changes 1 week after surgery. By two weeks after surgery, there was a significant increase in CTX II in the saline controls. By four weeks post surgery, biomarker concentrations of CPII, C12C and CTX II had decreased to pre-surgical concentrations in the saline controls, although C2C levels were increased. One week after injection with TA, C2C, C12C, and CPII concentrations were all significantly increased compared to saline controls. In contrast, CTX II was significantly lower than saline controls. By 3 weeks after TA injection, C2C concentrations continued to increase.
CONCLUSIONS: Arthroscopic surgery and TA administration had mixed effects on cartilage metabolism.

GS-12

Investigation of Genetic Risk Factors in Tarsal Osteochondrosis of Standardbred Horses

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Osteochondrosis (OC) is a manifestation of developmental orthopedic disease that affects weanling and yearling horses across breeds. OC is known to be a complex disease with many interacting factors influencing risk. Heritability estimates based on pedigree analysis in Standardbreds have been reported as high as 0.52, suggesting that as much as 50% of the risk for developing OC in this breed is due to genetics. We hypothesized that detectable allele frequency differences between Standardbred yearlings with radiographic evidence of OC and those without radiographic evidence of disease would allow for identification of genomic segments harboring alleles associated with OC risk, leading to identification of positional candidate genes for OC.

DNA was extracted from whole blood samples from 94 sire-matched Standardbred yearlings from a single breeding farm and submitted for analysis using the Illumina 54k Equine SNP chip. Subsequent statistical analysis identified potential regions of association on ECA6 and 14. The candidate gene HDAC4 was prioritized for follow-up. Sanger sequencing of 5 individuals has resulted in discovery of 8 novel SNPs to date. Resequencing is hindered by incomplete annotation of the HDAC4 gene structure in the equine reference genome. RNAseq data was used to identify several putative exons as well as 5'UTR and 3'UTR. Sequencing of these regions is ongoing. Variations identified in HDAC4 in this initial analysis will be followed up in the larger study population to confirm segregation with disease status. It is expected that variations in several genes will be identified as contributing to risk.

GS-13

Expression Profiles of MHC B-Locus Genes in the Domestic Turkey

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The major histocompatibility complex (MHC) is a highly polymorphic region of the genome containing genes essential to the immune response. In the turkey (*Meleagris gallopavo*), the MHC is divided into two genetically unlinked regions on microchromosome 18. The "classical" avian MHC or *B*-locus includes approximately 40 genes. Many *B*-locus genes are involved in antigen processing and presentation, encompassing class I and class II loci and accessory genes such as the *TAPs*. Other genes in the avian MHC have non-immune or unknown functions. The compact physical organization of these genes (~200kb), their tight genetic linkage and potential for co-evolution led to the concept of the *B*-locus as a "minimal essential" MHC. Given this close proximity, it is possible that some of the *B*-locus genes are co-regulated. Expression of these genes is likely to vary among different immune or non-immune tissues. However, evidence for expression of the turkey MHC is limited and there are no locus-wide expression studies in any avian species. Steady-state *B*-locus gene expression patterns can provide indications of potential functions, evidence for co-regulation, and background for MHC expression in immunological challenge studies. We investigated a panel of 10 immune and non-immune tissues by RT-PCR to identify basal transcription and qualitatively scored amplicons to characterize the expression profiles of 29 *B*-locus genes. As a whole, the genes of the turkey MHC *B*-locus were broadly expressed across tissues. Expression patterns varied widely, even among gene families such as the *TRIMs*, and expanded the knowledge of expression for predicted genes such as *Hep21* and *BTN2*. Identification of these expression profiles for *B*-locus genes provides insight into the varied functions of the MHC.

GS-14

Differential neural stem cell proliferation during Herpes Simplex encephalitis

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Herpes Simplex virus-1 (HSV-1) is the most common cause of sporadic viral encephalitis. Despite effective treatment with acyclovir, >50% of patients develop neurological deficits subsequent to herpes simplex encephalitis (HSE). The brain responds to inflammatory damage by stimulating neurogenesis, which is mediated by a small population of cells called neural stem cells (NSC). Using a murine model of herpes encephalitis we investigated the response of endogenous NSC to HSV-1 infection. Changes in the NSC population within neurogenic regions of infected brains was measured at 3, 6, 10, 15, and 30 days post infection (d.p.i) by flow cytometric analysis of CD45 and nestin, a myeloid and NSC marker respectively. A significant increase in the nestin(+)CD45(-) population was observed at 6d.p.i relative to control. This increase in the NSC population was transient and decreased significantly at 15 and 30 d.p.i relative to control. The nestin(+) cell population in the brain is intrinsically self-renewing, so proliferation was investigated. Interestingly, proliferation was significantly increased relative to sham inoculated controls at 3 d.p.i. This increase was also transient, with the proliferating cells consistently decreasing through 30 d.p.i. To identify factors responsible for the decrease in proliferation during chronic HSE, gene expression profiles of infected and sham-inoculated brains were analyzed at 15 d.p.i using a neurogenesis PCR array. The majority of genes that were differentially expressed were neurotrophic factors or genes associated with metabolism. In particular, bone morphogenic protein-4 (BMP4) and fibroblast growth factor-2 (FGF2) were substantially (3.35 and 5.83 fold respectively) down regulated at 15 d.p.i. Studies are underway to further characterize the changes observed in proliferative responses due to HSV-1 infection and to investigate the impact of decreased neurogenesis during HSE. The insights generated by these studies may lead to new therapeutic and preventative strategies for combating neurological damage associated with viral encephalitis.

GS-15

Role of incretin biology in equine metabolic syndrome

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Introduction: Equine Metabolic Syndrome (EMS) is a metabolic phenotype characterized by increased adiposity, insulin resistance, hyperinsulinemia, and laminitis. Laminitis is a debilitating and often life-threatening condition. A key feature of EMS is abnormal insulinemic responses to glucose or feeding challenges and the resultant hyperinsulinemia likely contributes to development of laminitis. In mammals, glucose-stimulated insulin secretion is greatly augmented by the action of incretin hormones released from enteroendocrine cells in response to oral glucose. The two main incretins are glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The half-life or incretin hormones are very short due to rapid inactivation by the protease dipeptidyl peptidase IV (DPP-IV).

Hypothesis: We hypothesize DPP-IV activity and GLP-1 responses to oral glucose challenge differ significantly between EMS horses and unaffected animals, and that these differences in incretin responses are associated with SNPs in proglucagon (GCG) which encodes the GLP-1 peptide and DPP4.

Study design: A cohort of ~300 EMS cases and matched controls were analyzed for association of laminitis and increased insulin response to oral glucose with polymorphisms in GCG and DPP4.

Results: Laminitis status is significantly associated with GCG genotype (p value <0.05). Insulin response to oral is significantly higher in heterozygous carriers of the GCG risk allele than non-carriers (p value=0.05). However, the DPP4 polymorphism does not demonstrate a significant association with laminitis status or insulin response to oral glucose.

Conclusion: Genetic variation in the proglucagon gene may contribute to the pathophysiology of EMS through dysregulation of the incretin response.

GS-16

Genetic Determinants of Melanoma Susceptibility in Gray Horses

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Melanomas are tumors of the skin that originate from melanocytes. A correlation between the gray coat color in horses and melanoma development has long been recognized with up to 80% of gray horses older than 15 years developing melanoma. Graying with age and melanoma formation has been linked to a duplication in the Syntaxin 17 gene, with STX17 genotype affecting melanoma grade and severity. The ASIP mutation, responsible for bay/black coat color modifies melanoma risk in gray horses; with increased melanoma severity potentially due to an increase in MC1R pathway signaling. We have identified a population of gray Quarter Horses (QH) that appear to have a *decreased* melanoma risk. Dermal melanoma is very low to non-existent is some pedigrees, suggesting there are other genetic influences in addition to gray coat color that play a role in melanoma development. The reason for the decreased incidence of melanoma in these horses is unclear, however based on the importance of increased MC1R signaling in melanoma susceptibility with the *ASIP* mutation; we believe that the cellular mechanism responsible for decreased melanoma susceptibility may also involve this pathway. We hypothesize that the decreased incidence of melanoma in gray QH may be related to a decreased level of MC1R signaling resulting from the high prevalence of the MC1R chestnut coat color allele in QH. We have phenotyped 265 gray QH for melanoma. Melanoma incidence in this population was 16.07%, compared to incidences of 50% and 31.4% in the Lipizzaner Camargue breeds, respectively. Similarly the melanoma incidence in QHs >15 years old (61%) was lower than the incidence published in Lipizzaner (75%) or Camargue (68%). The mean melanoma grade in our population is 0.35 (scale 0 to 4; 0= no melanomas, 4= ulcerated/metastatic melanomas). We are genotyping all the horses for the MC1R, ASIP and STX7 mutation to analyze the effect of the MC1R mutation in melanoma development and severity. At this time we have genotyped 264 horses for the MC1R mutation (59.7% are homozygous for the mutation and 36.36% are heterozygous for the mutation). These findings confirm a low melanoma incidence and a high prevalence of the MC1R mutation in gray QH.

GS-17

Modulation of 17β-estradiol causes a down regulation of TRPV1 in DRG neurons

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While it has been demonstrated that women experience greater cancer pain and pain severity than men, the potential role of estrogen in regulating nociception particularly as it relates to tumor pain remains unclear. Current data indicate that estrogens act directly on the nervous system to affect the severity of pain, but the mechanisms underlying this effect are unclear. A major nociceptive mechanism associated with the development of cancer pain is activation of the transient receptor potential vanilloid-1 (TRPV1) channel. TRPV1 is a Ca2+-permeable ionotropic receptor activated by multiple sensory stimuli including heat, acid, and protons. Antagonism of the TRPV1 channel attenuates nociception in a mouse bone cancer pain model. Both estrogen (ER) and TRPV1 receptors are known to be expressed on dorsal root ganglion (DRG) neurons, whose axons transmit pain from the periphery to the spinal cord. This raises the question of whether estrogen can alter TRPV1 receptor expression or function particularly in pain states like cancer pain. The present study was designed to evaluate the effects of estrogen on pain sensitivity and TRPV1 expression in the DRG of male and female C3H mice in a mouse model of bone cancer pain. One goal was to determine whether estrogen modulates nociceptive receptors in the DRG in a manner consistent with its effects on pain sensitivity. This study demonstrated that exposure to 17β-estradiol in gonadectomized males (ORCH+E), but the lack of this same hormone in gonadectomized females (OVX), significantly reduced the mRNA levels of TRPV1 in lumbosacral DRG neurons and this correlated with a reduction in tumor-induced nociception. Our results suggest that modulation of 17β-estradiol causes a down regulation in TRPV1 expression in DRG neurons, which may be associated with a reduction in fibrosarcoma-induced mechanical allodynia in both male female mice.

GS-18

Effect of water level on flexion and extension of the distal limb joints of healthy horses walked on an aquatic treadmill

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We hypothesized that walking horses in an aquatic treadmill would effectively increase the range of motion (ROM) of the distal limb joints when water is added, and that water level would influence each joint differently. The objectives of this study were to calculate the maximum flexion, maximum extension, and ROM of the fetlock (fore and hind), carpus, and hock of healthy horses walked in an aquatic treadmill with four different water levels. Nine sound adult horses were included in this study. Zinc oxide was used as skin marker in four different anatomical locations of the left fore and hind limbs (pastern joints, fetlock joints, ulnar carpal bone/talus, and mid-radius/tibia). Data was recorded by one digital camera and analyzed using 2-dimensional motion-analysis software. After 5 sessions of training and acclimatization, horses were recorded on the aquatic treadmill at the walk (0.9 m/s) with 1 cm of water (AT1), water up to the fetlock (AT2), water up to the hock (AT3), and water up to the stifle (AT4). Five complete gait cycles were utilized for analysis. Maximum and minimum joint angles and ROM were calculated for each joint in each stride. Descriptive statistics were performed and data was compared with repeated measures ANOVA using a Tukey's post hoc test. Maximum flexion and ROM were significantly greater for all joints when water was at the level of the fetlock joint (AT2, $P<0.01$) and above (AT3 and AT4, $P<0.001$) compared to 1 cm of water (AT1). Maximum extension was also significantly higher with AT2, AT3 and AT4 ($P<0.001$) in all joints except for the hock when compared to AT1. Carpal ROM was significantly higher with AT3 ($P<0.001$) compared to the other three water levels. Fore and hind fetlocks had the greatest extension with AT2 ($P<0.001$), and the greatest flexion with AT4 ($P<0.001$), although ROM was not statistically different. In conclusion, the aquatic treadmill is an effective rehabilitation modality to improve flexion and extension of the distal limb joints in the horse. Water level should be considered when designing the rehabilitation program of each particular injury.

GS-19

Compensatory effects of induced lameness in single and multiple limbs in horses demonstrated by kinetic changes

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Reasons for performing study: Previous equine lameness research has used subjective analysis with kinematic and sometimes kinetic data, but has only shown some evidence of compensatory mechanisms for single forelimb and hindlimb lamenesses.
Objectives: To describe kinetic methods of compensation horses use during induced lameness in single and multiple paired limbs by altering vertical and longitudinal ground reaction forces in affected and non-affected limbs.
Materials and methods: 8 clinically sound horses had lameness induced, using a hoof clamp, on single or paired limbs, and force plate analysis was performed. Percent change from baseline was determined for all lamenesses and data points were categorized to determine increasing or decreasing trends in all limbs.
Results: Small magnitudes in percent changes from baseline resulted in visible lameness in limbs with hoof clamp(s) tightened with evidence of compensation by non-lame limbs. In general peak vertical force, vertical impulse and breaking impulse decreased in the lame limb, and propulsion impulse increased. This trend was reversed when a bilateral hindlimb lameness; in this instance breaking increased and propulsion decreased. In single limb lameness, the majority of compensation occurred through increased vertical and breaking impulses in the contralateral limb. In paired multiple limb lamenesses, more vertical compensation tended to occur through force increases in the contralateral forelimb than hindlimb. Breaking forces, however, tended to be compensated for by increases in both contralateral forelimb and hindlimb. Less compensation was evident with bilateral forelimb or hindlimb lamenesses.
Conclusions and potential relevance: Definite trends in compensation were seen, and can help equine practitioners understand how horses alter their ground reaction forces in response to single and multiple limb lamenesses. This may be useful to help determine primary versus compensatory change, and to clarify some of the complexities of multiple limb lamenesses.

GS-20

Misfolded Y145Stop catalyzes the conversion of full-length prion protein.

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A point mutation in *Prnp* that converts tyrosine (Y) at position 145 into a stop codon leading to a truncated prion molecule in an inherited transmissible spongiform encephalopathy (TSE), Gertsmann-Straüssler-Schiencker syndrome, suggests that the N-terminus of the molecule (spanning amino acids 23-144) likely plays a critical role in prion misfolding as well as in protein-protein interactions. We hypothesized that Y145Stop molecule represents an unstable part of the prion protein that is prone to spontaneous misfolding. Utilizing protein misfolding cyclic amplification (PMCA) we show that the recombinant polypeptide corresponding to the Y145Stop of sheep and deer PRNP can be in vitro converted in presence or absence of preexisting prions. In contrast, recombinant protein full-length PrPC did not show a propensity for spontaneous conformational conversion to protease resistant isoforms. We found that seeded or spontaneously misfolded Y145Stop molecules can efficiently convert purified mammalian PrPC into protease resistant isoforms. Furthermore, we show that prion seeding activity present in in-vitro converted Y145Stop triggered accumulation of protease-resistant prion protein in a transformed deer cell line (MDB). These results establish that the N-terminus of PrPC molecule corresponding to residues 23-144 plays a role in seeding and misfolding of mammalian prions.

GS-21

Influenza A virus genetic diversity in immune pigs

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Hemagglutin (HA) protein is the main antigenic protein in influenza viruses. Changes in HA protein drive antigenic drift and immune pressure plays a role in part, in driving those changes. In this study we evaluated the genetic changes in HA of two groups of animals with either acquired immunity or passive immunity. The results from these analyses may help elucidate the mechanisms and causes of increased genetic diversity in influenza A viruses in swine and the possible generation of novel influenza A viruses that may cross species barriers. Virus sequences obtained from two different studies were analyzed to study HA changes in immune animals (with acquired immunity or with passive immunity) that had been subsequently infected with a triple reassortant influenza A virus of swine origin. Overall we observed more genetic changes in the sequences from viruses detected in the pigs with passive immunity than in the pigs with acquired immunity. Both non-synonymous and synonymous changes were observed, meaning that there were genetic changes that resulted in antigenic changes in the HA protein and some that did not result in predicted amino acid changes. In addition, changes appeared to happen in a very short time post infection, within a relative small population of animals, and with pigs of different immune status. Changes in the receptor binding site and other antigenic sites in the HA protein have been described before for influenza A H1 viruses. Some of the changes observed in this study corresponded to changes previously described in the literature. We are currently analyzing the substitutions observed in this study in the context to help elucidate the mechanisms and causes of increased genetic diversity in influenza A viruses in swine and the possible generation of novel influenza A viruses that may cross species barriers.

GS-22

Resolution of *M.bovis* phylogeny using genome-wide single nucleotide polymorphisms

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Piecemeal analysis of *Mycobacterium bovis* (MBO) genomes and conventional genotyping methods have not lent to a comprehensive resolution of its genetic diversity to explain the wide range of disease phenotypes caused by this zoonotic pathogen. Conventional genotyping methods target a small hypervariable region on the genome of MBO and provide anonymous biallelic information insufficient to develop MBO phylogeny. Genome-wide single nucleotide polymorphisms (SNPs) studies in *M. tuberculosis* have shown to have sufficient resolution to develop trait-allele interactions. Using the high throughput iPLEXTM Massarray (Sequenom), we interrogated the MBO genome for 350 loci including geneic (n =306) and intergeneic (n =44) regions for SNPs. A collection of 77 MBO isolates associated with bovine bTB outbreaks in the US between 1990-2009 and isolated from a variety of mammalian hosts – cattle, deer, elk, elephant, swine, and humans were used for the study. 62 *M. tuberculosis* isolates from human, primates, birds, and elephants were also included in the analysis. Based on 206 variant SNPs between the MBO strains, five major clusters consistent with epidemiologic and other strain-typing information were identified. All the MTB isolates were identical at the 350 loci. This SNP based phylogeny provides new insights into the evolution of MBO and a gateway for studying strain genotype-disease phenotype correlations that we undertook in an *in vitro* infection model of the disease with 4 virulent MBO strains isolated from human, cattle (2) and deer and investigated them for their virulence based on survival in macrophages and relative gene expression profile of various virulence genes at different time points post-infection. The preliminary results reveal a differential survival of 4 strains; however relative gene expression for mce4C, PE6, speE, mmpL12 did not reveal a differential expression pattern; we are currently evaluating more targets to draw specific conclusions including SNPs affecting intergeneic loci.

GS-23

Identification and characterization of a spore-like morphotype in chronically starved *Mycobacterium avium* subsp. *paratuberculosis* cultures.

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Mycobacteria are able to enter into a state of non-replication or dormancy, which may result in their chronic persistence in soil, aquatic environments, and permissive hosts. Stresses such as nutrient deprivation and hypoxia provide environmental cues to enter a persistent state; however, a clear definition of the mechanism that mycobacteria employ to achieve this remains elusive. While the concept of sporulation in mycobacteria is not novel, it continues to spark controversy and challenges our perceptions of a non-replication. We investigated the potential role of sporulation in one-year old broth cultures of *Mycobacterium* subsp. *paratuberculosis (MAP)*.
We show that dormant cultures of *MAP* contain a mix of vegetative cells and a previously unknown morphotype resembling a spore. These spore-like structures can be enriched for using sporulating media. Furthermore, purified *MAP* spore forms survive exposure to heat, lysozyme and proteinase K. Heat treated spores are positive for *MAP 16SrRNA* and *IS900. MAP* spores display enhanced infectivity as well as maintain acid-fast characteristics upon germination in a well-established bovine macrophage model. This is the first study to demonstrate a new *MAP* morphotype possessing spore-like qualities. Data suggest that sporulation may be a viable mechanism by which *MAP* accomplishes persistence in the host and/or environment. Thus, our current understanding of mycobacterial persistence, pathogenesis, epidemiology and rational drug and vaccine design may need to be reevaluated.

GS-24

Transcriptome mapping of pAR060302, a *bla*$_{\text{cmy-2}}$ positive, IncA/C broad host range plasmid.

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The multidrug resistance-encoding plasmids belonging to the IncA/C incompatibility group have recently emerged among *Escherichia coli* and *Salmonella enterica* of production animals and clinical human isolates in the United States. These plasmids have a unique genetic structure compared to other enterobacterial plasmid types, a broad host range, and propensity to acquire large numbers of antimicrobial resistance genes via their accessory regions. Currently, the basic biology of these plasmids enabling their rapid dissemination and success in bacterial populations is not completely understood. Using the prototype IncA/C plasmid pAR060302, we sought to define the baseline transcriptome of IncA/C plasmids under laboratory growth and in the face of selective pressure. Under growth in Luria-Bertani broth lacking antibiotics, much of the backbone of pAR060302 was transcriptionally inactive, including its putative transfer regions. A few backbone genes of interest were highly transcribed, including genes of a putative toxin-antitoxin system, a GntR-family transcriptional regulator, and an H-NS-like transcriptional regulator. In contrast, numerous genes within the accessory regions of pAR060302 were highly transcribed, including the resistance genes *floR*, *bla*$_{\text{CMY-2}}$, *aadA*, and *aacA*. Under antibiotic treatment with ampicillin, florfenicol, or streptomycin, very few genes were differentially expressed on pAR060302 compared to controls lacking antibiotics, suggesting that many of the resistance-associated genes are constitutively expressed at high levels. Overall, this snapshot of the transcriptome of pAR060302 suggests that it mitigates the fitness costs of carrying resistance-associated genes through global regulation with its transcriptional regulators.

GS-25

The Essential Protein, Gcp, is a Novel Repressor of Branch Chained Amino Acid Synthesis Pathway in *Staphylococcus aureus*

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Gcp is a conserved protein throughout all three kingdoms of life. Our previous studies have demonstrated its essentiality for the growth of *Staphylococcus aureus*. However, the essential biological function and mechanism of Gcp remains elusive. In this study, we employed proteomic approaches and revealed that the down-regulation of *gcp* expression significantly increased the expression of IlvA, IlvB, and IlvD proteins, which are located in the *ilv-leu* operon and responsible for branch chained amino acids (BCAA) biosynthesis in *S. aureus*. We further confirmed Gcp transcriptional regulation of the *ilv* operon by using an *ilv*-promoter-luciferase reporter fusion plasmid. We further defined the requirement of Gcp for survival in a methicillin resistant strain of *S. aureus* using *Pspac*-regulated gene expression technology. Moreover, we found that the down-regulation of Gcp dramatically enhanced the bacterial growth in chemically defined media lacking the BCAAs, isoleucine, leucine and valine. Taken together, the above data indicate that Gcp negatively regulates the BCAA biosynthesis pathway in *S. aureus*. To elucidate whether Gcp's negative regulation of the BCAA biosynthesis pathway contributes to its essentiality, we created an *ilv-leu* operon deletion mutant using the defined *Pspac*-regulated *gcp* expression strain and found that the deletion of *ilv-leu* operon did not diminish the bacteria's requirement for Gcp. This suggests that the essential nature of Gcp is not attributable to its repression of the *ilv-leu* operon. These new findings provide new insights into the biological function of the novel essential protein, Gcp, as well as the regulatory mechanisms of BCAA biosynthesis.

GS-26

A novel picornavirus in farmed baitfish of Minnesota and Wisconsin

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With the emergence of high profile fish diseases in the Great Lakes region, surveillance and regulatory inspections of fish populations have increased. This has resulted in a better understanding of known pathogens and isolation of many new pathogens. During routine inspection of an apparently healthy Fathead Minnow (*Pimephales promelas*) pond in Minnesota, a previously unknown picornavirus was identified. At least four other picorna-like viruses are known to occur in fish, with Bluegill picornavirus being the only one to occur in freshwater. Cytopathic effects were observed on the epithelioma papulosum cyprini (EPC) cell line, cultured at 200C, after six days of incubation. Small, round viral particles (~30-32 nm), with picorna virus-like morphology (almost featureless virions with no projections) were observed with negative contrast electron microscopy. Random amplification and sequencing of extracted RNA from the cell culture isolate confirmed it to be a picornavirus. Further analysis by complete 3D gene sequencing (gene involved in serotype differentiation) identified it as a novel virus in the Picornaviridae family. Subsequent investigation of archived viral isolates (from 2009-2011) identified this novel picornavirus in fathead minnows and white suckers (*Catostomus commersoni*) of both Minnesota and Wisconsin. Although all of our samples were collected from apparently healthy fish during routine inspection, the long term impact to the health of fish populations is unknown. Given the importance of these host species to the economy and ecology of the region, continued research is necessary.

GS-27

Novel PRRSV ORF5a protein is not immunoprotective but drives GP5 glycosylation

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus responsible for PRRS in swine; a disease with significant animal welfare and economic implications for which there is no specific treatment, and variable protection from vaccination due to viral genetic and antigenic diversity. Molecular mechanisms responsible for virulence, pathogenesis and protective immune response remain poorly understood. These factors limit progress towards development of effective measures for prevention and treatment of PRRS.

We have discovered a novel open reading frame (ORF) that is initiated upstream of and overlaps ORF5 encoding major envelope glycoprotein GP5. Presence of the ORF5a is evolutionarily conserved in all Arterivirus family members, and ORF5a protein is present in infected cells, incorporated into virions, and elicits antibody production in pigs infected with PRRSV. ORF5a protein has a highly conserved arginine-glutamine (RQ) rich motif arising from nucleotide sequence dually encoding the GP5 hypervariable glycosylation domain that is assumed to be driven by immunological selection. To investigate this paradox, 3400 PRRSV sequences were examined to determine codon usage and infer selective pressures on this region. We determined that purifying selection to maintain ORF5a protein drives GP5 reading frame variation through selective ORF5a RQ codon usage. This has implications for the variation in GP5 glycosylation pattern in this region where neutralizing epitopes have been described.
To determine if ORF5a was immunoprotective, pigs were immunized with ORF5a protein prior to virulent virus infection. Immunized pigs had consistent serologic responses which were not immunodominant. Antibodies did not neutralize virus, and robust antibody responses observed in some pigs did not translate into protection against viral challenge as evaluated by viremia. These findings indicate that ORF5a has functional significance but does not elicit protective immunity.

GS-28

The porcine antibody repertoire and its response to PRRSV infection

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Antibody responses are critical to effective immunity to viral infections. Thus, extensive efforts have been directed to characterize the antibody and neutralizing antibody responses to PRRSV infection, in the hope of elucidating key insights into protective and cross-protective immunity. Despite these efforts, the role of antibody responses in PRRSV immune protection remains poorly understood. To address this, we characterized the expressed immunoglobulin repertoires in healthy and JA142 PRRSV-infected pigs using amplicon-based 454 high-throughput sequencing. Bioinformatic analysis of approximately 450 thousand reads revealed preferential usage of CDR3s of specific lengths in the infected pool. These PRRSV-specific CDR3 lengths corresponded with unique sequences that accounted for between 11 and 35 percent of all transcripts in their respective CDR3 size class. Furthermore, these same sequences were rare (~0.1%) in the uninfected pool. Diversity analysis estimated the size of the porcine heavy chain immunoglobulin repertoire to be approximately $3.5x10^5$, an estimate similar to that reported in humans, suggesting that the swine antigen-binding repertoire is similarly complex, despite the apparent lack of diversity in the porcine heavy chain variable gene (IGHV) framework regions. As a consequence of their repertoires being dominated by a small number of sequences, pigs infected with PRRSV showed a decrease in their total repertoire diversity. Furthermore, PRRSV-specific IGHV gene segment usage was dominated by IGHV4/IGHV10, suggesting a possible immunogenetic component of PRRSV immunity. We expect the results of this research to open the door to development of therapeutic reagents to treat acute PRRS, genetic testing for PRRS resistance, and a mechanistic understanding of cross-protective immunity.

GS-29

Transcriptional profiling of a pathogenic and an attenuated homologous *Lawsonia intracellularis* isolate during *in vitro* infection.

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Lawsonia intracellularis is the causative agent of proliferative enteropathy, an endemic disease in pigs and an emerging concern in horses. Spontaneous attenuated isolates obtained through multiple passages in cell culture do not induce disease. Conversely, bacterial isolates at low cell passage induce clinical and pathological changes. The identification of genes differentially expressed between a pathogenic and an attenuated homologous *L. intracellularis* isolate can help to elucidate virulence factor-encoding genes involved in this infection. The current study used high-throughput sequencing technology to characterize the transcriptional profiling of a pathogenic and an attenuated isolate during *in vitro* infection. Bacterial RNA was harvested from infected piglet intestinal epithelial cells (IPEC-J2) five days post-infection (approaching peak of infection). A total of 319 protein-encoding genes were expressed in both pathogenic and attenuated isolates. These common genes between the two isolates are involved in metabolic, biosynthetic and cell motility pathways, such as chemotaxis and flagellar assembly. Only 10 genes in the chromosome and one in the plasmid LIB (*parA* – ATPase involved in chromosome partitioning) were uniquely expressed by the attenuated isolate. In contrast, 401 mapped genes were exclusive to the pathogenic isolate. Genes involved in ATP-binding cassette transporter synthesis, two-component system and protein export pathways were significantly responsible for this wider transcriptional landscape, which were distributed in the chromosome and three plasmids (LIA, LIB, LIC). In addition, only sequences from the pathogenic isolate could be mapped against the plasmid LIA, which may play an important role in the course of infection. We identified distinct genes and pathways between a pathogenic and an attenuated *L. intracellularis*. This information supports our hypothesis and opens a new research field for studying target genes involved in the ecology, pathogenesis and physiology of this intriguing intracellular organism.

GS-30

The development and validation of two non-invasive diagnostic screening assays for the detection of tuberculosis infection in non-human primates.

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The introduction of novel infectious diseases has become as a major threat to endangered primate populations. This is particularly true for habituated great ape populations, which have been conditioned for close encounters with human observers, and there is much evidence that exposure of primates to human pathogens, particularly those of respiratory origin, readily occurs. In an effort to sustain the health of habituated great ape populations, continued health monitoring of these populations is recommended. Unfortunately, health monitoring for some diseases is hampered by a paucity of sensitive, non-invasive diagnostic assays. Tuberculosis, a disease of high prevalence among humans in many African regions, is an example and poses a significant health risk for habituated great ape populations. The goal of this project was to develop and validate fecal and urine enzyme-linked immunosorbant assays (ELISAs) for the detection of host and pathogen-derived biomarkers of *Mycobacterium tuberculosis* (M.tb) infection in non-human primates. An ELISA was developed for the detection of a M.tb biomarker that is shed in the urine, lipoarabinomannan. This is an outer cell wall lipoglycan, specific for pathogenic mycobacteria of the M.tb complex (MTC). A second set of ELISAs were also developed to detect in feces, host antibodies to the highly antigenic proteins ESAT-6, Cfp10, and Ag85, all of which are specific to members of the MTC. ELISAs were developed using primate urine and fecal samples spiked with the target biomarkers. Validation of the assays will be carried out with the testing of known M.tb positive and negative macaques (*Macaca* spp.). Receiver operating characteristic analyses will be used to assess the diagnostic accuracy of the ELISAs.

GS-31

Sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity

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Influenza is a common respiratory disease of pigs caused by influenza A virus. Due to the relatively short duration of shedding and the potential absence of clinical signs, detection of influenza virus can be difficult. Oral fluids now provide an alternative means to detect pathogens in pig populations. The objective of this study was to assess the sensitivity of oral fluids for the detection of influenza virus in young pig populations with and without maternally derived immunity. Nine pens of 10 pigs (n=90) were each challenged with influenza virus via direct contact with an experimentally infected pig at 3 weeks of age. Three pens of pigs were seronegative and six pens had varying levels of maternally derived immunity to influenza virus. Nasal swabs and oral fluid samples were collected for a period of 13 days post-exposure. A nasal swab was collected from all pigs in each pen at each sampling occasion and one oral fluid sample was collected from each pen. A pen was considered positive if one nasal swab was positive or if the oral fluid sample was positive. All samples were assessed via matrix rRT-PCR. Agreement, sensitivity, and specificity were compared between nasal swab and oral fluid sample results at the pen level. The overall kappa coefficient for agreement (κ) between oral fluids and nasal swabs was 0.75 (95% CI 0.62-0.87). With nasal swab sampling as the gold standard, the sensitivity and specificity of oral fluids was 79% and 100%, respectively. The sensitivity of oral fluids increased to 93% when pen prevalence of infection was greater than 9%. This study indicates that pig age and maternally derived immunity may not have a large impact on the efficacy of pen based oral fluid sampling. This study provides additional evidence that oral fluid sampling is an effective and sensitive method for the detection of pathogens, including influenza virus, in swine populations.

GS-32

Evaluation Study of Interventions for Reducing the Risk of PRRSV Introduction into Filtered Farms via Retrograde Air Movement (Back-drafting) through Idle Fans

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Introduction: Airborne transmission of the virus is an important route of spread of PRRSV between farms. Several North American production systems have implemented air filtration systems. While preliminary results are promising, a major risk that currently exists in filtered herds under negative pressure ventilation is the retrograde movement of PRRSV-contaminated bioaerosols through non-filtered points i.e., inactive fans. To reduce this risk, several interventions have been developed but not validated. Therefore, the objectives of this study are to demonstrate that the risk of back-drafting of PRRSV-contaminated aerosols is a true risk and to validate commercially available interventions. **Materials and methods:** The study was conducted at the UMN SDEC production region model. Using an existing 25m² facility (void of pigs and ventilated via negative pressure) one of the two 30cm fans was intentionally stopped while the other continued to operate, creating retrograde movement of air into the facility via the inactive fan. This fan, located on the south end of the building was equipped with a standard plastic shutter commonly encountered in commercial swine farms. The operational fan was located at the north end of the facility. All other inlets to the facility were closed resulting in a static pressure of 2.45 Pa. Besides the standard plastic shutter, treatments tested included a plastic shutter plus a canvas cover, a nylon windsock, an aluminum shutter plus a windsock and a double shutter system involving both an aluminum and plastic shutters. All 5 treatments were challenged with 4 different aerosolized concentrations of PRRSV ranging from 1 to 7 logs of virus/L, generated via a cold-fog mister located exterior to the facility 46cm from the inactive fan. To determine whether aerosolized PRRSV could penetrate the treatments, a cyclonic collector was placed inside the facility 45cm from the inactive fan. Ten replicates were conducted per treatment, each replicate was 1 minute in length and air samples were tested for the presence of RNA PRRSV by PCR. **Results:** Retrograde movement of air in association with the introduction of PRRSV to the interior of the facility was observed during the assessment of the plastic shutter and plastic shutter plus canvas cover. PRRSV introduction to the facility was not observed following the application of the other interventions. **Discussion:** Under the conditions of this study, the introduction of PRRSV secondary to the retrograde movement of air was proven to be a true risk. In contrast, interventions such as double shutter systems or shutter plus windsock combinations appear to eliminate this risk. Therefore, a program to minimize the risk of retrograde movement of air into filtered facilities appears to be critical for reducing the airborne risk of PRRSV.

GS-33

Estimating Genetic Diversity at the MHC in a Population of Introduced Wild Turkeys.

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Genetic variation in the major histocompatibility complex (MHC) is known to affect disease resistance in many species. The antigen presenting class I and class II molecules located in this region of the genome are particularly known for their polymorphic nature, and investigation of the genetic diversity of these antigens has been performed on many species. Thus far, only limited studies of MHC haplotype variation in the turkey have been attempted, with variation assessed serologically, genetically (by restriction fragment length polymorphism, RFLP) and to a limited extent, by direct nucleotide sequencing. This study was designed to investigate MHC diversity in a collection of wild turkeys (*Meleagris gallopavo silvestris*) collected during population expansion following reintroduction in southern Wisconsin, USA. Single nucleotide polymorphisms (SNPs) were identified by sequencing select MHC class I gene regions in individuals with distinct MHC class IIβ Southern blot phenotypes. All individuals were subsequently genotyped by PCR/RFLP for haplotype analysis. To further characterize genetic diversity of the sampled birds, a portion of the mitochondrial D-loop was also sequenced. Results show that diversity predictions based only on class IIβ Southern blot analysis underestimate the number of MHC haplotypes.

GS-34

Comparison of intradermal and intramuscular influenza vaccinations for pigs

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The most common route of swine vaccination for influenza is by intramuscular injection, although recent improvements in needle-free injection technology have presented an opportunity to evaluate low-dose intradermal vaccination as an alternative option. Two farm-based studies were conducted to evaluate this method of vaccination. In the first study, H1N1-naïve growing-pigs were randomized to IM and ID vaccination groups to assess the feasibility of ID flu vaccine and to evaluate the humoral immune response induced by vaccination. In the second, adult, multiparous females with a clinical history of H3N2 infection were vaccinated via ID and IM routes with a herd-specific H3N2 vaccine to determine the booster effect of sow vaccination on serum antibody titers. Geometric mean H1N1 or H3N2 HI antibody titers (GMT) were calculated for all individuals at all time points. Differences between ID and IM groups calculated via two-sample t-tests assuming equal variances. For growing pigs, both ID and IM groups had a significant rise in serum antibody titers after receiving two doses of vaccine (p-value= <0.00001). The day 42 H1N1 antibody titers were numerically higher for pigs in the IM compared to the ID group (GMT 160 and 80, respectively), but this was neither statistically (p-value= 0.1) nor clinically significant. In the sow study, all sows had similar HI titers at the time of vaccination, consistent with prior infection with H3N2. There was a significant booster effect of vaccine for both ID and IM vaccinated sows. However, the rise in antibody titer was significantly higher (p-value= 0.01) for sows receiving 2.0 ml of vaccine IM compared to the ID vaccinated sows receiving 0.2 ml (GMT 402 and 268, respectively). This study, while limited in animal numbers and lacking stringent controls due to its on-farm "field" setting, highlights the need to determine the optimum volume of vaccine to be delivered ID to achieve the desired response. The results of these studies suggest ID vaccination against influenza is a viable, cost-efficient, and biologically effective option for growing pigs and adult swine.

GS-35

Estimation of the prevalence of *Mycoplasma hyorhinis* colonization in swine herds

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Mycoplasma hyorhinis has recently emerged as an important cause of mortality in nursery pigs. Approximately 50% of the cases with polyserositis received at the Minnesota VDL in 2010 had the involvement of this pathogen based on isolation or PCR. The objective of this study was to characterize the pattern of *M. hyorhinis* colonization in endemically infected herds. Three 6000 sow farrow-to-wean herds and their nurseries located in MN and SD were selected. These herds had a diagnostic history of recurrent mortality associated with *M. hyorhinis* isolation from systemic sites. Nasal swabs were collected from 60 sows, 60 piglets in each group of 1, 7, 14 and 21 days of age as well as 30 pigs in each group of 28, 35, 42, 49, 56, 63, 70 and 77 days of age. Oral fluids were also collected from the same post weaning pigs. Tissue samples were collected from ten clinically affected and ten clinically healthy pigs necropsied at the age of the peak of mortality. All samples were tested by a real time PCR developed in our laboratory. *M. hyorhinis* was detected in the nasal cavity of 5/60 sows in herd one, 3/60 in herd two and none in herd three. In herd one and two that had clinical cases suggestive of *M. hyorhinis*, the colonization prevalence in suckling piglets was low (avg=8%) and high in post-weaning pigs (avg=98%). In contrast, in herd three where *M. hyorhinis* clinical signs were absent, colonization in pigs was very low until the last week in the nursery. A total of 7/8 oral fluids tested *M. hyorhinis* positive in herd one and two, while 1/8 tested positive in herd three. Polyserositis was not observed in any of the healthy animals from all three herds neither in the diseased pigs from herd three. However, in herds one and two polyserositis was observed in 9/10 and 4/10 diseased pigs respectively. Isolation of *M. hyorhinis* from the pericardium was achieved only in herds one and two. *M. hyorhinis* was detected by PCR in the pericardium of 8/10 diseased pigs in herd one and 3/10 in herd two. In the healthy pigs only one sample tested PCR positive. In herd three *M. hyorhinis* was not detected in any of the necropsied pigs. In summary, *M. hyorhinis* can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. The pathogen can colonize pigs at day one of age; however, most of the pigs become colonized sometime in the nursery. High prevalence of *M. hyorhinis* nasal colonization in weaned pigs appears to be correlated to the presence of *M. hyorhinis* associated disease and the detection of the agent in polyserositis cases in nursery pigs.

GS-36

Detection of influenza virus in air from experimentally infected pigs

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Influenza A infected pigs shed the virus through nasal secretions for a period of approximately five to seven days and transmit the pathogen through nose-to-nose contact. In addition, influenza virus can also be transmitted through aerosols. However, literature about detection of airborne influenza virus is scarce. The objective of the project reported here is to determine the frequency of detection of influenza virus in air samples generated from experimentally infected pigs. Two groups of 11, 7-week-old influenza negative pigs were housed in two separate rooms at the University of Minnesota Animal Isolation facility. One “seeder” pig from each group of 11 pigs was infected with H1N1 influenza A virus in a separate room and commingled with the other 10 pigs when shedding in this pig was confirmed. Pigs were in contact for 8 days after commingling and individual shedding assessed by collecting individual nasal swabs from all pigs daily and tested for influenza RNA through RT-PCR. A cyclonic collector, capable of collecting 400L of air per minute, was used for air sample collection. Ten milliliters of minimum essential media (MEM) supplemented with 2% BSA were added to the cyclonic collector vessel and the collector was allowed to run for 30 minutes. Three air samples per day approximately every 8 hours were collected for 8 days after commingling. Air sample fluid was tested for influenza A virus RNA through RRT-PCR. Both seeder pigs and all in-contact pen-mates were confirmed to be shedding influenza virus for at least 4 days and a maximum of 6 days during the 8 day period. A total of 41 air samples were collected. Out of those 41 samples, 25 (52%) were positive to influenza virus. In both groups, influenza virus was detected for the first time 2 days after commingling when 4 out of 11 pigs were shedding virus. All air samples were positive for influenza from day three after commingling until the first sample on day seven. During these days, all nasal swabs tested positive by individual PCR. Our results are novel and confirm that detecting influenza A virus in air from experimentally infected pigs is possible and a frequent event under the conditions of this study. More studies are needed to further validate the cyclonic collector for influenza detection in field samples.

GS-37

Effect of changes in management practices on the risk of Johne's disease in Minnesota Johne's disease demonstration dairy herds

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Certain management practices have been recommended to minimize transmission of Johne's disease between infected and susceptible cattle. The objective of this study was to evaluate the risk of testing positive and its association with changes in recommended management practices in different birth cohorts. Eight dairy herds were enrolled in the Minnesota Johne's Disease Demonstration Herd Program. Herds were monitored for a period between 5 to 10 years. Annual testing for *Mycobacterium avium* subsp. *paratuberculosis* was performed for all cows that calved, using bacterial culture and serum ELISA. Risk assessments were performed annually to measure the level of implementation of the recommended management practices. Eight birth cohorts were defined based on the date of cow enrollment in the program. Birth cohorts -2 and -1 corresponded to cows that were born 2 and 1 year before the beginning of the program, respectively, and cohorts 0 to 5 corresponded to cows that were born 0 to 5 years after the beginning to the program. The annual risk assessment score was used to quantify the level of exposure by birth cohort and herd. A time dependent Cox's regression model was used to model the time to test positive, explained by herd, birth cohort and birth cohort exposure level. Compared to birth cohort -2, there was a reduction of the hazard ratio (95% CI) of bacterial culture positivity of 0.65 (0.49 to 0.85), 0.56 (0.42 to 0.73), 0.66 (0.48 to 0.90), 0.38 (0.26 to 0.58), 0.22 (0.14 to 0.35), 0.22 (0.14 to 0.34), and 0.20 (0.13 to 0.32), for birth cohorts -1, 0, 1, 2, 3, 4, and 5, respectively. Similar results were obtained for serum ELISA. The instantaneous hazard of testing positive for both tests increased with the level of exposure, however, the strength of this association decreased over time. There was a reduction in the transmission of Johne's disease associated with the level of implementation of the recommended management practices.

GS-38

Longitudinal Investigation of the Age-related Diversity in the Commercial Pig Distal Gut Microbiome

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Background: The importance of bacteria in the gastrointestinal tracts of animals is widely acknowledged as important. However, very little is known about composition and distribution of the microbial population in lower intestinal tracts of animals. Because most of bacterial species in pig intestines have not been cultured, it has been difficult to identify most bacterial species by conventional culture methods. Therefore, high throughput pyrosequencing of 16S rDNA libraries was used in our longitudinal study to explore the diversity of microbial communities of the pig distal intestine.
Methods: Fecal samples from the pigs (n=10) were collected 5 times at 3-week intervals starting when the pigs were 10 weeks of age. The sequences of the hypervariable V3 region of 16S rRNA were generated using the high throughput pyrosequencing. Sequences were quality assessed, and analysed using bioinformatics tools such as RDP classifier, Mothur, and FastUnifrac. Operational Taxonomic Unit definition at a similarity cutoff of 97% was used in this study.
Results: More than million sequence reads were generated by the pyrosequencing. The average Shannon-Weaver and Simpson (1-D) index values showed high diversity. While the bacterial communities of all samples were comprised primarily of *Firmicutes* and *Bacterioidetes*, which accounted for more than 90% of the total sequences, microbial ecosystems of pig distal gut continued to change as pigs aged. Group-based analysis of the sequences discovered a total of 18,711 OTUs. A small proportion of OTUs (558 core OTUs out of total 18,711 OTUs) were shared by all the groups, but the 558 core OTUs still contained the majority of the total sequences (>68%).
Conclusion: Microbial ecosystems in each pig continued to change and converged toward a certain profile characteristic of the gastrointestinal tract of adult pigs as pigs aged. The variations of bacterial population of the animals were caused mainly by the "rare" species present.

GS-39

Preliminary analysis of an ongoing study characterizing spatial patterns of white-tailed deer movement and cattle farm visitation in relation to bovine tuberculosis transmission risk in northwestern Minnesota

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The objective of this study is to characterize spatial patterns of white-tailed deer movement related to bovine tuberculosis transmission risk to cattle in northwestern Minnesota. Sixteen adult deer (12 females and 4 males) were initially captured in January 2011 and fitted with GPS collars just outside the BTB Management Zone in a study site with an approximate total area of 140mi2, that represents transitional deer habitat and where cattle operations are present (n ≈30). This area is similar in habitat composition to the BTB Core area in that deer have the option between moving east to the forest zone, west to the farmland zone, or remain resident in the transition zone. GPS collars were programmed to collect location information every 90 minutes (16x daily). Ground-truthing has been performed seasonally to assess the precise locations of fenced cattle, cattle feeding areas, and feed storage locations that could potentially be visited by deer. Due to unexpected high deer mortality (52.4%), mostly due to wolf predation (72.7%), a second capture effort was performed in early March, adding 5 deer (4 females and 1 male) to the study population. The current analysis includes only the remaining deer in the study (n=10) and data collected until August 8th. The preliminary descriptive analysis shows that 99% of deer visitation occurred in one farm, and 70% of overall visits occurred in areas with cattle and stored feed. Only two deer were responsible for 99% of the visits. A higher proportion of visits occurred in the morning (6 am to 12 pm - 62.86%), followed by night (12 am to 6 am - 32.86%). The study will continue until the spring of 2012, upon which GPS collars will be recovered and the complete data sets from all deer will be analyzed.

GS-40

Effectiveness of herd exposure methods to produce PRRSv-negative piglets from infected breeding herds

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Load-close-homogenize (LCH) has become the standard protocol to produce porcine reproduction and respiratory syndrome virus (PRRSv)-negative offspring from PRRSv-positive breeding herds.
Inoculation of resident live virus (LVI) and modified-live vaccine virus (MLV) are options to “homogenize”. The purpose of this study is to compare time to produce PRRSv-negative pigs at weaning (TTNP) from herds that used MLV to herds that used LVI. Day 1 of the stabilization program was the day that the herd was exposed to either the modified live or wild-type virus. PRRSv-monitoring started 12 weeks after day 1 and consisted of bleeding 30 piglets and testing serum samples for PRRS-RNA by RT-PCR. Herds were defined as producing PRRSv-negative piglets when they reached 90 days of consecutive monthly PCR-negative results. Preliminary descriptive analysis indicates that PRRSv shedding in farms going through herd closure is intermittent, indicating that PRRSv-monitoring must be done systematically over time. Moreover, farms had substantial variation in TTNP. In the MLV group the average TTNP period for 5 farms was 27.0 (range 12- 41) weeks, and for the LVI group, 7 farms had TTNP average and range of 26.4 (16 – 42) weeks. The enrollment of herds using modified live vaccine as the exposure method has been limited by the fact that most of the contacted herds using that strategy do not extend the closure period for the minimal of 180 days required by the study. Increasing the amount of data in the study’s database will enable us to better characterize the effectiveness of herd stabilization methods allowing producers to make informed decisions about PRRSv control and elimination.

GS-41

Enrofloxacin treatment affects the colonization status of *Haemophilus parasuis* in weaned pigs

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Introduction: A better understanding of the factors that affect *H. parasuis* (HPS) colonization is necessary for effectively preventing and controlling HPS infections. Antibiotics including enrofloxacin, are commonly used to treat HPS systemic infections. However, limited information is available on the effects of enrofloxacin on HPS colonization. Therefore, the objective of this study was to evaluate the effect of enrofloxacin in HPS colonization in weaned pigs. **Materials and Methods:** Twenty three weaned pigs positive for HPS by PCR were divided in two groups. On arrival blood samples, and nasal and tonsil swabs were collected from all pigs. Twelve pigs in the treatment group (TG) received a dose of enrofloxacin IM at 24 h post arrival. Eleven pigs in the control group (CG) received saline. Pigs were monitored for 15 days and were sampled every day by tonsillar and nasal swabs, which were tested by HPS qPCR. At necropsy, swabs were collected in duplicates, one for bacteriological examination and the other was tested by qPCR. ERIC-PCR genotyping and a PCR to detect HPS virulent gene (vtaA) were also used to characterize the HPS isolates obtained. Blood samples were tested using OppA-ELISA. Differences between the proportion of HPS positive pigs in treated vs control groups at each sampling time point were calculated using Fisher's Exact Probability Test, with Bonferroni correction ($\alpha = 0.003$). **Results:** Pigs in the CG tested positive throughout most of the study, while all pigs in the TG tested HPS negative by qPCR at 1 DPT and the treatment effect persisted partially until 12 DPT. The average of CFU/reaction/day for tonsil and nasal indicate that the daily bacterial load was less in the TG than in the CG. The proportion of positive pigs was statistically higher in the CG than in the TG (P-value < 0.003). Four HPS isolates were recovered at 15 DPT. Similar fingerprinting patterns by ERIC-PCR demonstrated that pigs were colonized with similar HPS strains, which were also negative for the vtaA gene. These results indicated that these isolates did not have the vtaA virulence factor. Serum antibody titers, as measured by ELISA S/P at the time of arrival and at the time of necropsy for both groups were under the cut-off value of 0.2. **Discussion and Conclusion:** Enrofloxacin treatment significantly reduced the number of pigs colonized with HPS and this effect was mostly seen during the first week post treatment. Enrofloxacin also reduced the presence and load of HPS on the nasal cavity and the tonsils of naturally colonized pigs, but was unable to completely eliminate the organism. Additionally, pigs did not seem to mount a humoral immune response to HPS colonization. Further research is needed to evaluate the lasting effect of enrofloxacin in HPS colonization patterns and disease dynamics.

GS-42

Effect of Intravaginal Progesterone Insert on GnRH-induced LH Surge, Follicle Growth, and Plasma Progesterone Concentrations.

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The objectives of this experiment were to evaluate the effect of treatment with a controlled internal drug release (CIDR) insert containing 1.38 g of progesterone (P4) at the time of GnRH injection on GnRH-induced LH surge, follicular growth and plasma concentrations of P4. Non-pregnant lactating Holstein cows were randomly assigned to one of three treatments after balancing for parity, body condition score and 305-d projected milk yield. The treatments were control (CON, n=7), 1GP4 (n=10) and 2GP4 (n=10). All cows were presynchronized with a CIDR insert for 5 days, one day before and upon CIDR removal cows received a 25mg PGF injection, and 2 d later a 100µg GnRH injection. The day of the GnRH injection was considered d0 of the estrous cycle. On d6, CON cows received 100µg of GnRH, 1GP4 cows received 100µg GnRH injection and a CIDR insert, and 2GP4 cows received 200µg of GnRH and a CIDR insert. Ovaries were scanned 0, 10, and 20h after the GnRH given on d6. Blood was sampled at 0, 15, 30, 60, 120, 240, 345, 600, and 1200 min after the GnRH given on d6. Data were analyzed by ANOVA for repeated measures. Although LH concentration from 0 to 345 min was greater ($P<0.01$) for 2GP4 cows (3.1±0.2ng/ml) than CON (2.1±0.3ng/ml) and 1GP4 cows (2.2±0.2ng/ml), that was mainly because at 60 (CON=2.6±0.4, 1GP4=2.7±0.3, 2GP4=3.7±0.3ng/ml) and 120 (CON=4.6±0.8, 1GP4=5.1±0.6, 2GP4=7.6±0.6ng/ml) min LH concentrations were ($P<0.01$) greatest for 2GP4 cows. Progesterone concentrations were smaller ($P<0.01$) for CON cows (1.9±0.3ng/ml) than 1GP4 (3.3±0.2ng/ml) and 2GP4 (3.4±0.2ng/ml) cows, but there were no ($P=0.82$) differences between 1GP4 and 2GP4 cows. There were no differences ($P=0.75$) among treatments in size of the dominant follicle at 10 and 20 h after the GnRH injection given on d6. Treating cows with intra-vaginal P4 concurrently with GnRH does not decrease LH concentration or peak, but treatment with 200µg of GnRH results in earlier rise in LH and greater LH peak concentration than treatment with 100 µg of GnRH.

PD-1

Activation of TRPV1 causes musculoskeletal hyperalgesia, similar to its effect on other types of nociception, but TRPV1 desensitization by RTX does not alleviate mechanical hyperalgesia

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The transient receptor potential vanilloid1 (TRPV1) is activated by an array of endogenous and exogenous physical and chemical stimuli. TRPV1 is involved in pain transmission; it's the chief detector of noxious thermal heat in the peripheral nervous system. TRPV1 expressing fibers also innervate muscle tissue, but less densely raising the question of whether TRPV1 activity is important in musculoskeletal pain. We tested the hypothesis that TRPV1 is involved in thermal (using hot plate) and musculoskeletal (using grip force) nociception. The TRPV1 agonist capsaicin transiently enhanced both musculoskeletal and thermal hyperalgesia. Pretreating animals with RTX (5 injections of 0.1 mg/kg sc) desensitized the TRPV1 receptor, as evidenced by blockade of capsaicin-induced thermal hyperalgesia, frequency of eye wipes produced by direct application of capsaicin to the eye, and even blockade of capsaicin-induced hypothermia, an effect highly resistant to desensitization. Yet in spite of this, RTX enhanced rather than inhibited musculoskeletal nociception. These data demonstrate that although RTX desensitized the TRPV1 receptors along the thermal and chemical nociceptive pathways, its use clinically as an analgesic may be hampered by a persistent increase in musculoskeletal nociception.

PD-2

Norepinephrine enhances the vaginal epithelial immune response to a staphylococcal superantigen

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Staphylococcus aureus is a Gram-positive pathogen that resides in the reproductive tract of approximately 20% of all women. It produces superantigen exotoxins which mediate toxic shock syndrome (TSS) by cross-linking antigen presenting cells and T cells to induce massive cytokine release. TSS toxin-1 (TSST-1) is the superantigen responsible for the majority of menstrual TSS cases. This toxin has pro-inflammatory effects in vaginal epithelial cells, and it is thought to induce migration of adaptive immune cells to the vaginal submucosa. We have previously reported (J. Neuroimmune Pharmacol., 5 (suppl. 1):S41, 2010) that cells immunoreactive for the norepinephrine (NE) transporter, a pharmacological target of cocaine, are present in human cervicovaginal mucosa and that NE delays wound healing in human vaginal epithelial cell (HVEC) monolayers. In this study, we tested the hypothesis that NE alters immune responses to TSST-1 in HVECs. Although it had no effect alone, 10 μM NE enhanced IL-8 release in response to TSST-1 (100 μg/ml). Propranolol and the β2-adrenergic receptor antagonist ICI 118551 inhibited this effect. Moreover, only the combination of TSST-1 and NE could induce a significant increase in intracellular cAMP levels, and this effect was also sensitive to ICI 118551. We are presently investigating the HVEC signaling pathways that mediate these effects. Psychostimulant drugs of abuse that act to increase NE levels may alter the ability of the vaginal mucosa to respond to pathogens and their associated exotoxins.

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PD-3

Whole Genome Association Study of Type 2 Polysaccharide Storage Myopathy (PSSM) in Quarter Horses

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We previously identified a mutation in the GYS1 gene in horses with Type 1 Polysaccharide Storage Myopathy (PSSM) that results in accumulation glycogen and amylase-resistant abnormal polysaccharide in skeletal muscle fibers. A proportion of horses with exercise intolerance that are diagnosed with PSSM based on the accumulation of largely amylase-sensitive aggregates of polysaccharide in skeletal muscle do not possess the GYS1 mutation. The high prevalence of this form of PSSM, termed Type 2 PSSM, in particular breeds such as the Quarter Horse, suggests a genetic basis may also exist for Type 2 PSSM. The purpose of this study was to identify the underlying genetic basis for Type 2 PSSM utilizing a genome-wide association mapping strategy to identify positional candidate genes. The genotypes of 124 Quarter Horse controls and 104 Quarter Horse Type 2 PSSM cases were analyzed in a genome-wide association study (GWAS) with 50,856 single nucleotide polymorphisms (SNPs). The results of a logistic regression test assuming a dominance model revealed associations with alleles of multiple SNPs (most significant p-value = 0.000008) on equine chromosome (ECA) 18 and a single SNP on ECA3. In the future, additional sequencing and genotyping will be done to evaluate the statistical significance of the region on ECA18.in the future.

PD-4

Obesity suppresses allergen-induced airway inflammation but not airway hyperresponsiveness

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Objective: To evaluate the effects of diet-induced obesity on allergic inflammation and lung function. **Methods:** C57BL/6 mice maintained on standard chow (normal diet [ND], 18% calories from fat) or a high fat diet (HFD, 60% calories from fat) were sensitized and challenged with cockroach antigen (CRA). Control mice received saline at the same time points. 24 hours after the last allergen challenge, bronchoalveolar lavage fluid (BALF) and lung tissue were collected to assess airway inflammation. Airway hyperresponsiveness (AHR) to methacholine and lung compliance were measured by invasive plethysmography. **Results:** The weight of HFD mice was 30% higher than ND mice. Baseline studies indicated no difference between HFD and ND mice in BALF total and differential cell counts. As expected, CRA challenge resulted in an influx of inflammatory cells to the airways (BALF and lung tissue) of mice on ND. Interestingly, significantly fewer cells were present in the airways of CRA-challenged HFD mice. Further, pulmonary eosinophil infiltration and epithelial mucus accumulation in CRA-challenged HFD mice were also significantly inhibited compared to ND mice. Decreased eosinophilic infiltration in CRA-challenged HFD mice was associated with reduced levels of inflammatory mediators such as leukotriene C4 as well as prostaglandins E2 and D2 compared CRA-challenged mice on ND. Despite this reduced airway inflammation, airway resistance remained elevated as in CRA-challenged ND mice. Further, even in saline-exposed HFD mice, dynamic lung compliance, a measure of lung distensibility, was significantly lower compared to saline-challenged ND mice and remained unchanged after CRA challenge suggesting that the lungs of the obese mice may be more rigid and inflexible. **Conclusions:** Diet-induced obesity suppresses allergen-induced airway inflammation but not AHR. Regardless of allergen challenge, HFD mice exhibit decreased dynamic lung compliance compared to ND mice.

PD-5

Regulation of eosinophil trafficking and migration by ORMDL3: potential role in allergic airway inflammation

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Using genome-wide association and microarray approaches, *orosomucoid 1-like 3* (ORMDL3) has been identified as a candidate gene for susceptibility to asthma; however, the function or mechanism by which this gene may contribute to asthma predisposition is not well known. Recent studies indicate that ORMDL3, which is predominantly expressed in the endoplasmic reticulum (ER), plays a role in ER-mediated Ca2+ signaling and sphingolipid homeostasis. Here, we show that allergen challenge results in the recruitment of inflammatory cells that express ORMDL3 to the airways and have investigated the potential role of ORMDL3 in regulating trafficking and activation of eosinophils (Eos) which constitute the predominant pro-inflammatory cells contributing to the exacerbation of allergic asthma. For the first time, we have established that ORMDL3 is expressed by both murine and human Eos at the mRNA and protein level. Confocal microscopy studies revealed that ORMDL3 is predominantly distributed in ER of Eos. Exposure of Eos to mediators of allergic inflammation such as IL-3 and eotaxin as well as alterations in intracellular Ca2+ levels induced ORMDL3 expression. While over-expression of ORMDL3 by Eos resulted in distinct cytoskeletal rearrangement associated with increased eotaxin-mediated chemotaxis and rolling on VCAM-1 under conditions of flow, knockdown of ORMDL3 by siRNA significantly inhibited chemotaxis as well as expression of CD48, a cell surface molecule which activates Eos and is known to be critically involved in allergic eosinophilic inflammation. Interestingly, IL-3 which induces expression of ORMDL3 by Eos has also been previously shown to induce CD48. These findings suggest that during inflammation (elevated eotaxin and IL-3) increased expression of ORMDL3 may participate in - (i) Eos trafficking and migration by regulating Ca2+ signaling and (ii) Eos activation via CD48 expression. Additional studies are underway to further examine the role of ORMDL3 in detail.

PD-6

Role of serotonin (5-HT) and its receptor 5-HT2A in eosinophil migration and airway inflammation

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While the association of 5-HT, a known neurotransmitter, with the pathogenesis of allergic and bronchial asthma is recognized, its role in promoting airway eosinophilia is not well understood. Our previous studies indicated that 5-HT induces chemotaxis/migration of human eosinophils (Eos) *in vitro*. Here, we investigated the potential role of 5-HT in regulating eosinophil recruitment during ovalbumin-induced allergic airway inflammation using 5-HT2A deficient (5-HT2A-/-) mice. Allergen-challenged 5-HT2A-/- mice exhibited significantly decreased recruitment of total inflammatory cells and specifically of Eos to the airways (bronchoalveolar lavage fluid) compared to WT counterparts. Next, we examined the importance of 5-HT and 5-HT2A interactions during eosinophil migration and trafficking as well as associated signal transduction events. Human Eos, AML14.3D10 cells (a human eosinophilic cell line) and bone marrow-derived mouse Eos were all found to predominantly express 5-HT2A. In addition to inducing migration, treatment of human Eos with 5-HT was found to induce distinct cytoskeletal and cell shape changes. Further studies indicated that 5-HT mediates trafficking (rolling and adhesion) of the human eosinophilic cell line AML14.3D10 on VCAM-1 *in vitro* and of mouse eosinophils within inflamed post-capillary venules of the mouse cremaster miocrocirculation *in vivo* although no significant changes in cell surface adhesion molecule expression were observed on Eos in response to 5-HT. Blockade of 5-HT2A with the selective antagonist MDL-100907 as well as inhibitors of signaling molecules such as MEK, PI3K and ROCK not only decreased 5-HT-induced migration of human Eos but also inhibited 5-HT-induced rolling and adhesion of AML14.3D10 human eosinophilic cells under conditions of flow *in vitro*. Further, these inhibitors prevented 5-HT-induced cytoskeletal/cell shape changes in AML14.3D10 human eosinophilic cells and mouse Eos. Overall, these data demonstrate the presence of functional 5-HT2A receptors on murine and human Eos and suggest that 5-HT/5-HT2A interactions mediate Eos trafficking and migration via MEK, PI3K and ROCK signaling.

PD-7

Human FcγR gene copy number variations are associated with asthma and atopy

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Objectives: Fcγ receptors (FcγRs) serve as a critical link between humoral and cellular immunities and the interaction between FcγRs and IgG immune complexes critically affects the human immune responses. FcγR single nucleotide polymorphisms are significantly associated with human inflammatory diseases. The aim of study was to investigate whether copy number variations (CNVs) in FcγR genes (*FCGR* genes) are associated with human asthma susceptibility. **Methods:** TaqMan real-time PCR assays were designed and validated to determine CNVs in five *FCGR* genes (*FCGR2A*, *FCGR3A*, *FCGR2C*, *FCGR3B*, and *FCGR2B*). CNVs in each *FCGR* gene were determined by at least two independent TaqMan probes (one in promoter regions and the other in exons or introns) specific for the gene. **Results:** We failed to find any CNV in *FCGR2A* by genotyping 282 subjects. We observed 11 individuals (0.8%) as one-copy *FCGR2B* carriers and 3 individuals (0.2%) as three-copy *FCGR2B* carries in 1395 subjects with mixed ethnic origins. Our data suggest that the *FCGR2B* CNVs were extremely rare (< 1.0%) in human populations. In contrast, extensive CNVs were detected in human *FCGR3A*, *FCGR2C*, and *FCGR3B* genes. We observed two major structure variations (deletion and duplication) were involved in the block of neighboring *FCGR2C* and *FCGR3B* genes. Furthermore, we observed that the subjects carrying CN ≤ 2 of *FCGR3A* is susceptible to atopy in humans ($P = 0.004$, OR = 2.54, 95% CI: 1.422-4.524). On the other hand, low copy number of *FCGR3B* (CN ≤ 1) has a protective role against atopy ($P = 0.0056$, OR = 0.387, 95% CI: 0.194-0.774) and asthma ($P = 0.0281$, OR = 0.324, 95% CI: 0.113-0.932) in humans. Our data imply that *FCGR3A* and *FCGR3B* have the opposing roles in the development of allergy and asthma. **Conclusions:** *FCGR3A* function has a protective role against allergy and *FCGR3B* function may be a risk factor for inflammation in allergy and asthma. *FCGR* CNVs are the important genetic markers for asthma and may have important biological consequences.

PD-8

The Equine Genetic Diversity Consortium: an international collaboration to describe genetic variation in modern horse breeds

Jessica L. Petersen, James R. Mickelson, Molly E. McCue
& 32 additional members of the Equine Genetic Diversity Consortium
Veterinary and Biomedical Sciences, Veterinary Population Medicine, & 20 international organizations

Our limited knowledge regarding the genetic diversity of horses impacts our ability to correctly define population-based issues, identify and preserve characteristics that define particular breeds, and decipher the history of the modern horse and the basis of numerous complex genetic traits. The Equine Genetic Diversity Consortium (EGDC) represents a collaborative, international community of equine researchers who are working to build a comprehensive understanding of genetic diversity among equine populations across the world. To date, tissue samples, DNA, and genotypes, contributed by members of the EGDC represent over 1900 horses more than 40 breeds. SNP genotyping of each horse will yield over 54,000 SNPs for use in statistical across the population. These data are being utilized to quantify diversity (e.g. Ne, FST, observed heterozygosity, FIS), define population relationships (e.g. STRUCTURE, ADMIXTURE and parsimony analyses), to identify ancestry informative markers (AIMs), and for use in identifying genomic regions targeted by selection. Analysis thus far show substantial variability among breeds; estimates of Ne range from 114 – 712, pairwise FST values (between breeds) from 0.002 – 0.26, and analysis of molecular variance (AMOVA) found that 8.6% of the observed variation is found among breeds. Sample and genotype collection is ongoing and these data will be updated as the project progresses. Once complete, this data set should stimulate new studies into the origins of breeds and breed-defining traits, and guide efforts to preserve genetic diversity.