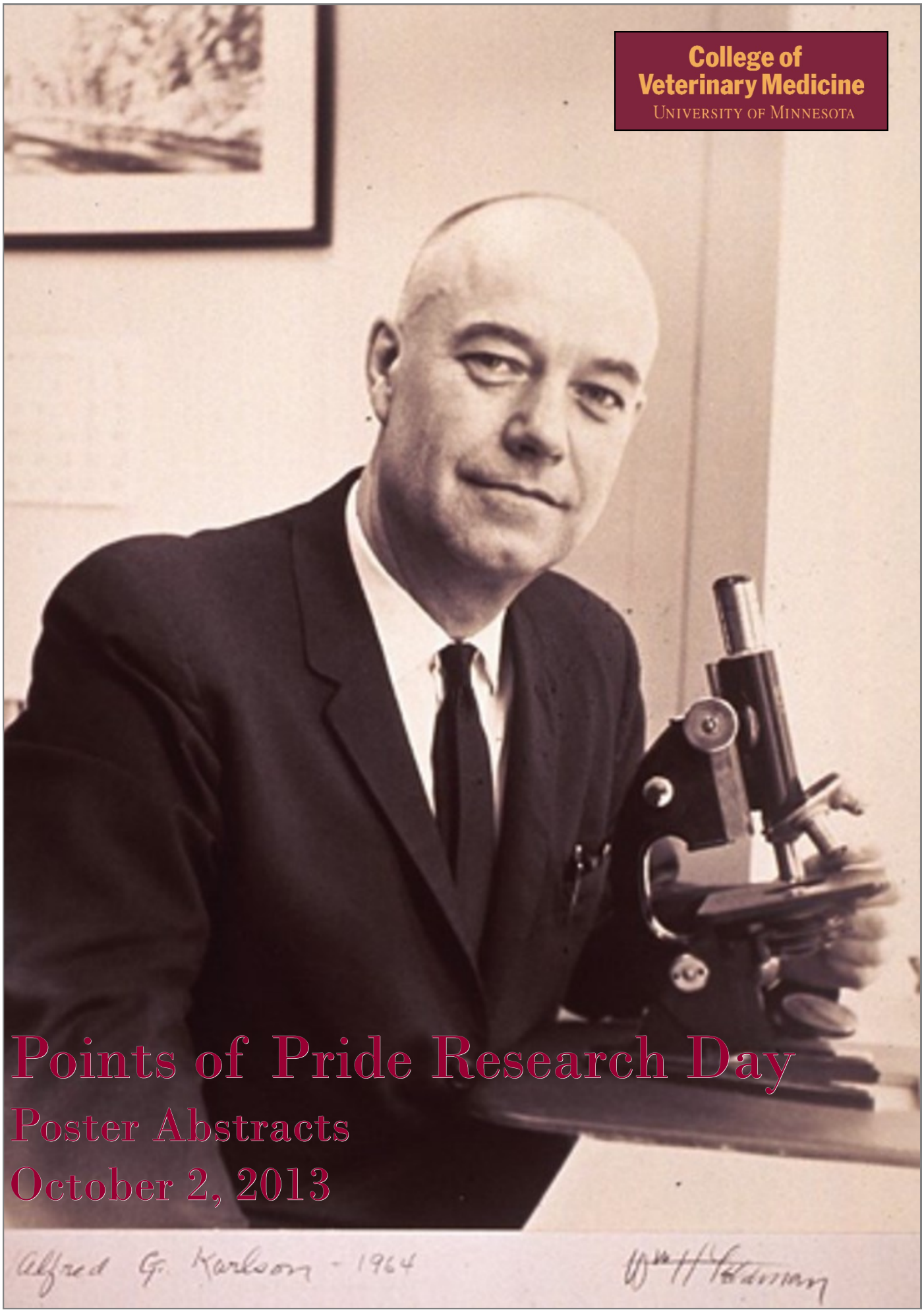
College of Veterinary Medicine
University of Minnesota




# Points of Pride Research Day

## Poster Abstracts

## October 2, 2013

*Cover Photo:*

*Dr. Alfred G. Karlson, who in 1942 earned the first PhD awarded by the College of Veterinary Medicine. Dr. Karlson, DVM, PhD, was a prominent microbiologist who excelled in understanding the biology and treatment of zoonotic Mycobacteria infections, and pioneered pasteurization to control M. bovis transmission to humans.*

# *Comparative Medicine Signature Program*

CM-1

## Muscle pain may be linked to thermoregulation as indicated by correlations between musculoskeletal hyperalgesia and body temperature in mice after daily forced swims at 26°C or 41°C

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Acute stress typically causes a transient antinociceptive effect in acute assays of pain. However, we found that an acute 15-min forced swim produced a transient (30 min) musculoskeletal hyperalgesia in mice as measured using the grip force assay and validated by its sensitivity to morphine. After the swim, the core body temperature of mice was influenced by the temperature of the water bath, i.e., their temperatures dropped transiently (30 min) below their basal temperature after a cold swim (26°C) but increased after a warm swim (41°C). Adaptation to this effect occurred over time as indicated by the smaller changes in body temperature after each subsequent swim. Therefore we investigated the relationship between thermoregulation (ability to resist changes in body temperature due to exposure to warm or cold ambient temperatures) and swim-induced musculoskeletal hyperalgesia over 15 daily swims. Prior to the forced swim, grip force responses did not correlate with the body temperature of mice. On day one of a warm swim, there was a strong positive correlation between thermoregulation and musculoskeletal hyperalgesia, i.e., the better mice were at defending against increases in body temperature, the lower the grip force response. On subsequent days the correlation was lost. In contrast, there was no correlation between thermoregulation and hyperalgesia after the first cold swim. Instead, body temperatures correlated negatively with grip force after the 15th daily swim such that the better mice defended against decreases in their body temperature, the greater the decrease in grip force responses. Together, these data suggest that thermoregulation is associated with modulation of nociception. Specifically, it is not the change in body temperature in response to ambient temperature challenges that is linked to hyperalgesia, rather it is the ability to defend against a change in body temperature - whether warm or cold - that correlates with enhanced musculoskeletal nociception.

CM-2

## Understanding the Behavior and Ecology of the Red-shanked Douc (*Pygathrix nemaeus*) in Central Vietnam: Notes from the Field

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The red-shanked douc (*Pygathrix nemaeus*) is an endangered species of Asian colobine, which inhabits east-central Lao PDR, central Vietnam, and northeast Cambodia. Colobines are folivorous Old World monkeys, that are anatomically, physiologically, and ecologically unique amongst the living primates. They possess specialized gastrointestinal (GI) systems similar to ruminants, including a multi-chambered stomach, allowing for the digestion and utilization of extremely high fiber diets. The complex gut and GI-associated microflora of colobine primates is thought to enable neutralization of digestive inhibitors and potential toxins present in plant materials, which constitute the majority of their natural diet. The purpose of this study was to better understand the behavior and ecology of the red-shanked douc, for which little is known. Specifically, we employed the following objectives: 1) Record feeding and non-feeding behaviors of wild red-shanked doucs and collect fecal and feed samples for laboratory analysis, 2) Establish a community-based conservation program in Danang City, Vietnam to protect the biodiversity of Son Tra Nature Reserve, and 3) Understand the cause and effect relationships between red-shanked douc diet composition and the gut microbiota. Presently, we have completed objective 1 and are currently in the process of completing objectives 2 and 3. Over the past year, Jonathan Clayton traveled to Vietnam where himself and his collaborators established the research site, surveyed the research area for red-shanked douc abundance, established two semi-habituated study groups, collected 130 hours of behavioral data via focal sampling, and collected 66 plant samples and 76 fecal samples for laboratory analysis. A successful outcome is translation of these findings into a comprehensive understanding of red-shanked douc behavior and ecology, identification of microbial biomarkers of douc nutritional health, and a scientific research model to study the gut microbial component of primate evolution.

CM-3

## MiR-708-5p regulation of CD38 expression in human airway smooth muscle cells

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The cell-surface protein CD38, through its enzymatic activity, generates cyclic ADP-ribose (cADPR) from β-NAD. Exposure of human airway smooth muscle (HASM) cells to TNF-alpha, an inflammatory cytokine, augments CD38 expression and cADPR-mediated calcium release during stimulation by spasmogens. The transcriptional regulation of CD38 expression in HASM cells involves activation of NF-kB, AP-1, MAP kinases and PI3 kinases, while the post-transcriptional regulation involves specific microRNAs. In this study, we investigated the role of miR-708-5p on CD38 expression in growth-arrested HASM cells. Web-based algorithms predict miR-708-5p binding sites within the 3'Untranslated Region (3'UTR) of CD38. Predicted binding sites for miR-708-5p were also found within the 3'UTR of MAP kinase phosphatase-1(MKP-1) and PTEN, a phosphatase that regulates PI3 Kinase signaling through Akt activation. HASM cells obtained from asthmatic (AS-HASM) and non-asthmatic (NA-HASM) donors were used and stimulated with TNF-alpha. Cells were transiently transfected with miR-708-5p mimic or control oligonucleotides. CD38 transcript (qRTPCR), CD38 enzyme activity (reverse cyclase), expression levels of MKP-1 & PTEN (western blot) and activation levels of MAPK & Akt (western blot) were measured. MiR-708-5p expression decreased in NA-HASM cells and elevated in AS-HASM cells on exposure to TNF-alpha. Transfection of HASM cells with miR-708-5p mimic, caused a concentration-dependent inhibition of CD38 expression, but had no effect on CD38 transcript stability. Such transfection also resulted in increased PTEN and MKP-1 expression with decreased Akt and JNK MAP kinase activation and also showed decreased Akt2 expression. Luciferase reporter assays with mutated target site revealed specificity in binding of miR-708-5p to 3'UTR of CD38. The results demonstrate that miR-708-5p regulates CD38 expression in HASM cells post transcriptionally by direct binding to 3'UTR to cause inhibition of translation and transcriptionally by decreasing the activation of JNK MAP kinase and Akt.

CM-4

## Unexpected selective sensory neuronopathy following virus mediated gene transfer

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Adeno-associated virus (AAV) vectors have emerged as powerful vehicles for genetic manipulation of primary sensory neurons, mediating efficient, stable, and nontoxic transduction. Differential targeting of subtypes of dorsal root ganglia neurons by AAV serotype 5 and AAV serotype 8 following intrathecal delivery by direct lumbar puncture has been demonstrated. Our goal in this study was to characterize the expression pattern of AAV serotype 9 -mediated gene transfer to sensory neurons by quantitative analysis of the neuronal subtypes expressing green fluorescent protein (GFP) after transduction. While successful sensory neuron transduction was evident with fluorescence microscopy, histological evaluation of affected dorsal root ganglia also revealed unexpected selective sensory neuron degeneration, loss, and replacement with aggregates of mononuclear cells. Here we present preliminary findings on the extent, time course, and dose relationship of apparent sensory neurotoxicity and AAV serotype 9.

CM-5

## Gene therapy: a new approach for preventing calcium oxalate stones

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Calcium oxalate urolithiasis is an important disease in companion animals and remains challenging for veterinarians to manage, because the precise etiological cascade of events leading to stone formation is unknown. Medical therapy to dissolve CaOx uroliths is currently unavailable. Therapies are unsuccessful and uroliths are commonly associated with lower urinary tract discomfort and potential life-threatening urethral obstruction. Hyperoxaluria, due to increased urine CaOx saturation, is an important risk factor for CaOx stone formation, since mammals are incapable of metabolizing oxalate. There are four major classes of enzymes and related proteins, found primarily in plants, fungi and bacteria, able to degrade oxalate. They are oxalate oxidase, oxalate decarboxylase (OXDC), oxalyl CoA decarboxylase and oxalate oxidoreductase. Our research goal is to develop safe and effective treatments to prevent stone recurrence, evaluating various approaches for gene therapy and their feasibility in a cell culture model system. The objective of this study is to evaluate oxalate-degrading enzyme gene expression and activity in a feline kidney cell line, in order to identify potential candidates for future gene therapy applications in dogs and cats. Our hypothesis is that kidney cells (Crandell-Rees Feline Kidney-CRFK) will stably degrade oxalate in vitro by expressing and secreting a functional oxalate-degrading enzyme into the media of transfected cells. We have cloned OXDC from *Bacillus subtilis* and *Flammulina velutipes*, that were grown, RNA extracted, cDNA synthesized and ORF fragments cloned into two mammalian expression vectors, pSG9M and pSECTAG, by infusion cloning method. We have optimized transfection methods for this cell line and expect to have expressed and secreted one or more oxalate-degrading enzymes in CRFK cell cultures that will stably degrade oxalate. A successful outcome will directly benefit companion animals that suffer recurrent CaOx stone attacks that are refractory to current therapies and, after further refinement, it may become a front-line option.

CM-6

## Shivers in Belgian horses: GWAS and whole genome sequencing

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Shivers is a common movement disorder that occurs in Draft, Warmblood and Thoroughbred breeds that distinctively affects backwards walking while forward gaits are normal. Horses, particularly tall males, present with abduction, hyperflexion and muscle tremors in the pelvic limbs during backwards walking. A genome-wide association analysis was performed across 54,000 single nucleotide polymorphisms (SNPs) on 51 affected (27 male, 24 female) and 98 control (40 male, 58 female) Belgian Draft horses using the Illumina Equine54KSNP platform. Genomic inflation was minimal ($\lambda GC=1.08$). Logistic regression was performed with sex as a covariate and an association signal was found between Shivers phenotype and a region on ECA8 spanning 166 kB that included four SNPs (highest $p_{unadjusted}$ 2.53 x 10-5; $p_{genome}$ 0.10 following 10,000 permutations). We then utilized next-generation sequencing (NGS) to further characterize polymorphisms within this region of ECA8 in our populations. Four male Belgians (2 Shivers-affected and 2 age-matched controls) were sequenced on the Illumina HiSeq 2000 using 100 bp paired-end reads (12x coverage). An average of 142 million reads/horse was obtained (10.5x coverage) with an average quality Q score of 30. Trimming was performed based on quality score and reads were mapped to the equine reference genome using BWA with an average of 96% properly paired. Variants (SNPs, indels) were called with Unified Genotyper within the genome analysis tool kit (GATK) package after locally realigning, removing duplicates and recalibrating base quality. An average of 5.9 million variants (SNPs and indels) were identified genome-wide, with 1,956 variants identified within the candidate region of ECA8. Of the genome-wide variants, 65% were intergenic, 30% were intronic, 2.5% were exonic and 2.5% were 500 bp upstream or downstream of an annotated gene. Of the variants in the 166 kb candidate region of ECA8, 1,908 were intronic (97.5%), 31 (1.6%) were located in non-coding RNA (ncRNA) and 17 (0.9%) were 500 bp upstream or downstream of the ncRNA.

CM-7

## Metabolic and Genetic Determinants of Calcium Oxalate Urolithiasis in a Spontaneous Canine Model

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Kidney stone disease is a significant problem affecting roughly 10% of the population. The majority of kidney stones are composed of calcium oxalate (CaOx), and idiopathic hypercalciuria (IH) is a common abnormality underlying stone risk. Both CaOx stones and IH are known to have high heritability (~50%). However, the complexity of the disease has made it difficult to identify susceptibility genes, and the pathogenesis of stone formation remains poorly understood. Novel research approaches are needed to elucidate genetic risk factors for this painful condition. Dogs offer a naturally occurring animal model of CaOx stones with a strong heritable component to disease. Our research characterizes metabolic factors associated with stone risk in dogs and uses this natural animal model to ascertain susceptibility genes for CaOx urolithiasis and IH.

The first objective of this study was to compare serum calcium and urinary metabolites (calcium and oxalate) between breed-matched CaOx case and control dogs in three canine breeds: the Miniature Schnauzer (MS), Bichon Frise (BF), and Shih Tzu (ST). We hypothesized that IH underlies stone risk in all three breeds. The second objective was to perform a genome wide association study (GWAS) to identify genetic determinants of CaOx urolithiasis in the Miniature Schnauzer. We hypothesized that we would locate the chromosomal segment containing susceptibility gene(s) and identify a causative mutation within this region.

Urinary metabolite data was obtained from 43 MS, 28 BF, and 13 ST dogs. Compared to breed-matched controls, MS and BF cases had higher serum calcium ($p<0.05$), and cases of all three breeds had higher urinary calcium levels ($p<0.005$). Urinary oxalate levels were not different. The GWAS included 62 case and 42 control MS dogs. A strong signal was present on chromosome 37 (p-raw = 8x10-6, p-genome = 0.01). Haplotype analysis identified a critical region that contains 18 protein-coding genes. Next-generation sequencing of two cases and two controls was performed, and analysis of discovered variants in currently underway.

CM-8

## Heparan sulfates regulate allergen-induced airway remodeling

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Heparan sulfates (HS) participate in many aspects of inflammation by virtue of their ability to bind to growth factors, chemokines, interleukins and extracellular matrix proteins. We have previously shown that endothelial-expressed HS promote allergic inflammation by regulating recruitment of inflammatory cells to the airways through leukocyte-vascular endothelium interactions. Here, we investigated the role of HS in airway remodeling, a characteristic feature of chronic asthma, in a murine model of allergen (ovalbumin [OVA])-induced airway inflammation. Mice deficient in endothelial and leukocyte expressed N-deacetylase/N-sulfotransferase-1 (Ndst1), a key enzyme involved in the biosynthesis of HS chains, and wild type (WT) mice were exposed to chronic intranasal challenge with OVA for 8 weeks and evaluated for the development of airway remodeling. Chronic OVA-challenged WT mice exhibited airway eosinophilia along with mucus hypersecretion, peribronchial fibrosis, increased smooth muscle mass and moderate peribronchial angiogenesis. In contrast, OVA-challenged Ndst1-deficient mice demonstrated significantly reduced eosinophil and macrophage infiltration in bronchoalveolar lavage fluid (BALF) and lung tissue along with attenuated airway mucus accumulation and peribronchial fibrosis. Further, a trend towards decreased airway smooth muscle mass and peribronchial angiogenesis was noted. Ndst-1 deficiency also resulted in decreased lung Th2 cytokines IL-4 and IL-13 as well as macrophage expression of remodeling-associated mediators such as TGF-β1 and FGF-2 in lung tissue. The ability of bone marrow progenitors from Ndst1 deficient mice to form fibroblasts in the presence of lineage-specific mediators in colony forming unit assays (CFU-F) was significantly compromised compared to WT cells. In addition to its role in promoting inflammatory cell recruitment, these studies suggest that HS contribute to the development of asthma-related airway remodeling most likely due to expression/retention of pro-fibrotic factors such as TGF-β1 and FGF-2 in the lung.

CM-9

## Vaccination with autologous glioma lysate induces tumor-reactive IgG antibody response and increases survival indogs with glioma.

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Gliomas are invasive primary brain tumors for which there is currently no cure or very effective therapy. In spite of widespread and intensive ongoing investigations for improved glioma treatments, there is disappointingly little progress in translational medicine. Pet dogs can serve as an outstanding comparative animal model for study of novel therapies because they develop spontaneous brain tumors that faithfully recapitulate human disease. We have undertaken a clinical trial using immunotherapy with autologous glioma cell lysate vaccines, combined with the TLR9 agonist (CpG oligodeoxynucleotides) to treat canine patients with glioma. The objective of this trial is to induce specific anti-tumor immunity directed at residual tumor and to reduce recurrent tumor after resection. Treatment groups consist of surgery and vaccination with or without gene therapy (intracranial injection of ad-INF-γ vector). Dogs treated with palliative therapy alone (historical controls) or with surgery and chemotherapy (temozolomide) served as control groups. No severe toxicities have been observed, validating the safety of the treatment. Our preliminary results demonstrate the ability of this vaccine to activate specific humoral antitumor responses with concurrent extension of survival times in the treated dogs compared to controls. Tumor-reactive IgG antibody responses were detected post vaccination in all dogs. Examination of immune responses in therapeutic trials of dogs with glioma can greatly aid in predicting outcomes of similar treatments in humans. Data from these trials also provide an opportunity to accelerate the development of more efficient immunotherapeutic strategies for treatment of intracranial malignances.

CM-10

## Gene Expression Profiling Reveals a Role for CXCR4/7 in Canine Hemangiosarcoma

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Hemangiosarcoma (HSA) is a common fatal cancer in dogs. We have shown that HSAs are organized hierarchically with a population of putative "cancer stem cells" at the apex. We can enrich these cells in culture by culturing them in defined serum-free media as non-adherent spheres (hemangiospheres). CXCR4 and CXCR7 are chemokine receptors. In normal bone marrow-derived cells, these receptors are necessary for hematopoiesis and regulation of the differentiation of multipotent progenitors. CXCR4 also appears to be necessary to sustain bone marrow stromal cells, a property that may be co-opted by tumors to establish a favorable microenvironment. We analyzed expression of CXCR4 and CXCR7 in a panel of HSA tumor samples as well as in HSA cell lines maintained under conventional monolayer culture conditions or grown as hemangiospheres. The expression of both receptors was variable among HSA tumor samples, but it was consistent across RNA-seq and microarray platforms. The expression of both genes in cell lines grown as hemangiospheres was higher than in the corresponding cells grown as monolayers, and CXCR4 protein levels also were predictably higher in the hemangiospheres. Ingenuity Pathway Analysis revealed activated biological functions of cellular movement and immune cell trafficking as well as attenuated cell proliferation in the hemangiospheres. Treatment of HSA cells with AMD3100, a selective inhibitor of CXCR4 reduced the fraction of CXCR4+ cells by approximately 50%. Experiments to define the biological consequences of CXCR4 and CXCR7 inhibition in HSA cells are in progress. Together, our data suggest that CXCR4 and CXCR7 are expressed by canine HSA cells and may contribute to the pathogenesis of this disease by regulating processes such as cell migration and tumor-microenvironment interactions. Additional work will define the potential to incorporate CXCR4 and/or CXCR7 blockade in new, innovative strategies to improve treatment outcomes for this disease.

CM-11

## Validation of a non-human primate cytokine multiplex assay

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The development of multiplexing technology has allowed for the measurement of multiple analytes in a single, small volume (25µL) sample. This advantage is tempered by the need to apply simultaneous validation strategies to multiple (and potentially diverse) analytes when evaluating these methods prior to adopting them for use in research investigations. This technology is attractive for use for cytokine measurement in various matrices because individual cytokine data are frequently considered in relation to other cytokines as part of a profile of associated values. A validation strategy that allows for simultaneous evaluation of multiple analytes is required to ensure the performance of the assay is fit for purpose. In this study, a Millipore multiplex kit for the simultaneous measurement of 23 cytokine concentrations was evaluated for use in serum collected from healthy cynomolgus macaques. The validation protocol included the evaluation of precision (intra- and inter-assay) and recovery for each analyte. Pre-established acceptance criteria were set at ≤ 20% coefficient of variation (CV) for intra-assay precision, ≤ 25% CV for inter-assay precision and 75-125% expected recovery rates. Analytes were categorized as accepted, marginal or rejected, based on these performance criteria. Of the 23 cytokines tested, 4 were accepted, 12 were considered marginal and 7 were rejected. The results for four of these analytes are presented to illustrate the performance variability. Validation data were accepted for IL-6; IFNγ was considered marginal; and validation data were rejected for IL-12/23(p40) and TNFα. Intra-assay precision, using repeated measurement (n=15), ranged from 7-74%, inter-assay precision (n=14) ranged from 9-29%, and average recovery varied from 29-91% in these four cytokines. Recovery data suggest that this assay consistently underestimates true concentrations by 25-75% at cytokine concentrations above 200 pg/mL. This performance variability illustrates the need to evaluate the 'fitness for use' of laboratory methods prior to interpreting research data.

CM-12

## Allelic variation in the porcine IGLV genes

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Production of a vast antibody repertoire is essential for protection of animals against pathogens. Variable region germline complexity is a standard feature of mammalian immunoglobulin loci, but functional V region genes are limited in swine. For example, the porcine lambda light chain locus is composed of 23 variable (V) genes and 4 joining (J) genes, but only 13 V and 2 J genes are functional. Allelic variation in V and J might increase overall population diversity, but might lead to repertoire holes in individuals lacking key alleles. Here we investigated the germline allelic variation of the porcine immunoglobulin lambda variable (IGLV) genes. 22 V genes were amplified and sequenced from 81 pigs. We identified amino acids alleles in each gene pool and determined the functionality of each allele. IGLV3 and IGLV8 gene families contributed to the majority of allelic variation. Some genes that were previously identified as functional contained non-functional alleles due to frameshifts and stop codons. Also, we discovered three recombination hotspots and related recombination motifs. These recombination hotspots had breakpoints located in framework 3 (FR3) and shifted complementarity determining regions 3 (CDR3s). Interestingly, the conserved recombination sequence motif differed by only 1-bp compared to humans, which has been intensively studied as a recombination-associated motif. This is the first report of the recombination hotspots in the porcine antibody repertoire. Taken together, we characterized extensive allelic variation in the porcine IGLV, showing the potential for substantial variation in the ability of individual animals to response to specific antigenic challenges. The discovery of porcine recombination hotspots suggests that non-allelic recombination is an alternative mechanism to generate germline diversity.

CM-13

## The effect of tibial plateau angle on cranial cruciate ligament strain: A ex vivo study in the dog.

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Introduction: Changing the tibial plateau angle (TPA) is commonly used for the treatment of dogs with complete rupture of the cranial cruciate ligament (CCL). Changing the TPA has been proposed for treatment of a partial rupture of the CCL. It has been suggested that the CCL has the ability to heal and prophylactic TPA techniques for dogs that predisposed to disease of the CCL may be protective. The objective of this study was to evaluate the relationship between TPA and strain in the intact CCL during axial loading.

Materials and Methods: Six, adult canine cadaveric knee specimens were collected. A bi-radial saw was used to perform the osteotomy and a custom designed plate was secured to the leg. Each knee was loaded and CCL strain and axial displacement were recorded. TPA was adjusted to -20, -10, 0, +10, +20 degrees of normal. Change in strain was assessed during the axial loading period.

Results: For all specimens linear displacement of the femur and CCL strain increased with increasing axial load. Mean change in strain was 4.41, 5.26, 6.02, 6.3 and 7.39 at -20°, -10°, 0°, 10° and 20°, respectively. The R-squared for the linear regression equation was 0.91 suggesting a predictable relationship between change in TPA and CCL strain.

Conclusion: The mechanical testing model used found an expected relationship between axial load, tibial translation and CCL strain. CCL strain increased with increasing axial load regardless of the TPA.

Acknowledgement: Supported by the VMC Small Companion Animal grant, University of Minnesota

CM-14

## Descriptive analysis of canine cacium oxalate, struvite and purine urolithiasis submitted to the Minnesota Urolith Center between 2009-2012

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The Minnesota Urolith Center (MUC) receives uroliths from around the world for quantitative mineral analysis. Calcium oxalate (CaOx), struvite and purine uroliths are the most common urolith types retrieved from dogs. The objectives of this study were to evaluate the descriptive information of the data from MUC between 2009-2012 and to investigate the percent change in submissions by year. The descriptive analysis was performed by analyzing the submission data of CaOx, struvite and purine uroliths between years 2009-2012 (n=174,076). Submission percentage, percent change of submission by year, age and gender were analyzed. CaOx was the most predominant urolith type in dogs (49.01%) followed by struvite (45.89%) and purine (5.10%). CaOx was more common in male dogs (M:F ratio = 3.59:1) and significantly older (8.46+0.01 years) dogs compared to other stone types. Struvite was overrepresented in females (F:M ratio = 6.42:1) and common in middle age dogs (6.17+0.01 years). Purine was predominant in males (M:F ratio = 4.65:1) and more common in younger dogs (5.48+0.03 years) and highly prevalent in dogs younger than 1 year. Between 2009-2012, the percentage of submissions for all stone types increased each year. However, percent change of submissions for each urolith type was variable. The percent of CaOx submissions dramatically increased in 2010 (14.09%) and then decreased (8.43% in 2011 and 5.5% in 2012).The percent of struvite submissions increased by 12.2% between 2009-2010 and then decreased to 7.55% in 2011 and slightly increased in 2012 (7.67%). Purine percent change dramatically increased between 2010-2011 (1.05% in 2010 to 5.73% in 2011) and decreased slightly in 2012 (4.35%). Explanations of the decrease of the submissions of CaOx in each year and increased rate of struvite submissions recently require further evaluation. If the rate of struvite submissions continues to increase surpassing CaOx, it would necessitate a change in urolith preventive measures from dietary control to control of urinary tract infections.

CM-15

## Disrupting CD16 shedding to augment the antitumor response of leukocytes

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Targeted immunotherapy with monoclonal antibodies (mAbs) has become critical for the successful treatment of many forms of cancer. Antibody-dependent cell cytotoxicity (ADCC) by Natural Killer (NK) cells is a major mechanism in the anti-cancer effects of mAbs. Interest in neutrophils recognizing tumor bound mAbs has increased as well, in part, because these leukocytes perform several effector functions dependent on antibodies, including ADCC. Therapeutic mAbs are recognized by Fc gamma receptors (FcγR) expressed by leukocytes. FcγRIII (CD16) exists in two isoforms encoded by two highly homologous genes. FcγRIIIa (CD16a) is a transmembrane protein expressed by NK cells that is critical for ADCC. FcγRIIIb (CD16b) is a GPI-linked protein expressed exclusively by neutrophils. Upon cell activation, both forms of CD16 undergo ectodomain shedding from the cell surface, resulting in their rapid down-regulation in expression, which is associated with impaired antibody recognition. We demonstrate for the first time that the membrane-associated metalloprotease ADAM17 plays a critical role in cleaving CD16a and CD16b upon cell activation. We hypothesize that blocking CD16 shedding will enhance the anti -cancer activity of neutrophils and NK cells in the presence of therapeutic mAbs. Using mass spec on cleaved CD16a and CD16b from activated NK cells and neutrophils, respectively, we have determined the ADAM17 cleavage site in CD16. Using site-directed mutagenesis to exchange amino acids at the cleavage site, we have disrupted CD16a and CD16b shedding. Non-cleavable CD16a has been expressed in a NK cell line and their ability to mediate ADCC is being investigated. Our objective is to eventually express non-cleavable CD16 in genetically modified leukocytes derived from human pluripotent stem cells to enhance their performance in combination with therapeutic mAbs.

CM-16

## Germ-line risk factors are associated with upregulation of genes mediating cell cycle arrest and stem cell activity in canine hemangiosarcoma

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A recent genome wide association study (GWAS) in dogs with and without hemangiosarcoma (HSA) identified two germ-line regions that were associated with risk for this disease. Independently, gene expression profiling showed activation of genes associated with multipotency in canine HSA tumors. We examined the relationship between these two findings using two gene expression data sets. One included 22 HSA samples that had high-risk (n=8) or low-risk (n=14) genotypes at one locus identified in the GWAS, analyzed using next generation RNA sequencing (20 M paired end reads). The other included HSA cell lines that were maintained in conventional culture conditions (n=12) or grown as free-floating spheres in the absence of serum (a condition which supports enrichment of cancer stem cells, n=6), analyzed using Agilent 4x44K microarrays. The GWAS risk groups showed differential expression of 116 genes ($p<0.05$, >2-fold change), however statistical significance was not observed with multiple testing corrections, suggesting analysis was underpowered. Because the genes with lowest unadjusted p-values seemed to have a common biological signature as cancer drivers, we used Ingenuity Pathway Analysis to define molecular and cellular pathways specifically enriched in this set of genes and potential upstream transcriptional regulators. We observed preferential activation of the RB and p53 checkpoint control pathways in the high-risk group, whereas CylA and the E2F family were inhibited. Similar, significant activation of the RB and p53 pathways was seen in HSA sphere cultures. The data suggest that the RB and p53 pathways remain intact in canine HSA. Tumors from dogs that carry greater inborn risk are potentially enriched for cells that engage these pathways and maintain gene expression profiles consistent with cancer stem cells. Additional work to determine the significance of these findings in relation to disease progression and outcome are ongoing.

CM-17

## Mast cells in the CNS modulate a variety of nociceptive modalities in mice

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Mast cells contribute to maintaining a heightened sensation of pain, a protective mechanism, however, many conditions lead to a chronically elevated pain that serves no additional protective purpose. Because stress hormones activate mast cells and increase many types of pain, and because mast cells are capable of promoting pain, we hypothesized that mast cells located in brain might similarly regulate pain intensity. Although there is a relationship between migration of mast cells to brain and increased pain, the present study aimed to show a cause and effect relationship. Using mice, we found that pain, as indicated by the animals' behavioral response, correlated with activity of mast cells in the thalamus, but only when pain is elevated. Drugs that stabilize mast cells also inhibited heightened pain. For example, the intensity of behavioral responses to CHEMICAL PAIN correlated positively with thalamic mast cells activity. When the mast cell stabilizer cromolyn was injected centrally, it inhibited the number of behavioral responses to chemical pain. TACTILE SENSITIVITY, determined by withdrawal of the paw from being poked by a small fiber, was enhanced by superficial injection of solutions that cause arthritic inflammation in the hind paw. Cromolyn injected centrally inhibited this behavior. In a similar fashion, tactile sensitivity was also increased by injections of compounds known to enhance mast cell activity. Enhanced activity correlated with tactile sensitivity of mice and central injection of cromolyn inhibited these behaviors. MUSCULOSKELETAL pain, as indicated by a decreased ability to hold onto a bar, was increased by central injections of a compound that promotes mast cell activity and by intramuscular injections of carrageenan, an inflammatory compound. Central injection of cromolyn inhibited effects of carrageenan. The present data indicate that enhanced chemical, tactile and muscle pain responses seem to depend on the activity of mast cells specifically in the brain.

CM-18

## Whole-genome approaches to identification of genetic risk factors for osteochondrosis

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Osteochondrosis (OC) is a disease characterized by a failure of normal cartilage development at the end of long bones (in the legs) and vertebrae (in the neck and back). OC affects large numbers of young horses across breeds, with prevalence estimates greater than 60% in some radiographic surveys of yearling performance horses. While the precise cause of OC is unknown, both environmental and genetic risk factors are known to play a role in disease development. However, although it is estimated that up to 50% of disease risk is due to genetics, specific genes and alleles underlying OC risk in the horse are completely unknown. We hypothesized that one or more genes of major to moderate effect underlie OC susceptibility in horses, and further, that these risk loci are shared across breeds.

Initially, a genome-wide association study (GWAS) was performed in GEMMA using data from 45,703 single nucleotide polymorphisms (SNPs) in 162 Standardbred horses (60 affected with OC, 102 unaffected) born and raised on a single breeding farm. Relatedness within the population was accounted for using a genotype-based relationship matrix. Regions of association with OC were identified on equine chromosomes (ECA) 2, 14, and 15 ($p < 9 \times 10^{-5}$). To follow up on these regions of association, 18 individuals (9 affected, 9 unaffected) were selected for whole-genome sequencing. Quality control, sequence alignment, and variant discovery were carried out using the University of Minnesota Supercomputing Institute's Galaxy platform. After initial quality control, 103,785 simple variants were identified within the regions of interest on ECA2, 14, and 15. Of these, 19,211 variants (18.5%) were predicted to occur within or immediately upstream/downstream of an annotated gene. Only a few variants with predicted functional effect also segregated with OC status. A repeat GWAS with 20 additional horses strongly supported the region of association on ECA14; thus, the 4 segregating variants of predicted functional effect in this region will be prioritized for follow-up in a larger population of horses.

CM-19

## Regulation of CXCR2 expression and neutrophil recruitment by ADAM17

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The CXC chemokine receptor 2 (CXCR2) has a critical role in directing neutrophil migration out of blood vessels at sites of inflammation. Excessive inflammation, however, results in impaired neutrophil recruitment and host defense, which is associated with decreased surface levels of CXCR2. Cell surface levels of CXCR2 are rapidly down-regulated by internalization upon ligand binding and proteolytic cleavage following neutrophil activation. For the latter process, little is known about the primary protease involved and its significance in regulating neutrophil migration. We report that the metalloprotease a disintegrin and metalloprotease 17 (ADAM17) cleaves cell surface CXCR2 on neutrophils in mice and humans, which was determined using a selective ADAM17 inhibitor, a unique ADAM17 function blocking mAb, and conditional ADAM17 knockout mice. During pronounced inflammation, circulating ADAM17-null neutrophils maintained higher levels of CXCR2, and their infiltration into the inflammatory locus was significantly increased. A CXCR2 inhibitor was found to block the enhanced recruitment of neutrophils in conditional ADAM17 knock-out mice, implicating CXCR2 cleavage in controlling neutrophil recruitment. Taken together, our data demonstrates that ADAM17 contributes in regulating the surface levels of CXCR2 and neutrophil recruitment, suggesting that the protease might provide a therapeutic target to diminish neutrophil dysfunction during excessive inflammation.

CM-20

## Genome wide association study for equine exertional rhabdomyolysis in Standardbred horses

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Exertional rhabdomyolysis (ER) is a clinical syndrome characterized by muscle pain, stiffness and cramping with exercise. Recurrent exertional rhabdomyolysis (RER), a form of ER most commonly seen in Thoroughbred (TB) and Standardbred (STDB) racehorses, is thought to have an underlying genetic basis. Recent work by our group has suggested that genetic risk loci for RER in the TB are not shared with the STDB. The objective of this project was to identify genomic regions contributing to RER risk in STDB horses by performing a genome-wide association study (GWAS) in a cohort of 148 individuals (89 cases and 59 controls). RER cases were defined as horses with a history of one or more episodes of ER identified by the trainer and/or referring veterinarian. Controls were defined as individuals without documented episodes of ER. All horses were genotyped with either a 54,602 or a 65,153 single nucleotide polymorphism (SNP) marker genotyping array. Only 45,321 markers are shared by the arrays, therefore genotype data from the RER cohort was combined with data from 323 additional STDB horses for genotype imputation. Following quality control and data pruning in PLINK (Hardy-Weinberg equilibrium, SNP and individual missingness rates, discordant sex information or abnormally high heterozygosity), imputation and haplotype phasing was performed using BEAGLE, yielding a total of 74,595 SNP genotypes. To control for spurious association due to relatedness in the RER cohort or confounding factors such as gender, gait and age, a relationship-matrix constructed from identity-by-descent estimation and these covariates were included in a mixed logistic regression model using Genome-wide Efficient Mixed Model Analysis (GEMMA) software. A significant association was noted across a 583.9 kb region on chromosome 14 (ECA14), with p-values from 2.58e-4 to 2.71e-6. Haplotypes phasing and haplotypic association on ECA14 are underway to allow for identification of individuals for whole genome sequencing and variant discovery.

CM-21

## M2 macrophages stimulate neural stem/progenitor cell proliferation via a Wnt 5a dependent pathway: Implications for viral encephalitis

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Activation of macrophages and microglia is a critical component of the host response following brain damage. Previous studies in our laboratory demonstrated that HSV-1 brain infection stimulates neural stem/progenitor cell (NSC) proliferation between 3 and 6 d p.i., concurrent with macrophage infiltration. In the present study, we examined the role of macrophage activation phenotypes on NSC proliferation. Evaluation of infiltrating macrophages [CD45(hi)CD11b(+)] revealed that 68.6±3.3% of the cells were Ly6C(hi) at 5 d p.i. Expression of an M2 marker, CD206, was five-fold higher than CD86, an M1 phenotype marker, in Ly6C(hi) macrophages at 5 d p.i, indicative of an alternative activation phenotype. To determine if macrophage polarization modulated neurogenesis, NSCs were cultured with supernatants from M1 or M2 polarized bone-marrow derived macrophages. NSC cultures treated with M2 conditioned media (M2CM) had 4-fold more proliferating cells compared to those cultured in control media. Cells in all treatment groups maintained their stem cell phenotype [≥95% nestin(+)] at collection. M2CM treated NSCs continued to increase in number at 96 h post-treatment with significantly higher numbers of cells in the G2/M phase of cell division. Transplantation of M2 macrophages into the lateral ventricles of uninfected mice resulted in a 15% increase in Sox2(+) NSCs proliferation and the total number of Sox2(+) NSCs doubled compared to saline or heat-killed cell controls, at 5 d post-treatment. Interestingly, Wnt5a expression was significantly increased in M2 polarized macrophages and treatment with either dickkopf-1, a Wnt5a inhibitor, or Wnt5a neutralizing antibody suppressed NSC proliferation, to control levels. Finally, Wnt5a gene expression in HSV -1 infected brains increased at 3 d p.i., coinciding with the increase in neurogenesis and M2 macrophage infiltration into the brain. Results from these studies suggest that M2 macrophages promote neurogenesis following herpes virus -induced brain damage and may provide an avenue for therapeutic intervention.

CM-22

## Gut barrier defense systems are compromised following infection with EcoHIV and exacerbated with morphine: a novel murine model of HIV and drug abuse

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Compromised gut barrier function, which is exacerbated by opiates, is believed to be integral for early pathogenesis of HIV; however no mouse infection model currently exists to study epithelial and immune functional changes in the gut. EcoHIV was developed to simulate HIV pathogenesis by genetically altering HIV to infect mouse cells by substituting gp80 from Murine Leukemia Virus for gp120 of HIV. We show that chronic morphine in EcoHIV treated mice additively enhances bacterial translocation from the gut to systemic tissues beyond what is seen in either treatment alone; this replicates human studies which implicate LPS in serum as a marker for translocation. Epithelial cells are a major physical barrier against bacteria and we observed that tight junctions are modulated severely in combined morphine and EcoHIV treated animals. Interestingly, goblet cells that normally secrete mucous decrease in count with morphine and further decline in the small intestine of EcoHIV treated animals. This likely allows the epithelial cells greater access to bacteria resulting in TLR activation. Examining the treatment effects in TLR2 or TLR4 knockout mice, we see bacterial translocation drastically reduced in all groups compared to wildtype mice. Cumulatively, these results imply morphine and EcoHIV cause a severe deficiency in the first line defenses that typically keeps bacteria outside the normally sterile body cavity and is a potential mechanism for how bacterial translocation is exacerbated in human HIV patients who abuse opiate drugs.

CM-23

## Concentrations of cytokines, growth factors, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in serum and synovial fluid following injection of autologous conditioned serum into equine osteoarthritic distal inter-phalangeal joints

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Autologous conditioned serum (ACS), also known as "IRAP", is a novel treatment for lameness attributable to osteoarthritis. ACS is processed from blood harvested from the patient. Monocytes within blood are activated when exposed to a borosilicate surface during incubation and centrifugation. The resultant "conditioned" serum is proposed to have increased levels of the anti- inflammatory cytokine: interleukin-1 receptor antagonist protein (IL-1ra). Potentially, altered levels of other indirect biomarkers (cytokines, growth factors, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs)) may also occur. The ACS product is then administered intra-articularly to the patient for treatment of inflammation associated with osteoarthritis. There are positive anecdotal reports from veterinarians regarding the therapeutic benefits of ACS in clinical practice. However, review of the literature reveals a lack of knowledge of the specific composition of ACS and its effects on the joint. This study will collect serum and synovial fluid samples from 12 horses with distal inter-phalangeal joint osteoarthritis that are subsequently treated with ACS therapy. Collected serum and synovial fluid samples will be analyzed for changes in concentration of various cytokines, growth factors, MMPs and TIMPs that are active during inflammation. The specific aims of this study are to measure: (1) cytokines, growth factors, MMPs and TIMPs concentrations in equine ACS and (2) evaluate ACS effects on cytokines, growth factors, MMPs and TIMPs in synovial fluid 7 days, 14 days, and 21 days after intra- articular administration. We hypothesize that there will be an increase in concentration of the anti-inflammatory biomarkers within the ACS product compared to control serum. We also hypothesize that we will see an increase in anti-inflammatory biomarkers in synovial fluid, compared to pro -inflammatory biomarkers.

CM-24

## cMYC transactivated miR-17-92 cluster plays a crucial role in osteosarcoma progression by targeting tumor suppressor driver genes

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Osteosarcoma is the most common primary bone malignancy affecting children, adolescents and young adults.Around 30% of patients with localized osteosarcoma and 70% of patients with metastasis will experience treatment failure within 5 years of diagnosis. Such dismal outcome has remained static for 20 years leading to an urgent need to identify novel targets and therapeutic agents that will improve overall patient survival and minimize immediate and long-term side effects of current chemotherapeutic agents. Additionally, osteosarcoma is commonly found in canines, with approximately 10,000 new cases each year. The complex biology of osteosarcoma and tumor heterogeneity makes it very difficult to identify effective new gene targets and therapeutic agents. We have found that a unifying feature of osteosarcoma is the overexpression of miR-17-92 microRNAs. We have deciphered that deregulation of the 14q32miR/cMYC network leads to overexpression of the miR-17-92 cluster in osteosarcoma. This deregulation is associated with more poor outcomes in osteosarcoma and is preserved in canine osteosarcoma suggesting a conserved disease mechanism. Additionally, our data suggest that upregulation of miR-17-92 miRNAs contributes to osteosarcoma progression by targeting potential tumor suppressor osteosarcoma driver genes such as *PTEN*. Additionally, our studies show that Minnelide - a prodrug of triptolide, inhibits cMYC expression and downregulates miR-17-92 miRNAs resulting in upregulation of several tumor suppressor driver genes including *PTEN*. Together, our data suggests that upregulation of miR-17-92 miRNAs contributes to osteosarcoma progression and Minnelide reduces tumor burden in osteosarcoma by inhibiting miR-17-92 expression.

CM-25

## Morphine treatment increases *Citrobacter rodentium* virulence and disrupts infection induced IL17a immune response in mice

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Opioids induce immunosuppression and bowel dysfunction leading to increased susceptibility to bacterial and opportunistic infections (The American Journal of the Medical Sciences 2012, 343:277). It is unclear how opioids modulate bacterial virulence and mucosal host defense against intestinal infection. *Citrobacter rodentium* is a natural mouse pathogen that models intestinal infection by enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) in humans and causes attaching and effacing (A/E) lesions and colonic hyperplasia (Journal of Clinical Microbiology 2000, 38:4343). The expression of most virulence genes in *C. rodentium* is controlled by a regulator, Ler, a member of the histone-like nucleoid structuring (H-NS) protein family (PNAS 2004, 101:3597). Our study shows that morphine treatment resulted in increased expression of the virulence factor, Ler. Furthermore, we observed increased infiltration/adherence of bacteria to the gut epithelium by fluorescence in situ hybridization (FISH) on intestinal cryostat sections. Meanwhile, *C. rodentium* intestinal infection induced IL17a expression was attenuated in morphine treated animals compared to placebo treated groups. This is the first study to demonstrate that morphine modulates virulence factor-mediated adhesion of pathogenic bacteria and induces disruption of mucosal host defense during *C. rodentium* intestinal infection in mice.

# *Emerging and Zoonotic Diseases Signature Program*

EZD-1

## Use, characterization, and potential zoonotic organisms among dogs using dog parks in the Twin Cities metropolitan area

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**Introduction:** Dog parks are becoming increasingly popular venues for dog owners. In 2010, there were an estimated 2200 dog parks in the United States. Considering the large numbers of dogs visiting these parks there are opportunities for increased dog to dog and dog to human interactions. These extensively used and popular areas can permit the spread of disease causing organisms and potential zoonoses. Our aim was to characterize dog parks, the owners, and dogs visiting Twin City metropolitan dog parks. **Methods:** Owners were surveyed to collect information regarding frequency of visits, vaccination and deworming status, and health of their dog. A site survey was done to collect information on the size, posting of rules, and presence of poop bag dispensers at the dog park. Owners were asked permission to collect dog feces for testing of *Campylobacter*, *Salmonella*, and *Escherichia coli* (*E. coli*). **Results:** 22 dog parks were visited during July and August, 2013. 47% of owners visited a dog park 5 or more times per week. 186 fecal samples were collected. 58 (53%) of 109 fecal samples were *Campylobacter* positive and 1 (.5%) of 186 samples were *Salmonella* positive. 46 (25%) of *E. coli* samples were AmpC positive and 4 (2%) samples were extended spectrum beta-lactamase (ESBL) producers. A number of these isolates were classified as multidrug resistant, harboring plasmids that confer this phenotype. Isolates from different dogs in the same dog parks harbored similar antimicrobial susceptibility profiles. **Conclusions:** Surveyed dog owners frequented metropolitan dog parks. Potential zoonotic agents and antimicrobial resistant organisms were isolated. Veterinarians need to be aware of the potential for infectious disease transmission among dogs using dog parks and the possibility of zoonotic consequence.

EZD-2

## Piloting a Tool for Assessing Public Health Risks from the Importation of Wildlife into the United States: the Case of Rodent Importation From Latin America

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An inherent risk of the wildlife trade – both formal and informal – is the risk of disease introduction and/or emergence into the United States. Numerous different proposals have aimed to prevent or control this risk by banning importation of select species, or by creating 'white lists' of species that are cleared for importation. These approaches could cause economic harm to certain private sectors, such as the pet industry, and would potentially place substantial burden on importers to provide proof of low risk for importation of individual species. There is thus a need for creation of an unbiased, scientifically-based, risk assessment tool that can be easily implemented by governmental agencies, NGOs and industry, in order to inform these critical policy and import decisions. As a feasibility study, we built a risk analysis framework following the general OIE guidelines, and applied it to a specific group of animals (rodents), a specific geographical location of origin (Latin America), and a specific outcome (risk of zoonoses entering the United States). This subset was chosen for the pilot given the lack of any health requirements for rodents coming into the United States from Latin America, and potentially the small number of traded species and zoonotic diseases to identify. This framework will be expanded to other taxa and geographic locations to ultimately inform policy.

EZD-3

## Molecular characterization of *Mycoplasma hyorhinis* field isolates

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*M. hyorhinis* associated disease has been one of the main concerns of the U.S pork industry. It appears that differences in virulence of the infecting *M. hyorhinis* strain, the host immune response, and concomitant infections may play a role on disease manifestation. There are currently no genotyping tools available for the characterization *M. hyorhinis* isolates circulating amongst swine populations. The molecular typing of *M. hyorhinis* would aid in better understanding transmission routes, in assessing sources of infection and also in evaluating interventions such as vaccination and use of antibiotics. The objective of this study was to develop and validate a multi-locus sequence typing (MLST) protocol for the characterization of *M. hyorhinis* field isolates. Thirty-nine *M. hyorhinis* field isolates together with one reference ATCC strain were utilized. The genome sequences of four *M. hyorhinis* isolates were utilized to identify potential target genes. Primers were designed with MEGA 5.2.1. PCR was carried out and agarose electrophoresis was performed on the amplified products. PCR products were bidirectionally sequenced by standard Sanger sequencing. Quality of the generated sequencing data was evaluated and sequences were aligned utilizing ClustalW and trimmed to equal sizes. Phylogenetic analysis was carried out using MEGA 5.2.1. A total of 25 genes were evaluated as potential target genes. Genes where discarded when the sequence of all 4 genomes where identical, when primers could not be designed due to high variability of the sequences, when no PCR amplification product was obtained, or when there was low quality and poor reproducibility of the amplified product. Finally, a total of 5 target genes were included in the MLST protocol: *ung*, *pdhB*, *mtlD*, p3, *p95*. Within each gene the percent informative sites ranged from 0.5% to 20%. There was evidence of variation at the nucleotide level amongst the 39 isolates tested, with varying degrees of nucleotide difference between isolates within each gene. The concatenated tree showed clustering of isolates by system.

EZD-4

## Presence of porcine circovirus type 2 antibodies and virus in finishing pigs after widespread use of PCV2 vaccination

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Porcine circovirus 2 (PCV2), one of the most economically important pathogens of pigs, is the causative agent of porcine circovirus associated disease (PCVAD). Widespread availability and use of PCV2 vaccines, starting in 2006, ameliorated PCVAD in finishing pigs so successfully that nearly all pigs in the US are currently vaccinated around weaning. Vaccination of piglets eliminates disease, decreases the serum PCV2 level, and increases production performance, but does not eliminate infection. Thus, it is possible that nearly all finishing pigs are infected with PCV2. Alternatively, widespread use of PCV2 vaccination may decrease the PCV2 viral load in pigs, thus leading to generation of PCV2-negative animals over time. The aim of this study is to examine and compare the PCV2 viral load and antibody levels in finishing pigs today, after widespread vaccination starting in 2007, to that of samples obtained in 2006, prior to vaccine. Serum samples were collected as part of the USDA NAHMS Swine 2012 study and a subset were examined for both PCV2-specific antibody and viral levels. PCV2 viral loads were similar between animals on the same farm, but between farms, viral loads varied from barely detectable to low viral levels. High viral levels were not observed in any farms, contrary to 2006 sample viral loads. PCV2 capsid-specific antibodies were present in all animals, but at lower levels than were observed in 2006. Antibodies to the PCV2 replicase protein were low, with high levels of antibodies in a few pigs. Widespread use of PCV2 vaccines has greatly decreased, but not eliminated PCV2 virus in swine herds throughout the US. PCV2 viremia today is at low or undetectable levels in finishing pigs, whereas in 2006 high levels of viremia were observed in all finishing farms in the majority of animals. PCV2-specific antibodies remain present in the majority of animals, but at lower levels than were observed previously. Thus, widespread PCV2 vaccination has decreased the PCV2 viral load in the US finishing herd, in addition to providing solid protection against PCVAD.

EZD-5

## In vitro propagation of U.S. strains of porcine kobuvirus

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Enteric disease is an important cause of morbidity and mortality among domestic pigs. The porcine kobuvirus was first identified in Hungary in 2007. Since then, the virus has been reported from both healthy and diarrheic pigs in Hungary, China, Thailand, Japan, Korea, and the USA. The cultivation of porcine kobuvirus in vitro has not been yet reported. The aim of this study was to determine if porcine kobuvirus can be grown in vitro using three different cell types as well as to develop a serum neutralization test for detecting antibodies against this virus in infected pigs. In a previous study, porcine kobuvirus was detected by RT-PCR in intestinal contents from diarrheic piglets submitted to the Minnesota Veterinary Diagnostic Laboratory (MVDL) from 15 different states. The virus was also found in apparently healthy pigs from Minnesota. In the current study, four samples from healthy pigs and six from diseased pigs (that were positive for porcine kobuvirus by RT-PCR) were grown in three different cell lines e.g., PK-15 (porcine kidney), porcine alveolar macrophages (PAM), and Vero (African green monkey kidney) cells. The virus was successfully isolated from five samples using PK-15 cells. Two samples were from apparently healthy pigs and three from diarrheic pigs. The presence of virus was confirmed by RT-PCR and electron microscopy. Using the newly isolated virus, we developed a serum neutralization test to detect antibody against porcine kobuvirus. This study shows that PK-15 cells are most susceptible for the propagation of porcine kobuvirus. The newly developed serum neutralization should be helpful in conducting seroprevalence studies in swine.

EZD-6

## Over-expression of *Staphylococcus aureus* YhcR Results in Greater Staphopain B Expression and Survival in Human Blood

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Two component systems (TCS) are utilized by bacteria to sense and adapt to their environment and play an important part in gene expression regulation. The two-component system *yhcSR* is essential in the human and animal pathogen *Staphylococcus aureus.* YhcS is redox responsive and phosphorylates or de-phosphorylates its cognate response regulator, YhcR, in response to available oxygen and other oxidants. YhcSR is a known regulator of metabolic genes, but little analysis has been done to investigate if it plays any role in virulence and survival during infections. We wanted to investigate how altering the expression of YhcSR impacted *S. aureus* survival in human blood.

We found that altering the expression of *yhcSR*, either by antisense RNA technology or protein over-expression, decreased or enhanced, respectively, survival of *S. aureus* in human whole blood. We hypothesized that the enhanced survival phenomenon is due to altered virulence factor production in YhcR Over-expression strain.

Preliminary analysis of culture supernatants identified the cysteine protease, SspB, to be over-represented in YhcR over-expression culture supernatants. SspB is involved in innate immune evasion by promoting atypical cell death of phagocytic cells and phagocytosis of neutrophils and monocytes by macrophages.

Further analysis found transcription from the *sspABC* promoter to be up-regulated during YhcR over-expression as well as much greater production SspB protein in culture supernatants as measured by gelatin zymography.

In summary, the essential YhcSR TCS regulates the expression of the cysteine protease, SspB, and the over-expression of YhcR leads to over-expression of SspB which allows Staphylococcal bacterial cells to survive for a longer period of time in human whole blood. It remains to be determined if the regulation of SspB by YhcSR is direct or indirect. This data links metabolically important oxygen-sensing YhcSR TCS to the production of virulence factors in *S. aureus*.

EZD-7

## The succession of the bacterial microbiome in the turkey gut – implications for health and development

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It is well established that the gastrointestinal tract is key to animal health, and the establishment of indigenous microflora in animals plays a critical role in overall gut development. However, we only have a gross knowledge of the succession of microbes in the turkey gastrointestinal tract, and the implications of these microbes in overall health and development. The purpose of this study was to examine the bacterial populations in the turkey ileum and cecum over time using 16S rRNA microbiome analysis, and to identify correlations between bacterial succession and overall weight. We analyzed 60 individual turkeys aged 1-12 weeks and their surrounding litter environment in a research farm mimicking typical commercial turkey production settings. At selected timepoints, birds were euthanized and weighed, and ileum and ceca contents were comparatively analyzed for their bacterial community content. Overall, a predictable subset of bacterial taxa were identified that were indicative of gut microbiome development, and correlations between certain bacterial taxa and positive weight gain were identified. “Predictor” bacterial taxa that were markers of gut microbiome development included Lactobacillus aviarius, Lactobacillus salivarius, Lactobacillus reuteri, and Candidatus division Arthromitus. The ability to modulate these microbes may result in improved turkey performance in the absence of antibiotic agents.

EZD-8

## Characterization of a Growth Enhancer for Improved Cultivation of *Francisella tularensis*

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*Francisella tularensis* is categorized as a Class A select agent by the United States government and is difficult to culture in the laboratory. We are investigating a method to increase the *F. tularensis* growth rate and enhance cultivation methods for this pathogen. We have developed a two-step enrichment process for improved cultivation of *F. tularensis* mixed in food and environmental matrices, which will aid current diagnostics. We have found that *F. tularensis* is a dormant bacterium that requires a signal from its environment to induce growth. We detected enhanced growth in the Francisella spent medium. Addition of 10% spent culture medium resulted in growth enhancement and robust growth was observed in 1/9000 and 1/27000 dilutions at 24 h. The growth enhancer is not heat labile and survived heat treatment at 90$^{o}$C at 10 min. Size exclusion chromatography was performed and growth enhancer was determined to be between 750-7000 Da. Furthermore, the growth enhancer bound to anion exchange columns indicating that the substance is negatively charged. Future directions for identification of the growth enhancer from spent medium include comparisons from spent medium from multiple species of Francisella against non-related bacteria that do not cause stimulation of *F. tularensis* growth. The chemical composition of this growth enhancer is being identified using mass spectrometry. In addition to improved cultivation with *F. tularensis* supernatant, we have developed 10 DNA aptamers against *F. tularensis*, which are capable of specific capture of *F. tularensis* in mixed bacterial cultures. We propose that the spent supernatant and DNA aptamer technology may be combined to 1) enrich for *F. tularensis* (especially at low initial inoculums) and 2) selective capture of *F. tularensis* in a high background of other environmental bacteria.

EZD-9

## Dissecting the regulation of IncA/C plasmid conjugation

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Multidrug resistant bacterial infections are a growing threat to public health. The alarming rate at which resistant organisms emerge and spread is, in part, due to horizontal gene transfer (HGT). Often times, HGT is mediated by conjugative plasmids. IncA/C plasmids are a group of plasmids that are able to be transferred and maintained in a broad range of Gram-negative bacteria. IncA/C plasmids have been significant contributors to the spread of clinically relevant resistance genes, including genes that confer resistance to third-generation cephalosporins and carbapenems. Despite the threat they impose, little is known about the basic biology of IncA/C plasmids. We set out to identify and characterize genes involved with the regulation of plasmid transfer. Our hypothesis was that because IncA/C plasmids have a broad host range, they would encode transcriptional regulators, which control the expression of the conjugative machinery. Using prototypical IncA/C plasmid pAR060302, we screened mutants using a conjugation assay that measures the frequency of transfer events.We identified ORFs 183-188 as being critical for transfer. Our analyses showed that ORF183 likely represses transcription from a promoter upstream of ORF184. Furthermore, our data show ORF186 and ORF187 are likely the positive regulators of the genes encoding the conjugative machinery and that ORF188 represses multiple loci on the plasmid. Taken together, these data indicate that IncA/C plasmids encode a complex regulatory network that governs conjugative transfer. Further work is needed to elucidate the cues given by either the host microbe or environmental conditions where plasmid transfer is derepressed. Understanding these biological processes and the mechanisms that drive them will aid efforts to curtail the spread of multidrug resistance.

EZD-10

## Molecular epidemiology of global *Brachyspira hyodysenteriae* isolates

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Swine dysentery was largely eliminated from the U.S. in the 1990s, although it continued to cause significant economic losses globally. Recently however, this disease has re-emerged in swine herds across the U.S. To investigate the strain diversity, epidemiology and phylogeny of *Brachyspira hyodysenteriae* within the U.S. and globally, we conducted a multi-locus sequence typing (MLST) analysis. A total of 341 global *B. hyodysenteriae* isolates originating from 10 countries over five decades were analyzed by an established MLST scheme based on seven housekeeping genes. This included 59 recent isolates from 42 farms, 17 production systems and nine states in the U.S. All isolates were analyzed for their diversity, population structure and phylogenetic / evolutionary relatedness. A total of 110 nucleotide sequence types (STs) and 67 amino acid types (AATs) were identified in the global population, and amongst these, all STs and some AATs from the U.S. were unique as compared to those identified globally. Within the U.S., each farm had only one ST and in general, a common ST was found in farms belonging to a common production system. A predominant strain, ST93, was identified in approximately 40% of the U.S. farms evaluated. The global isolates showed spatial and temporal clustering, and in general each ST was specific to a country. The *B. hyodysenteriae* population showed a heterogeneous but clonal structure, both at the global and country level. A primary founder type, AAT9, was identified in nine countries including the U.S., which might represent a potential ancestral strain for the global population. In addition, three secondary subgroups were identified which might represent the divergence of three potential strain lineages. The U.S. isolates represent a genotypically distinct subset of the global *B. hyodysenteriae* population. In addition to the use of nucleotide information for epidemiology, diversity and population structure analyses; this study highlights the use of amino acid information for studying the evolution and ancestry of this pathogen.

EZD-11

## Using network modeling to investigate rabies spread through a raccoon population

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The number and duration of contacts made between individuals in wildlife populations can be highly heterogeneous. These differences can be at the individual level, with some individuals tending to make more contacts than others. Also, there can be dramatic changes in contacts throughout the whole population, for example during different seasons. This heterogeneity in contact patterns is important when considering the spread of an infectious disease through a population. We used raccoon social interaction data, collected using proximity logging collars in suburban Illinois, to construct adjacency matrices specifying the connections made between raccoons over one year. These matrices formed the basis of our network model, which we used to simulate the spread of rabies through the raccoon population. Raccoons act as a reservoir for rabies, with raccoon rabies epidemic across the eastern United States. Raccoon interaction patterns were highly seasonal, so we created separate networks for the breeding and non-breeding seasons. In addition, we observed two types of contacts made by the raccoons: a) long-term, stable interactions that occurred regularly throughout a season, and b) short-term, random contacts. Both types of interactions were found to have a significant impact on disease dynamics, and more specifically, on the incidence and spread of rabies throughout a raccoon population. These results have important implications for potential rabies control measures.

EZD-12

## Broadly neutralizing antibodies against Porcine reproductive and respiratory syndrome virus, a rapidly evolving RNA virus

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Neutralizing antibodies are a critical part of the immune armory for defense against viruses, and are the mechanism by which many effective vaccines work to protect against viral infections. However, infections by rapidly evolving and genetically diverse viruses are often characterized by ineffective neutralizing antibody responses. Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly genetically diverse RNA virus that causes the most significant disease (PRRS) of pigs worldwide. The prevailing view of immunity to PRRSV is characterized by delayed and ineffectual production of neutralizing antibodies lacking cross-reactivity that is necessary for vaccine efficacy. We sought to examine PRRSV neutralization characteristics from serum of animals from herds with a history of multiple exposures to PRRSV over time, either through natural infection, modified live virus vaccination, or serum inoculation. Fluorescent focus neutralization (FFN) and ELISA-based serum neutralization (SN) assays were used to screen sow serum against a panel of diverse PRRSV isolates for quantification of anti-PRRSV cross-neutralizing activity. Sera from previously infected commercial sows had high levels of neutralizing activity against diverse PRRSV strains, including genotypically distinct type 1 PRRSV. Fifty percent cross-neutralization titers in excess of 1/1024 were observed. Cross-neutralization activity was dose-dependent and was maintained in the immunoglobulin fraction. The presence of high-titered, anti-PRRSV cross-neutralizing antibodies in pigs is strong evidence that highly conserved neutralization epitopes are present in genetically disparate PRRSV. These findings provide a new model to help elucidate mechanisms of antibody production and maturation that target inapparent conserved neutralization epitopes in rapidly evolving viruses.

EZD-13

## Tick-Borne Diseases in Minnesota: Increased Incidence and Projected Change in Geographic Distribution

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Lyme and other tick-borne diseases are serious public health threats with important links to environmental conditions which affect vector habitat. We used two decades of disease case data from Minnesota Department of Health to model the spread of Lyme disease, Anaplasmosis, Babesiosis, and Ehrlichiosis across Minnesota relative to landscape and climatic factors. Infection rates were strongly associated with tick habitat and climate factors. We used risk mapping and spatial simulations to demonstrate that northwestern Minnesota faces the greatest risk of increasing tick-borne disease as climate warming continues to increase survival rates of these important disease vectors in northern forests.

EZD-14

## Comparative Pathogenicity of Turkey Arthritis Reoviruse (TARV) in Poults

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Turkey arthritis reoviruses (TARV) have been isolated from the gastrocnemius tendons and tibiotarsal joint fluid of lame, >12-week-old male turkeys in the Midwest. Two experiments were conducted to compare the pathogenicity of three TARV, turkey enteric reovirus (TERV) and chicken arthritis reovirus (CARV) for turkeys. In blind studies, 200 ul of each virus was inoculated by different routes (oral, intratracheal, and footpad) into 6-day-old poults raised in isolator units. Poults were necropsied at 1 and 4 weeks postinfection (PI) in experiment 1 and at 2 and 4 weeks PI in experiment 2. Reovirus was detected by RT-PCR and virus isolation in tendons from TARV poults at 1, 2 and 4 week PI. TARV generally produced a lymphocytic gastrocnemius and digital flexor tenosynovitis without inflammation of the tendons proper. TARV-MN2 and TARV-O'Neil produced significantly higher histologic inflammation scores than TERV-MN1 and CARV-MN1 in experiment 1 and higher scores than control in experiment 2. Koch's postulate was fulfilled when reoviruses isolated from tendon of inoculated poults, were sequenced and matched with TARV-MN2 and TARV-O'Neil. Reproducible results of the two experiments indicate that TARV have a unique ability to induce tenosynovitis, and that administration of TARV-O'Neil through oral or intratracheal routes would be an excellent model to study pathogenesis of TARV infection.

EZD-15

## Detecting Leptospirosis in wild rodent populations

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Leptospirosis has been said to be the most widespread zoonosis in the world, but it relies heavily on water in the environment to sustain itself outside of host species. Although it is most common in less developed countries, Leptospirosis poses a serious threat to people and animals that come into contact with the bacterium here in the United States. Leptospirosis can be transmitted between animals and humans through contact with environments, namely bodies of water, which have been contaminated with the urine of infected individuals. It is important to study variation in Leptospirosis across landscapes and seasons to understand how differences in water availability, vegetation, and host species impact risk of infection. We evaluated potential environmental reservoirs for the Leptospirosis bacterium within Cedar Creek Ecosystems Science Reserve. Field teams spent three, week-long sessions during late spring and summer trapping rodents using capture-mark-recapture methods. Data were used to estimate populations across plots varying in vegetation, water content, and histories of human alterations. After the final week, 76 individuals of the genera *Peromyscus*, *Microtus*, *Clethrionymus*, and *Zapus* were culled. Liver samples were obtained from these individuals and sent to the Veterinary Diagnostic Laboratories at the University of Minnesota for PCR testing to detect the presence of Leptospirosis. We report the number of individuals within each genera that are infected with Leptospirosis, and the distribution of these among the varying sampling plots.

EZD-16

## The Evaluation of Molecular Epidemiological Relatedness within ST5 *Staphylococcus aureus* Isolated from Swine Veterinarians in the USA

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The livestock associated *Staphylococcus aureus* (LA-MRSA) ST 398 has been isolated frequently from livestock, especially pigs, worldwide since first detected from pigs and pig farmers in the Netherland in 20051. Subsequent studies are revealed greater genetic diversity in LA-MRSA using molecular epidemiologic characteristics. Several studies reported that ST9 isolates are predominate among MRSA isolates from pigs in Asian countries, while both ST398 and ST5 lineages have been found in pigs and livestock workers in North America3,4. Unlike ST398, which is rarely involved in significant human infections, the occurrence of ST5 sequence type in pig industry is of some public health concern as this ST5 lineage has long been associated with human MRSA infections related the USA100 group (defined by sma1 PFGE)2,5. In a pilot study of pig farms in Minnesota, the predominant spa types found were t034 (ST398), and t002 (ST5), comprising 37% and 29% of isolates respectively (all MSSA). The aim of this study was to investigate the prevalence of ST5 *S. aureus* nasal swabs in US swine veterinarians, and to evaluate the diversity of spa type t002 isolates using PFGE with SmaI. Similar to our findings in pigs, the most prevalent spa types found in US swine veterinarians were t034-ST398 and t002-ST5. ST9 spa types (t337, t3446, t2498) were also common. Sixty (19%) of 308 MSSA isolates and 6 (19%) of 32 MRSA isolates detected were spa type t002 (ST5). Other ST5 MSSA spa types found included t045 (14), t062 (6), t570 (3) and t2049 (1). Sma1 PFGE analysis of 23 isolates indicated that t002 isolates from the swine veterinarians were not clonal but heterogeneous between isolates. Fifteen distinct pulsotypes were seen, and eight isolates were classified as USA100. Given previous reports of t002-ST5 MRSA in North American pigs, our findings of diverse ST5 spa types as well as genetic diversity within spa type t002, provide improved understanding of ST5 lineage, which may have long association with pigs overtime after introduction from human. Further investigation of ST5 lineage is warranted to better understand their potential implications for human health.

EZD-17

## Survival of porcine epidemic diarrhea virus in fresh feces and slurry

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Infection with porcine epidemic diarrhea virus (PEDv) is characterized by severe enteritis, vomiting and watery diarrhea in swine. For epidemiological studies, it is important to determine the length of virus survival in fresh feces (that represents the risk posed by transport) and slurry (old feces representing a risk of manure spreading). Many factors can influence virus survival in the environment, amongst them temperature and relative humidity (RH) are the most important. To study the survival of PEDv, we collected samples of fresh feces and slurry from PEDv-free farms. These samples were seeded with PEDv (homogenate of mucosal scrapings from a PED-positive pig). Aliquots of virus-spiked samples of feces and slurry were placed in sterile tubes (in triplicate) followed by storage at three different temperatures; 40ºC, 50ºC, and 60ºC for feces and 25ºC, 4ºC and -20ºC for slurry. Three RH levels (30%, 50%, and 70%) were used in all experiments except for slurry stored at -20ºC. Three tubes of each sample were removed at various time points (0, 1, 3, 7, 14 and, 28 days) and the virus was eluted by suspending the aliquots in 3% beef extract-0.05M glycine buffer. After centrifugation, the supernatants were removed and pooled for each triplicate sample. Serial 10-fold dilutions of the supernatant pools were tested by real time RT-PCR in an effort to determine the time at which no nucleic acid is detectable. The results show that the PEDv RNA can be detected in fresh feces for 3-7 days at the three temperatures tested but there was no effect of RH on 'survival' of RNA. In slurry, the viral RNA 'survived' for up to 28 days under all conditions of temperature and RH. Because PEDv is not currently amenable to propagation and titration in vitro in cell cultures, we plan to inoculate all samples in 2-week-old piglets to determine the time at which virus is inactivated. The survival of a related coronavirus (transmissible gastroenteritis virus) was determined under similar conditions using ST cells for virus titration. The survival results of TGEV paralleled those of PEDv RNA indicating that bioassay results of PEDv in piglets would probably be similar to those of RT-PCR.

EZD-18

## The roles of host TrkA signaling pathway in the influenza A virus replication

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Influenza virus is a major global pathogen that causes annual epidemic infection and occasional severe pandemics. Antiviral drugs are important therapeutic interventions needed to control influenza virus infections. However, influenza variants resistant to current antiviral drugs occur and spread rapidly. Targeting host signaling and/or factors important for the influenza viral replication represents an alternative approach. Characterization of the specific roles of signaling pathways in the influenza A virus infection may therefore provide novel targets for antiviral therapeutics. We have shown that specific inhibitors of TrkA receptor tyrosine kinase can effectively inhibit the synthesis of all three influenza viral RNA species, block the nuclear export but not nuclear import of the viral nucleoprotein complex, and reduce the level of viral particle release from the infected cells. By over-expression and shRNA-mediated knockdown methods, we have shown that the TrkA signaling plays important roles in the replication of influenza virus. Furthermore, we provide evidence that inhibition of TrkA signaling provides therapeutic benefits against influenza virus infection in vivo using the influenza-mouse model. In summary, our study has not only characterized the novel functional roles of host TrkA signaling pathway in distinct stages of influenza viral life cycle but also revealed a potentially new therapeutic approach against flu infections.

EZD-19

## Deep sequencing analysis of PRRSV genetic variation among cell types

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus with an extremely high mutation rate estimated at ~$10^{-2}$/site/year. Mutations can arise from viral RNA polymerase infidelity, genomic recombination, and host cell mutator activity. However, the frequency of nucleotide variations across individual sites in the viral genome, which might help address the contribution of various mutational mechanisms, has not been investigated in permissive cells or host animals. Since biological and antigenic variation arising from these mutations may contribute to disease severity, incomplete effectiveness of vaccination, and prolonged infection, we examined this question using ultra-deep sequencing. Strain variation has been examined extensively through consensus ORF5 sequencing analysis, however, nucleotide sequence variation across the entire genome of individual viral genomes in a viral population has not been evaluated. We sequenced three independent virulent PRRSV strains grown in two different permissive cell types at an average redundancy between 6,000- and 50,000-fold. Fifty bp, paired-end reads were mapping to the corresponding reference genome and single nucleotide polymorphisms were detected across the genome. Our preliminary results show that the highest mutation frequencies were detected in nonstructural protein 2 coding region (nsp2), nsp3, and nsp11. Overall nucleotide substitution patterns were random, but at frequencies higher than 1%, A to G and G to A substitutions were over-represented, suggesting the potential editing activities of a cytoplasmic form of the apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3 (APOBEC3) family. PRRSV whole genome SNP analysis showed that the mutational spectrum was dependent on both virus strain and permissive cell type, either porcine macrophages or MA-104, a simian epitheliod cell line. Overall, host cellular anti-viral mechanisms appear to have a limited effect on the PRRSV mutation rate, suggesting that antigen-specific adaptive immunological responses may play a dominant role in driving PRRSV mutation.

PS-1

## Preliminary data on the effect of the Electrostatic Particle Ionization (EPI) system on decreasing artificially generated PRRSV aerosols

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**Introduction** Porcine reproductive respiratory syndrome virus (PRRSV) is one of the most significant swine pathogens airborne transmitted. The EPI system is a technology capable of reducing airborne agents due to its ability to clump and settle airborne particles. However, this effect is influenced by distance to the source of ions and environmental characteristics of the air. The objectives of this study were to determine the effect of the EPI system on particle size distribution and quantity of PRRSV in artificially generated aerosols. **Materials & methods** The EPI system was installed at 3 levels (1, 2, 3m from pig level) in a 35 m3 isolation unit at the UMN. Artificially generated PRRSV aerosols were sampled using an Andersen Cascade Impactor capable of collecting particles by size (8 stages, 9 to 0.4$\mu$). Air samples were collected with the EPI system on and off for 30 min and analyzed by qPCR. Three replicates per level were performed. Differences in viral quantities were obtained by size. All results were fit into a regression model to predict the efficiency of the system in removing virus particles from the air and adjusted for the conditions of this study. **Results** A total of 144 air samples were analyzed for the study. The PRRSV qPCR analysis of each Andersen Impactor stage demonstrated a significant decrease in the quantity of PRRSV from the air when the EPI system was on. The reduction was significant for largest particle sizes and the increase in the distance from the EPI lines. **Conclusion** This study investigated the effect of the EPI system on artificially generated PRRSV aerosols. Our results indicated that particle size and distance to the source of ions interacted in the efficiency of the system. Further studies are needed to corroborate these results with aerosolized viral particles generated by infected pigs. Decreasing the infectious viral load of PRRSV in the air of positive pig farms could decrease the likelihood of dissemination of airborne pathogens to neighboring pig sites.

PS-2

## Socio-Economical and Political Aspects of Spent Lead Ammunition

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Lead has been known to be toxic for humans and wildlife for thousands of years. Although lead shot was banned for waterfowl hunting on federal lands and on most state-owned hunting lands in the United States in 1991, lead ammunition is still used for hunting deer and other cervids, small game and upland birds. There is scientific evidence of the connection between lead intoxication in eagles and deer hunting in the Midwest, but any attempt to raise awareness of this issue has historically caused a negative response among the hunting shooting sports. An interdisciplinary team of experts met in 2012 to analyze this issue from different perspectives (economic, policy, epidemiology, public health, conservation, social, and cultural). Using a bottom-up approach, this team organized an event at an outdoor sporting goods retailer to create awareness about lead intoxication in eagles among the hunting community and discuss non-toxic ammunition alternatives. Although the event was carefully planned, an inaccurate media headline resulted in the event being significantly altered. Despite the disappointing outcome of the event, there has been huge progress on the issue of lead intoxication by using a holistic bottom-up approach. In addition, important lessons were learned about messaging and transparency in the communication with all stakeholders. Thanks to these initiatives, there is ongoing work with the goal of solving the issue of lead intoxication in wildlife.

PS-3

## Needlestick injuries in livestock workers and prevention programs

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**Introduction:** Veterinary medicine and agriculture have historically lacked needlestick injury (NSI) research, education, and mitigation. Depending on the procedure/pharmaceutical used, these injuries may include mild/severe bacterial or fungal infections, lacerations, local inflammation, vaccine/antibiotic reactions, amputation, miscarriage, and death. The study objective was to identify published case reports and case series/surveys on human needlestick exposure to veterinary biologics, and to review literature describing needlestick prevention strategies for agricultural workers and veterinarians. **Methods:** An electronic database search was conducted using key search terms in PubMed© and CABI©. Article inclusion criteria were those detailing NSI in agricultural workers only. Abstracts of all search results were read and relevant articles compiled into a RefWorks© database. References cited within articles were examined to locate additional articles. **Results:** Fifty-six articles were identified. Literature consisted of case reports (n=14), survey/case series articles (n=11), prevention guidance documents (n=6), and background articles (n=25). Forty-eight cases were found. Twenty-four identified injury location: 13 (54.2%) NSI to the hands: three to the right, eight to the left, and two were not specified. Eight injuries were to the legs (33.3%): five to the right and three were not specified. Of the 48 cases, 11 (22.9%) involved oil-adjuvanted vaccines. The remaining products included: other vaccines, antibiotics, analgesics/sedatives, and hormones. Forty-six (95.8%) of 48 cases reported seeking medical attention. Of the 11 survey/case series articles: two focused on oil-adjuvant products, one on Brucellosis RB51 vaccine, three on tilmicosin, and five were non-specific. General recommendations from guidance documents were included. **Conclusion:** NSI in agriculture workers and veterinarians can result in injury and loss of work. It appears that NSI awareness is limited among workers. There is a need for comprehensive programs to prevent NSI on livestock operations.

PS-4

## Secure Egg Supply (SES) Plan: Tri-State Implementation Workshop Efficacy Analysis

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**Background:** The Secure Egg Supply (SES) Plan promotes food security and animal health through continuity of market planning for a highly pathogenic avian influenza (HPAI) outbreak. **Introduction:** To facilitate the dispersal of the SES Plan to stakeholders within Iowa, Minnesota, and Wisconsin, a regional implementation workshop was developed. This workshop acted as the "beta-test" to help establish an approach to enhance regional implementation. It was the hope that this type of workshop could be implemented in a similar fashion to multiple U.S. regions. **Purpose:** The goal of this workshop was to discuss permitted movement of egg products out of a control area in the event of a HPAI outbreak. Four objectives were established: 1) practice permitted movement using the APHIS – HPAI SES Plan 2) to understand each states "intrastate" and "interstate" movement of egg products 3) identify the gaps within state and between the three states in relation to permitted movement and 4) to make connections and establish relationships between industry and states agencies. **Methods:** A planning committee was convened consisting of state animal health officials from the three states. The planning started in August of 2012 and the workshop was held on March 12, 2013. **Results:** An evaluation was developed to assess the objectives. Analysis of the evaluation (n=29) results indicated an average response of 3.47 / 4.0 (1=disagree, 4 = strongly agree) for each question. Gaps in implementation knowledge were identified including: a difference in information management between states, how to handle multi-state truck routing movements, lack of understanding export implications, stakeholders missing from the conversation, and proper risk communications. **Conclusions:** Analysis indicated that participants felt the objectives for the workshop were met. Participants were also able to identify gaps in knowledge intrastate, interstate, and internationally. Steps forward include implementing this regional discussion in other parts of the country and introducing and engaging trading partners.

PS-5

## Understanding transmission pathways in chronic wasting disease in Minnesota farmed cervids

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The objective of this study was to examine the known transmission pathways for chronic wasting disease (CWD) through construction of a case series from the descriptive epidemiology of CWD in farmed cervid herds in Minnesota and performing a systematic review of published scientific literature involving CWD transmission in cervids. The case series and systematic literature review involved multiple cervid species including: white-tailed deer, mule deer, Rocky Mountain elk, red deer, reindeer, and fallow deer. Five CWD- positive farms were detected in Minnesota between 2002 and 2012. CWD likely was introduced to several of these farms through farmed cervid movements. The last CWD-positive farm was the first naturally-occurring case of CWD in red deer and the epidemiology does not provide a clear pathway for CWD introduction on the farm. The systematic literature review identified thirteen scientific articles on experimental transmission of CWD in cervids. These reports indicated that multiple species of cervids are susceptible to CWD through multiple exposure routes including: oral, aerosol, intravenous, and environmental exposures. CWD infection can be transmitted through various inoculums, such as brain, blood, and saliva. CWD is a threat to the farmed cervid industry in the United States. Without federal funding available for control programs including indemnification after depopulation of detected herds, additional research is needed to identify disease management options.

PS-6

## Improved Methods for Detection of *Mycoplasma hyopneumoniae* Infection in Live Swine

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The objectives of this study were to develop alternative serology methods (ELISAs) to detect *Mycoplasma hyopneumoniae* (*M. hyop*) specific antibodies in live pigs and to use western blot as a possible test to confirm infection. For this study 23 pigs were housed in isolation rooms for 28-days divided into two experimental groups; one group consisting of twenty one pigs and one control group of two pigs. On day 0, pigs were sampled (oral fluids, tracheal-bronchial lavage (TBL) and blood) followed by the inoculation of the infected group with *M. hyop*. Sampling was done at 2, 5, 9, 14, 21 and 28 days. Three ELISAs were developed in this study. For the detection of IgM in serum the IDEXX platform was adapted to detect IgM. Tween-20 *M. hyop* coated plates (ISU) were adapted to detect IgA (in oral fluids, TBLs and lung lavage) and IgG (in TBLs and lung lavage). For western blots, *M. hyop* was grown, lysed, run on a gel and transferred to a PVDF membrane. Individual serum samples were coated on *M. hyop* -PVDF strips. Bands were analyzed using BioNumerics. *M. hyop* infection in all inoculated pigs was confirmed using PCR. IgM detection in blood occurred as early as day 14. Detection of secretory immunoglobulins (IgA and IgG) showed similar results; post-mortem lung lavages were significantly more sensitive than the samples taken from live pigs. For western blots most pigs showed no discernible differences over the 28-day sampling period with three exceptions, these pigs expressing unique bands at 73kDa and/or 30kDa on days 14, 21, and 28. IgM in serum proved to be a more sensitive method, however, IgM is transient in any immune response, and regular blood sampling would be required for this to be a viable assay. It was found that TBLs and oral fluids are poor sampling methods for detection of IgA and IgG. Lung lavages are best for detection of secretory immunoglobulins but this requires euthanizing the pig. Western blots have been suggested as a possible confirmatory test for infection, but in this study it was determined to be generally not a very sensitive assay.

PS-7

## The Society for Quality Assurance University Speciality Section

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The mission of the Society for Quality Assurance (SQA) University Specialty Section (USS) is to maximize the information exchange between organizations, universities and industries enhancing the quality of university-based research studies. This is accomplished by addressing unique quality assurance challenges that are inherent to regulated and non-regulated studies conducted in an academic environment. The USS promotes SQA as the main resource for QA professionals at universities, serves as a forum for open communication among the QA profession, university faculty, students and administration, and provides recommended continuing education resources. Additionally, the USS creates liaisons and enables discussions with other professional organizations within quality assurance. The SQA encourages student participation by featuring a significantly reduced membership process and fee structure (25.00). Consider joining the SQA and incorporating research quality assurance into your training program! During the 29th SQA Annual Meeting, the USS is sponsoring sessions that focus on the strategies for implementing and maintaining quality systems at universities. The USS will also sponsor a SQA Quality College entitled "Developing a GLP-Compliant Program in a University Setting" including several current hot topics related to regulated studies in a university setting throughout the conference. USS members are currently focusing their efforts to address the importance of implementing a quality system early in the drug development phase. The majority of members are university QA professionals, but USS also includes industry QA representatives and consultants – a valuable resource as they guide academics through the regulated processes. The USS, with over 70 members from several countries, welcomes anyone with an interest in gaining insight for conducting and managing studies in an academic setting that require compliance with quality regulations.

PS-8

## Influenza A virus infection and transmission in wean to finish pigs

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Influenza A virus infections are worldwide distributed in many animal species, including humans, and its epidemiology within and between species is complex and not completely understood. The pig has been identified as a mixing vessel for IAV, but little is known about IAV evolution, prevalence and incidence in endemically infected swine populations. The objective of this study is to characterize IAV infection and transmission in pigs after weaning to identify determinants that may be associated with IAV persistence in swine populations. A cohort of 132, 3-week old pigs were randomly selected at arrival to a commercial wean to finish facility. Nasal swabs were collected from each pig on a weekly basis for 15 weeks and each sample was tested for IAV by RT-PCR. Additionally serum samples were collected at arrival and every 4 weeks there after, and tested for antibodies to IAV by ELISA. Twenty five pigs out of 132 (19%) tested positive by PCR for IAV at arrival. The period prevalence to IAV was 98%, and the weekly prevalence ranged between 4% and 64.4% ($p<0.05$). We identified two epidemic curves of IAV during the study period, at 2 and 7 weeks post weaning with a prevalence of 64.% and 55% in each peak respectively. Eighty two percent of the pigs that tested positive became re-infected. The mean and standard deviation of the sample to negative ratio (SN) at weeks 0, 4, 8 and 12 were 0.550 (0.227), 0.695 (0.186), 0.191 (0.109), 0.224 (0.1426) respectively ($p<0.05$) which indicated a decay on the maternal immunity followed by the subsequent seroconversion due to active infection. In conclusion our results indicate that IAV can be maintained in growing pig populations at a low prevalence and that pig to pig transmission can occur even among previously infected immune pigs. We speculate that persistence at the population level is the result of virus adaptation as a result of pig to pig transmission, and the diversity in the levels and type of immunity found in the pigs. Acknowledgments: National Pork Board (NPB)

PS-9

## Molecular typing of *Mycoplasma hyopneumoniae* in clinical samples using MLVA

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*Mycoplasma hyopneumoniae* (*Mhp*) is the cause of enzootic pneumonia, an important swine disease. The aim of this study was to standardize an assay for the differentiation of *Mhp* strains and to investigate the genetic variability of *Mhp* circulating in the US. A total of 210 clinical samples submitted to the UMN-VDL were used in this study. DNA was extracted from the clinical samples and a Multilocus Variable Number Tandem Repeat Analysis (MLVA) was standardized by modifying a previously established protocol. The assay targets the number of repeats in 2 adhesin proteins (p97 and p146) of *Mhp* were studied. Fluorescently labeled primers were combined with a touchdown PCR was developed to amplify the target loci. Agarose electrophoresis was performed to confirm size of the amplicons. Ten microliter of each sample was diluted 1:32 and submitted for capillary electrophoresis at Roy J.Carver Biotechnology Center- University of Illinois. Samples that failed in the capillary electrophoresis analysis were sequenced by standard Sanger sequencing, and loci repeats were manually counted for inclusion in the analysis. MLVA patterns were analyzed in BioNumerics. The two loci were clearly identified on electropherograms according to their size ranges and colors, and converted to categorical values based on the number of repeats. The Simpson's diversity index for the assay was D=0.908 for p97, D=0.929 for p146 and D=0.979 when both loci were combined. Analysis of the combination of 2 loci revealed 87 MLVA types. The most frequent MLVA types were: 9-26 (7.2%), 15-25 (6.2%), 15-21 (5.2%), 11-15 (4.7%) and 14-21(4.3%). No clustering was observed on the basis of geographical location. In the state of MN 54 MLVA types were found, suggesting that multiple *Mhp* variants are circulating. Further analysis of samples collected longitudinally from diverse geographic locations and disease types are necessary to investigate if a nonrandom distribution of genotypes is present among highly pathogenic strains.

PS-10

## Detection of Porcine Epidemic Diarrhea Virus in air samples at varying distances to epidemic farms in Oklahoma

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Porcine Epidemic Diarrhea (PED) was first detected in the United States in 2013. The disease is financially devastating for a farm due to high preweaning mortality. A cluster of farms in the Oklahoma panhandle were all infected within such a short period of time and with no common sources that aerosol spread was suspected. Coronaviruses similar to PEDv are relatively stable in arid conditions. These conditions may also increase potential for aerosol spread. There is a need to determine if PEDv can be detected in air samples.

Air collection was performed with a high-volume air sampler run for 30 minutes with 11 ml of transport media at each sampling location. Air was sampled at varying distances from farms experiencing a PED outbreak. Bio-assay of the positive PCR air samples to determine viability of the viral particles collected was performed by inoculating 15 pigs allocated to 13 isolation rooms. We included 1 positive control sample, 1 diluted positive control sample, and 1 negative control sample. All pigs were euthanized 48 hrs post-inoculation and their tissues were tested by PEDv PCR.

PED PCR-positive air samples were detected outside of multiple mechanically-ventilated sow units and naturally-ventilated finishing units. Distances of 30 ft, 60 ft, 300 ft, ¼ mile, ½ mile, 1 mile, 2 miles, 3 miles, 5 miles, 10 miles, and 15 miles downwind of known positive farms were sampled yielding 64 samples in total with 11 PCR positives. The only pig to show diarrhea on bio-assay was the pig inoculated with the low-Ct positive control sample with positive intestinal samples. All other pigs were clinically and PCR negative.

PEDv can be detected in the air as much as 10 miles from a herd. Negative bioassay results of the air samples may indicate the following:

-The low concentration of virus in this study may have been below the minimum infectious dose for PED in 10 day old piglets.
-There was no live virus in the PCR-positive air samples.
-Piglets exposed to a low concentration of virus may need more than 48 hours for replication to be detected in bio-assay procedures.

PS-11

**A simulation model for Influenza A virus dissemination in a growing pig herd with waning immunity**

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Swine influenza A virus has become a major pathogen, causing respiratory disease in swine including wean-to-finish herd. Waning immunity against influenza A infection after growing-pig infection has been poorly addressed. The objective of this study is to create a model of the effect of waning growing-pig immunity on susceptible, infected, and cumulative infected proportion over the 20 weeks of the wean-to-finish period. Simple deterministic SEIR models were constructed for such population. Ten infected weaned pigs were introduced in a barn with 1000 pigs ($\mu$=0.01429 day$^{-1}$) with 5.3% mortality. Transmission rate $\beta$ was 2.18. Exposure period was 1.9 days ($\kappa$=0.526 day$^{-1}$). 0.5% of infected piglets died from influenza infection was assumed ($f$=0.005). Three scenarios were simulated ($\pi$=0.0, $\pi$=0.05, and $\pi$=0.10, of piglet population recovered day$^{-1}$ becoming susceptible for scenario 1, 2, and 3 respectively). Results showed after the 40$^{th}$ till the 140$^{th}$ day post influenza A virus introduction, the proportions of susceptible growing pigs remaining in the herd were 10.8%, 10.6%, 10.6% but the proportions of infected growing pigs remaining in the herd were 5.4%, 18.9%, 27.0% (scenario 1, 2, 3 respectively). Susceptible growing-pig proportions for three scenarios were similar over a course of Influenza A virus dissemination. In contrast, infected growing-pig proportions for three scenarios were different in proportion. In addition, among infected pig subpopulations, the pig proportion without waning immunity ($\pi$=0.0) has sharply increased since 16$^{th}$ till the 30$^{th}$ day post introduction but consistently increased both 5% immunity wane ($\pi$=0.05) and 10% immunity wane ($\pi$=0.10). In conclusion, the waning rate of wean-to-finish pig immunity affected only the proportion of infected pigs, but not for the proportion of susceptible pigs. Among infected pig subpopulation, pigs without waning immunity has sharply increased infections after 16$^{th}$ till the 30$^{th}$ day post introduction, then this became stable in Influenza A virus transmission in the wean-to-finish barn.

PS-12

**Effect of antibiotic treatment on the development of *Haemophilus parasuis* disease and seroconversion**

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**Introduction:** Antimicrobials are widely used to control *Haemophilus parasuis* (Hps) disease in swine, but little is known about how this treatment may affect the development of immunity to Hps. **Hypothesis:** Antimicrobial treatment interferes with the development of a protective immune response against Hps. **Objective:** To evaluate the effect of enrofloxacin on seroconversion and resistance to challenge in an Hps infection model. **Study design:** Pigs (3-week-old, n=10/group) were either treated with enrofloxacin 3 days prior (ABT/EXP), or 3 days after (EXP/ABT) controlled exposure (EXP) with a low dose ($10^6$ CFU/ml) of virulent Hps strain at day 0. Control groups included: exposure only (EXP), antibiotic only (ABT), challenge only (CHA) with a high dose ($10^8$ CFU/ml) of virulent Hps and negative (NEG). All groups, except NEG, were challenged. **Results:** Clinical signs of Hps disease were observed in 6 pigs from EXP and ABT/EXP groups starting on day 4 post-exposure. Acute septicemia due to Hps was confirmed in two pigs by systemic Hps isolation. The other affected pigs were treated with enrofloxacin and recovered. After challenge, only pigs from CHA, EXP/ABT and ABT groups became ill. Fourteen pigs from these groups presented polyserositis at necropsy. Pigs from NEG, EXP and ABT/EXP groups remained healthy. Seroconversion was observed in groups EXP and ABT/EXP after controlled exposure and in groups CHA, EXP/ ABT and ABT after challenge. **Discussion:** Virulent Hps, even at low dose, was able to cause disease in 60% of the pigs from groups EXP and ABT/EXP. Interestingly, disease in ABT/EXP group started 3 days after onset of disease in group EXP, which might reflect a decay of enrofloxacin levels in that group and delay in infection. Seroconversion after controlled exposure was associated with survival to challenge. Enrofloxacin treatment in group EXP/ABT interfered with protection against challenge. Therefore, time of antimicrobial treatment in relation to Hps infection is important to determine the outcome of clinical signs and development of disease and protective immunity.

PS-13

## Initial assembly and analysis of the MHC *B*-locus of the ocellated turkey

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The ocellated turkey (*Meleagris ocellata*) is a distinctive galliform native to the Yucatán Peninsula of Mexico, Guatemala and Belize. Due to hunting and habitat loss, the range of the ocellated turkey has been reduced to less than 50,000 sq. miles. Therefore, unlike their widespread relative, the wild turkey (*M. gallopavo*), ocellated turkeys have a near-threatened conservation status. Understanding the structure and diversity of the major histocompatibility complex (MHC) can have important implications for conservation efforts and lead to hypotheses on MHC evolution. Whole genome sequencing (WGS) was performed on the Illumina HiSeq, producing 100 bp paired-end reads. After trimming and filtering, approximately 168M paired-reads remained, of which over 3M read pairs and 4M single reads aligned to the domestic turkey MHC *B*-locus (*MHC-B*). Alignment resulted in an average sequence depth of 1,659 reads/reference nucleotide, due to areas of extremely high sequence coverage in repetitive regions. Twenty known gene families from the *MHC-B* were represented in the assembled sequences. Contigs were aligned and compared to the domestic turkey and other galliforms; a number of polymorphisms and insertions unique to the ocellated turkey were identified. This analysis provides insight into gene content and evolutionary conservation of the ocellated turkey MHC and provides a framework for further investigation of MHC variability.

PS-14

## Isolation and characterization of S class gene segments of a new turkey arthritis reovirus

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From 2011- 2013, we isolated nine turkey arthritis reoviruses (TARVs) from cases of lameness, arthritis and rupture of gastrocnemius tendons in 15-18 weeks old tom turkeys. Two isolates were received from another lab. In this study, we report on the complete characterization of S class segments of these 11 TARV isolates. Primers were designed from 5' and 3' untranslated regions (UTRs) and amplified by one-step RT-PCR. Amplified PCR products were purified and sequenced. Based on phylogenetic analysis of S2, S3 and S4 genome segments, all TARVs grouped into two lineages. On the basis of S1 gene segment, however, they all grouped in a single lineage. All TARVs had 95% to 100% nucleotide identity based on sigma C protein while nucleotide identity varied from 90% - 100%, 89%-100% and 88.7%-100% based on S2, S3, and S4 genome segments, respectively. Based on sigma C protein, TARVs had the same amino acid identity as nucleotides which indicates that occurrence of non-synonymous nucleotide substitutions more than the synonymous. The TARVs grouped together under genus avian orthoreovirus in the family *Orthoreoviridae* but formed a different group among avian reoviruses (different from chicken, duck and goose reoviruses). However, no clear cut differentiation was observed between TARVs and TERVs (turkey enteric reoviruses). Point mutations along with possible reassortments throughout the S class indicate the importance of conducting epidemiological studies on TARVs. Surveillance studies in hatcheries and commercial farms are indicated to determine the types of TARVs circulating in the field, which should be helpful in designing prevention and control measures.

PS-15

## Characterization of air contaminants associated with type of swine production facilities.

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Workers in the swine industry are frequently exposed to respiratory hazards including organic dusts, bioaerosols, gases, and endotoxin. As production practices in the swine industry continue to evolve to meet growing demand and in response to consumer and food industry preference, the change in practices may cause a change in the working environment. The extent to which these air contaminants vary by the type of swine production facility is not clear. The objective of this research is to characterize air contaminant concentration and variability associated with differing swine rearing practices. This ongoing project characterizes air contaminants in a swine facility with parallel sow/farrow rearing systems and finishing pens using dry and wet feed delivery systems. Time-weighted average respirable dust and endotoxin concentrations were measured at stationary locations. Real-time measures of respirable dust, hydrogen sulfide ($H_2S$), ammonia ($NH_3$) and carbon dioxide concentrations ($CO_2$) and temperature and relative humidity were measured to assess temporal and spatial variability throughout the site. The contaminant concentrations are summarized to estimate average, peak, and spatial distributions and compared between the type of production system. Measured variables were compared between different rearing and feed delivery systems. Preliminary findings indicate airborne exposures vary by season and sow housing configuration and animal activity levels may impact air contaminant exposure levels. Additionally, temperature and humidity levels can reach levels where worker and animal health may be affected. Characterizing airborne contaminants in swine production operations by type of facility will identify the potential impact facility type has on workers, which can then be evaluated along with animal welfare needs. Understanding variability of exposure by type of operation will inform future research on the control of air contaminants within these systems.

PS-16

## Minnesota Baitfish Industry: Understanding a complex network.

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The goal of this pilot study was to characterize the movement of baitfish throughout Minnesota. The baitfish industry in Minnesota is the second largest in the nation and largely unregulated. There are a large number of stakeholders represented in this industry including fish farmers, bait shop owners and anglers, who are connected through a network of fish harvesting and sales. It was hypothesized that the movements of fish through this network are vast and complicated. A survey was sent to producers and bait shop owners inquiring about the locations of source lakes and distribution regions of their wild-harvested and licensed water-raised baitfish, specifically inquiring about Fathead minnows (*Pimephales promelas*) and Spottail shiners (*Notropis hudsonius*). Survey recipients were asked the amount, in gallons, of fish harvested from the wild and raised in licensed waters. Bait shops were asked how far, once purchased, the baitfish traveled from their shop. The survey resulted in a response rate of 21% with 63% of the responses received from producers and 37% from bait shop owners. Bait shop owners indicated that baitfish, once purchased, traveled approximately 20 miles from their place of business. Results of the survey indicated the size of producers varies extensively from 200-2000+ gallons of Fathead minnows and Spottail shiners raised and harvested per year. Larger producers indicated multiple source and distribution regions in Minnesota while smaller producers indicated just 1 or 2. Our pilot study concluded that the movement of baitfish throughout Minnesota was even more complex and untraceable than was anticipated, with each sub-watershed potentially distributing fish to multiple other sub-watersheds due to the common practice of producers to mix fish from multiple sources before distributing them along the network. A formal network of the movement of baitfish throughout Minnesota could not be designed due to limited information. Continuation of this research requires interest and support from regulatory agencies and members of the industry itself.

PS-17

## *Risk factors* for calcium carbonate *urolithiasis* in *goats*: (368 cases: 1984 - 2012)

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*Objectives*-To determine the predominant mineral composition of naturally occurring goat uroliths; to determine whether age, breed, sex, reproductive status, geographic location, season, and anatomic location were risk factors associated with urolith formation in goats; and to determine whether the rate of urolith submissions to the Minnesota Urolith Center varied over time. *Design*- Case series and case-control study. *Animals*-834 goats from the Minnesota Urolith Center of which 368 had calcium carbonate uroliths, and 16,366 control goats from the Veterinary Medical Database. *Procedures*-Information on breed, age, sex, reproductive status, season of submissions, geographic location and anatomic location in goats were used to identify risk factors. Changes in the yearly urolith submission frequencies were also evaluated. *Results*- Breeds of African descent (Pygmy, Boer, Anglo-Nubian, and Nigerian Dwarf) and mixed breeds had a significantly higher risk of developing calcium carbonate uroliths (65%; n =239) than the combination of other breeds evaluated in this study (35%; n = 129). Neutered male goats had a significantly increased risk of developing calcium carbonate uroliths compared with control goats. A significant association was found between increasing age, geographical location, season and anatomic location and the detection of calcium carbonate uroliths. Calcium carbonate urolith submission rates significantly increased during the study period. There was an increase of 3% observed from 1984 to 1990. There was an increase of 12% from 1991to1997. There was an increase of 30% from 1998 to 2004. There was an increase of 41% from 2005 to 2009. There was an increase of 43% from 2010 to 2012. *Conclusions and clinical Relevance*-The results of this study suggest that the prototype goat with calcium carbonate uroliths was a neutered male, 2 to 6 years- old and of African descent. While these observations indicate risk factors for calcium carbonate urolithiasis in goats they do not represent a cause- and- effect association.

PS-18

## Comparison of two sample types for detection of *Mycoplasma hyopneumoniae* in naturally infected pigs

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This study was conducted to compare nasal vs. laryngeal swabs for detection of *Mycoplasma hyopneumoniae (Mhp)* in naturally colonized and infected pigs at different ages. Paired nasal and laryngeal swabs were collected randomly from 108 piglets at weaning age in a sow farm. Paired nasal and laryngeal swabs were collected randomly from 60 pigs in two finishing sites. Nasal swabs were collected by introducing a swab into each nostril at 45o angle latero-medial towards the nasal septum, and rotating the swab clockwise and counterclockwise. Laryngeal swabs were collected by opening the mouth with a mouth gag, depressing the tongue using a laryngoscope, and introducing a swab guided by the laryngoscope blade to swab the laryngeal area. In older pigs, a sanitized BIC® pen fits snug in the cap of a swab, and acts as an extension to reach the laryngeal area. Samples were submitted to the UMN-VDL for real time PCR (rt-PCR) testing. At weaning age, 33/108 pigs (31%) were positive by nasal swabs, 13/108 (12%) were positive by laryngeal swabs, and 41/108 (38%) by the combination of both sample types. At market age, 10/60 pigs (17%) were positive by nasal swabs, 18/60 (30%) were positive by laryngeal swabs, and 18/60 (30%) by the combination of both sample types. The most common sample type used to detect *Mhp* in the field is the nasal swab. On the other hand, experimental trials and field studies indicate that more sensitive sampling sites are located in the lower part of the respiratory tract, specifically the trachea and the bronchi. Results at weaning age showed that nasal swabs were more sensitive than laryngeal swabs, and the combination of both sample types increased the level of detection by 7%. At market age, more positive pigs were detected using laryngeal swabs. Results from this study suggest that a greater sensitivity in detection of *Mhp* could be obtained by selecting a sample type based on disease progression stage, which may be associated with the location of the pathogen in the respiratory tract of the pig.

PS-19

## Impact of dietary components on phenotypic measurements for Equine Metabolic Syndrome

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Previous studies in healthy horses have demonstrated that dietary adaptation has an impact on many of the phenotypic variables measured and used as diagnostic criteria for horses with presumptive equine metabolic syndrome (EMS). However, the relationship between dietary intake and measured responses in EMS horses is less understood. Our objectives were to evaluate the relationships between dietary components and phenotypic variables used as EMS criteria and to determine if the relationship differs based on obesity or previous laminitis status. We have collected biochemical and dietary data from 634 horses from 167 farms located throughout the United States and Canada. Biochemical variables were measured in the fasted state (insulin, glucose, triglycerides, NEFA and leptin concentrations) and after an oral sugar test (OST, glucose and insulin concentrations). For each individual, total daily dietary consumption was calculated by weighing the daily rations of hay and concentrate and estimating pasture consumption based on hours of pasture access/day. Concentrate, hay and pasture samples were analyzed by Equi-Analytical (Ithaca, NY). Total daily digestible energy (DE), crude protein (CP), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), and starch were calculated as a % body weight in dry matter intake. Correlations with dietary components adjusted for obesity/previous laminitis status, age, breed, sex, and exercise were determined using a mixed effects model with farm as a random effect. Interaction terms were included in the model to detect differences in the correlations between dietary components and EMS phenotypic measurements dependent on obesity/previous laminitis. Dietary components were found to be correlated with biochemical variables and the relationships did vary dependent on obesity/prior laminitis status, however these dietary correlations only explained a small portion of the total variation in EMS phenotypic measurements. Further research is needed to identify causes for individual differences in phenotypic responses to diet.

PS-20

## Dynamics of *Mycoplasma hyosynoviae* detection and clinical presentation

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This investigation was proposed with the objectives to: 1) Evaluate *Mycoplasma hyosynoviae* (*Mhs*) detection dynamics across a naturally challenged flow within a production system. 2) Assess the correlation of diagnostic data and incidence of clinical signs. A production system with a history of *Mhs* challenges was chosen for this study. Sampled groups were: GDU at 0, 4, 8, 12 &16 weeks post placement; sow farm 30 gilts/sows, and one piglet of their progeny. Piglets were serially tested at 0, 3, 5 & 7 weeks of age. Pigs at 10, 13, 16, 19 & 22 weeks of age were sampled cross-sectionally. Ten oral fluid and 30 tonsil swabs/group were collected and lameness evaluation was performed. Swabbing was directed at lame pigs. Clinical evaluation of gait was performed on pen/barn basis, and was expressed as a function of severity and percentage of lame pigs. Samples were assayed by real-time PCR for *Mhs* at the UMN-VDL. The correlation between diagnostics and clinical presentation was evaluated by Pearson's analysis. Sixty percent of sows tested positive, while their progeny were negative at 1-2 days of age, and 13% tested positive at 3 weeks of age. The decline in % positive samples in week 5 could be the result of antibiotic treatment. Oral fluids yielded consistently lower Ct values and greater % positive samples, suggesting it is a more sensitive tool for detection of *Mhs* colonization. Tonsil swabs were correlated with lameness score (R=0.82). Piglets were colonized prior to weaning, making it difficult to source *Mhs* negative piglets from a positive sow farm, and suggesting that colonization in the sow farm may play a role in the clinical presentation downstream. In this pig flow the GDU is operated as a continuous flow, causing potential exposure, shedding and propagation of the pathogen into the sow farm with the arrival of gilts. An increased % positive oral fluids and tonsil swabs at week 19, with a mild increase in clinical presentation suggests that surveillance and subsequent intervention may be a means of preventing severe clinical presentation in finishing.

PS-21

## The risk of Highly Pathogenic Avian Influenza (HPAI) transmission associated with the movement of turkey hatching eggs

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The emergency response in the event of highly pathogenic avian influenza (HPAI) outbreak in the United States would include quarantine and movement controls for poultry products. Such measures may adversely impact business continuity of turkey hatcheries given the limited holding capacity and the loss in hatchability of eggs with extended holding. The draft Secure Turkey Supply (STS) Plan provides guidelines for the movement of turkey hatching eggs during an HPAI outbreak and aims to support business continuity while minimizing disease spread. The key outbreak measures from the STS Plan include active surveillance, holding time before egg movement, sanitization of eggs, personnel biosecurity, and cleaning and disinfection of vehicles and equipment. We evaluate the risk of HPAI spread associated with the movement of turkey hatching eggs from flocks in a Control Area. Simulation models of HPAI disease in breeder flocks and detection via active surveillance were utilized in the risk evaluation. Qualitative methods were used to evaluate exposure pathways for horizontal transmission of HPAI virus from eggs to day-old poults via movements of people and equipment in the hatchery. A survey of turkey industry practitioners participating in the STS workgroup was conducted regarding the perception of risk associated with hatching egg movement and past outbreak experiences. The risk assessment results, outbreak experiences and practitioners' perceptions were consistent and indicate a low risk of HPAI spread due to the movement of turkey hatching eggs when outbreak control measures are strictly followed.

PS-22

## Molecular Bases of Type 2 Polysaccharide Storage Myopathy

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Polysaccharide Storage Myopathy (PSSM) is a muscle glycogen storage disease in horses characterized by accumulation of excess and abnormal skeletal muscle glycogen and clinical myopathy. A dominant mutation in *GYS1* has been described as a causative mutation in one form of PSSM (PSSM1). However, a significant portion of PSSM horses cannot be attributed to this mutation and evidence points to a second heritable form of PSSM (PSSM2). The genetic mutation responsible for PSSM2 is unknown. Genome wide association analysis (GWAS) was performed in a cohort of 104 PSSM2 Quarter Horse (QH) cases and 124 QH controls, genotyped with the Illumina Equine SNP50 Beadchip. Basic and structured association tests identified 6 SNPs on equine chromosome 18 (350 kb region) highly associated with PSSM2. The region encompasses 2 annotated genes and a novel-protein coding mRNA. Attempts to sequence key functional elements in the region by Sanger sequence were not entirely successful and were hampered by apparent poor annotation and assembly, segments of highly repetitive sequence, and the likely presence of a large structural variant(s). Therefore we have applied a combination of approaches to evaluate sequence in this region that include next-generation sequencing (NGS) and de novo assembly of BAC clones, and long-range PCR to produce amplicons for NGS from 11 PSSM2 cases and 11 controls. Further, whole genome NGS of 3 PSSM2 cases and 3 controls has been performed (100 bp paired-end, 12x coverage, Illumina HiSeq). The amplicons and the whole genome sequencing data were mapped to the equine reference genome and no significant difference in variant frequency was observed between cases and controls. A de novo assembly was created from the BAC clones and encompasses the entire region of interest. The de novo assembled sequence contains 233kb through the promoter and 5' UTR of one gene of interest and encompasses 4 gap regions present in the reference genome with a total size of 1087 bp. Sequence reads are being mapped to the new contig to identify variants in the region.

PS-23

## National PRRSv Incidence Project

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Porcine Reproductive and Respiratory Syndrome virus (PRRSv) continues to devastate the United States (US) swine industry with losses approximated at $664 million annually (Holtkamp et al., 2013). In the fall of 2011, we began the first effort to describe the incidence and prevalence of PRRS infections United States. The program currently consists of a sample of 371 sow farms, representing approximately 1.2 million sows in 14 production systems across 15 states. Veterinarians working with these farms report weekly PRRSv status using the AASV classification system (Holtkamp et al., 2010) for each farm over a four year period from July 2009 – June 2013.

This project has revealed a strikingly repeatable pattern of PRRSv infections during the study period. During the last four years, new infections increased gradually in September, followed by a dramatic increase at the end of October, then plateauing in late February. Each year, 29-38% of the herds in the database have reported a new infection. Additionally, an exponentially weighted moving average of the weekly incident cases indicates the onset of the annual PRRSv epidemic in mid to late October every year. The project may be used to identify significant predictors of new infections and may allow for unique spatio-temporal analyses using Geographic Information Systems (GIS) to identify clusters or disease hot spots in different regions across the US and potentially study movement patterns.

This is the first scientific effort to study the incidence of PRRS virus in a large sample of US sow herds. Due to the voluntary nature of the participants this cohort may not be representative of the entire US sow herd; however, preliminary results are extremely consistent across four years of data and between systems.

The authors appreciate the willingness of the participating farms and veterinarians to share their data. They would also like to acknowledge ongoing funding and support from the National Pork Board and the USDA PRRS Coordinated Agricultural Project for providing the initial project funding.

PS-24

## Capturing the cough: Understanding the impact of respiratory disease on a habituated population of great apes.

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Infectious disease is increasingly recognized as a threat to free-living populations of great apes. In particular, respiratory disease outbreaks have contributed to significant levels of morbidity and mortality among habituated great ape populations. Accordingly, a review of 47 years of data on a habituated community of chimps in Gombe National Park, Tanzania revealed that almost half of all health-related mortalities were respiratory in nature. In response to these findings, the Gombe Ecosystem Health Project (GEHP), a syndromic surveillance system, was created to collect baseline and outbreak health data on two habituated chimp communities. An analysis of 8 years of GEHP data is underway to better understand the impacts of respiratory disease and the efficiency of monitoring methods in capturing health data. From 2004-2012, the baseline occurrence of respiratory disease in these communities, characterized by any combination of cough, sneeze, or rhinorrhea, was fairly low, with an average of less than one chimp per month showing any of these signs. The most frequently observed respiratory sign in both communities was cough. During the analysis period, 4 large respiratory disease outbreaks occurred. Two outbreaks affected the Mitumba community alone, one the Kalande community, and the fourth started among a small number of chimps in Mitumba and quickly spread throughout much of Kalande. The outbreaks averaged a 2-week transmission duration (duration of new case identification), with a maximum of 4 weeks of observed clinical signs. No mortalities were associated with the outbreaks although observed morbidity averaged 49% (± 30%, range: 30-100%) in each community. Further analysis for clusters of chimps with respiratory signs exceeding the upper 95% confidence limit of the monthly baseline average revealed 2 additional smaller scale outbreak events in each community. These and ongoing analyses will be used to generate recommendations for outbreak response, including recommendations for targeted, noninvasive diagnostic sampling and enhanced veterinary monitoring.

SS-1

## Investigation of putative functional alleles underlying gait in the Standardbred horse

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The Standardbred horse, best known for its use in harness racing, is separated into two distinct lines based on the gait in which they are trained to race: trotters and pacers. The trot is a two-beat diagonal gait in which front and hind legs on opposite sides of the horse's body move together, while the pace is a symmetrical gait in which the front and hind legs on the same side move forward and back at the same time. A genome-wide association (GWA) analysis of 494 Standardbred trotters and pacers revealed multiple regions in the equine genome that were significantly associated with gait. To investigate these regions more thoroughly, eighteen horses (9 pacers and 9 trotters) were selected for whole genome sequencing. Quality control, sequence alignment, and variant discovery with BWA and GATK, respectively, were carried out using the University of Minnesota Supercomputing Institute's Galaxy platform. Variants found within the regions of interest were filtered to exclude intergenic variants and variants that did not segregate with gait, leaving 4,865 genetic variants for further analysis. Of these variants, 45 had predicted functional effect based on the current annotation of the equine genome. On equine chromosome 17 (ECA17), 712 variants segregated perfectly with gait in the 18 sequenced horses, demarcating an ~5 Mb region. Follow-up genotyping for five variants with putative functional effect in this ~5Mb region on ECA17 was performed using Sanger sequencing or restriction fragment length polymorphism (RFLP) in 384 Standardbreds, including a subset of the original GWA population (n = 224) as well as an independent population (n = 160). All five variants were found to be significantly associated with gait ($p < 2.2 \times 10^{-16}$). Manual annotation of the equine genome is underway in the regions of interest to determine if additional potentially functional variants exist at loci that are incompletely annotated in the published equine genome.

SS-2

## The relationship between depression and thermoregulation using the forced swim test

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Although depression is a common disorder, further research is necessary to completely understand the mechanism behind it, thus improving diagnosis, treatment, and prevention. In studies of depression, mice are commonly subjected to a forced swim where immobility is a reflection of depressive behavior. The severe hypothermia that develops is stressful and believed to be necessary to unmask or reveal depressive behavior. Repeated daily swims initially increase immobility over the first few days, but then tolerance to this effect develops. Our hypothesis is that the regulation of mood and thermoregulation share common neurotransmitters and, as a result, body temperature in response to a cold stress (i.e. defense against cold) is a reflection of depression. To test the ability of cold verses warm stress to create a model of depression and to assess the importance of warm verses cold stress in detecting depressive behavior, mice were monitored for body temperature and immobility during a daily cold or warm swim occurring over an extended period. On the last day of the study, the temperature of the swim was switched and body temperatures and immobility were monitored once again. Through comparisons of immobility and body temperature, we will determine whether chronic cold or warm stress increases depressive behavior and correlational analyses will test the relationship between depression and thermoregulation.

SS-3

## Overexpression of CuZnSOD in median preoptic nucleus attenuates chronic angiotensin II hypertension

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Despite ongoing advancements, hypertension is still one of the most damaging cardiovascular problems in the world. The central nervous system plays an important role in blood pressure regulation by monitoring angiotensin II and osmolality levels and adjusting sympathetic nervous system and hypothalamic-pituitary output accordingly. The brain receives these signals through circumventricular organs including the forebrain subfornical organ (SFO) that is directly connected to the median preoptic nucleus (MnPO). Previous studies have shown that overexpression of superoxide dismutase via adenoviral vectors in the SFO attenuated chronic low dose angiotensin II hypertension. Therefore, the current hypothesis is that overproduction of O2•- in the downstream MnPO is a signaling factor and an underlying mechanism for the long term hypertensive effects of chronic angiotensin II. To test this hypothesis, adenoviral vectors capable of transgene expression of human copper/zinc superoxide dismutase or containing an empty control vector were injected into the MnPO. Rats were implanted with telemetric transmitters in their aortas for recording of heart rate and mean arterial pressure. After a three day control period of saline infusion, rats were then infused with angiotensin II for ten days. Preliminary data showed that rats overexpressing superoxide dismutase in the MnPO had a blood pressure increase of only 10 mmHg after seven days of angiotensin II infusion while the blood pressure rose approximately 30 mmHg in control rats. These results support the hypothesis that O2•- in the MnPO has a role in the hypertensive response to chronic angiotensin II.

SS-4

## Evaluation of bacteriophages to inactivate antibiotic resistant shiga toxigenic O26 and O103 *E. coli* strains

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Background: The purpose of this study is to evaluate bacteriophages as an effective biological control method, to reduce the load of Shiga Toxin producing *Escherichia coli* (STEC) in the food supply, under typical storage conditions (4°C), abuse refrigeration temperature (8°C) and at room temperature (23°C). The study also aims to determine whether antibiotic resistance provides additional survival advantages against bacteriophages. Methods: *E. coli* O26 and O103 serotypes (susceptible and multi-antibiotic resistant strains) were grown overnight, washed, diluted and inoculated ($10^6$ CFU/mL) into tryptic soy broth (TSB). A phage cocktail (N=7) was previously made (Stelios et al. 2011), and added to the tubes to achieve a multiplicity of infection (MOI; PFU/CFU) of 10, 100 and 1000. Tubes were incubated at 4°, 8° and 23°C. Samples were collected at 0, 0.5, 1, 24 and 48 h and bacterial counts were determined. Results: At 4°C, all *E. coli* strains showed similar survival kinetics with phage treatment. *E. coli* counts did not change at MOI 10 or 100 at 4°C. At 1000 MOI, a 1 log reduction (CFU/mL) in the O26S strain and 0.75 log (CFU/mL) reduction in the O103S strain was observed after 24 and 48 hours of treatment, respectively. O26R and O103R remained constant at 1000 MOI. At 8°C, about 4 log (CFU/mL) reductions were achieved for O26S, and 2 log (CFU/mL) reductions were observed for the O26R at 1000 MOI. At 23°C, bacterial counts of the R and S strains declined 3-5 log (CFU/mL) within the first hour; however, after 24 h, the strains showed resistance to phage treatment and populations began to rebound. Conclusion: At 4°C and 1000 MOI, the phage cocktail was more effective at lysing the susceptible strains than the resistant strains. Though, at 4°C, the phages did not appear to be an effective method of control. However, at 8°C and 1000 MOI, the phage cocktail was found to be effective. At 23°C, the phages were able to reduce the *E. coli* population for a short period of time but did not maintain the population at a reduced level for longer periods (24h and 48h).

SS-5

## Transcriptome comparison of equine adipose tissue from the tailhead, omentum and nuchal crest

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In humans, a wealth of data suggests that the location of adipose tissue deposits differ in metabolic activity and function, with certain depots, such as omental fat, strongly linked to metabolically unhealthy phenotypes, including metabolic syndrome (MetS). Likewise, a similar syndrome in horses, termed equine metabolic syndrome (EMS) is characterized by differential and abnormal fat distribution; however, little is known about the metabolic activity of various equine adipose tissue depots. As a first step in understanding differences in adipose tissue depots in adult horses, differences in the transcriptome and gene expression in three adipose tissue depots were explored. For this comparison, total RNA was extracted from tailhead, omental, and nuchal crest fat depots of four normal horses. For each adipose tissue sample (n=12 total) mRNA was extracted and sequenced using Illumina HiSeq (RNAseq, 100bp, paired-end, 20 million reads per sample). RNA-Seq reads were mapped to the annotated equine genome using Tophat, and transcript and gene expression was compared using the Cufflinks-Cuffdiff pipeline. Differences in gene expression among individuals and among adipose deposit locations were determined using DEGseq. Exploration of the equine transcriptome relating to metabolic disorders may provide greater insight to gene function and differences between adipose tissues. This project was supported by the National Center for Research Resources and the Office of Research Infrastructure Programs of the National Institutes of Health through Grant Number T35 OD011118.

SS-6

## Evaluation of *Neospora caninum* abortions and serology in Minnesota from 1991 to 2011

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*Neospora caninum* is a parasitic disease that is known to cause abortion in cattle and neurologic disease in canid species. The actual transmission patterns of *N. caninum* in Minnesota are not fully understood and require further research. A University of Minnesota (U of M) study is just starting, aiming to evaluate the prevalence of *N. caninum* within select Minnesota cattle herds and within both domestic and wild canids. The Veterinary Diagnostic Lab (VDL) at the U of M has collected data regarding *N. caninum* abortions and serology over the past 21 years. Veterinary anatomic pathologists at the VDL used microscopic analysis of fetal diaphragm tissue to diagnosis the abortion cases. For this study, summary statistics were calculated using the database of VDL sample submissions and *N. caninum* cases. These statistics were then evaluated for annual or seasonal trends, and used to determine variation between breeds. Between the years of 1990 and 2011, 6950 abortion cases were submitted to the VDL for pathological analysis. Of those cases, 310 were diagnosed as abortions caused by *N. caninum*, which is 4.5% of the total abortion submissions. Between the years of 2001 and 2011, 919 samples were tested for *N. caninum* exposure using an indirect Enzyme-Linked Immunosorbent Assay (ELISA). Of the 919 tested samples, 168 samples tested positive for *N. caninum*, which is 18.2% of the total tested samples. Both the abortion submissions and serology submissions exhibited trends of increased positive samples in June and July, respectively, suggesting the potential for an increase in *N. caninum* parasitism in the summer. Further work needs to be completed to characterize seasonal trends of *N. caninum* in the state of Minnesota. In particular, age data needs to be collected to make conclusions regarding serology and time of exposure. Determining the prevalence of *N. caninum* across domestic and wild populations in Minnesota will provide valuable insight into the economic impact of this disease, particularly in the dairy and beef industries.

SS-7

## The genetic basis of calcium oxalate urolithiasis in Miniature Schnauzers

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Breeding practices that are responsible for desirable traits in dogs can inadvertently propagate deleterious genetic mutations that result in breed-specific predispositions to disease. Miniature Schnauzers are at high risk for calcium oxalate (CaOx) urolithiasis (urinary stone disease) relative to mixed breed dogs, and a genetic component to disease is suspected. The purpose of this study was to execute a genome-wide association study (GWAS) to identify the genetic basis of calcium oxalate urolith formation in Miniature Schnauzers. The GWAS consisted of 72 CaOx stone-forming cases and 42 stone-free controls. A logistic regression was performed using sex as a covariate. A strong statistical signal was found on canine chromosome 29 ($p_{raw}$ = 1.9x10^-5, $p_{genome}$ = 0.05), located 168 Kb upstream from a solute carrier gene, *Slc26a7*. NextGen sequencing of 1 case and 1 control was performed to evaluate the gene. There were two variants of note, a 10 bp deletion and a 5 bp deletion in intron 3. We are now genotyping the study population plus an additional cohort for these deletions to test their strength of association with CaOx urolithiasis. We are also sequencing cDNA to determine if the variants affect splicing. *Slc26a7* is a transporter of chloride, sulfate, and oxalate and is expressed almost exclusively in the kidney. Additionally, solute carrier mutations are responsible for a genetic risk for urate urolithiasis in Dalmatians (*Slc2a9*) and cystine urolithiasis in Newfoundland dogs (*Slc3a1*). The strong association signal together with the known function of *Slc26a7* makes it an ideal candidate gene. Funding was supplied by the University of Minnesota College of Veterinary Medicine, the Morris Animal Foundation, and the Merial Veterinary Scholars Program.

SS-8

## Characterization of age-related susceptibility of macrophages to Porcine Reproductive and Respiratory Syndrome Virus

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Porcine Reproductive and Respiratory Syndrome (PRRS) is the most economically important disease affecting swine production in the United States today. Age dependent resistance to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has been observed, with young pigs experiencing longer periods and higher levels of viremia compared to older pigs. PRRSV targets pulmonary alveolar macrophages (PAMs) and cellular surface receptors CD163 and CD169 have been identified as important for infection. However, mechanisms underlying age-related differences as well as the specifics of the immune response to PRRSV remain unclear. Preliminary evidence suggests PAMs belonging to older pigs are more resistant to PRRSV compared to those from younger pigs. We hypothesized that age-related resistance to PAM infection results from decreased infection of cells due to differential expression of surface receptors for PRRSV. PAMs isolated from six pigs of different age groups (3 days old, 10-12 weeks, and adult) were infected with PRRSV. Level of PRRSV infection and expression of CD163 and CD169 were analyzed by flow cytometry at 12 hours post-infection (HPI). Viral replication was compared at 12, 24, and 48 HPI by qRT-PCR. A higher percentage of PAMs from younger pigs were infected and yielded greater amounts of virus compared to those from older pigs. Level of infection for PAMs from 10-12 week old pigs was more similar to that of PAMs from the adults. CD163 and CD169 expression was not different between age groups. In conclusion, PAMs isolated from older pigs are more resistant to PRRSV infection compared to those from younger pigs. This age-related PAM resistance to PRRSV infection is not due to differential levels of CD163 and CD169 expression. In the future, we hope to identify differences responsible for the age-related resistance. These may include cellular receptor polymorphisms, innate anti-viral gene response, and differential macrophage polarization.

SS-9

## The safety and efficacy of a nutraceutical in the control of pain associated with osteoarthritis

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Osteoarthritis is a disease that affects up to 80% of dogs over eight years of age. The severity of the disease is variable as are the methods used in treatment. Nutraceuticals, considered food additives by the FDA, receive little oversight addressing their safety or efficacy. However, because of nutraceutical availability to the public, their usage is prevalent for the treatment of osteoarthritis. The objective of this study was to assess the safety and efficacy of a commercially available nutraceutical for the treatment of pain and lameness in dogs with osteoarthritis. This was a randomized, blinded, placebo-controlled pilot study with 20 canine participants. Prior to enrollment, participants could not be treated with steroids, non-steroidal anti-inflammatory drugs, analgesic medications, and other glucosamine and/or chondroitin products. The dogs remained off of these products for the duration of the study. Patients with joint pain secondary to osteoarthritis were enrolled in the study; the presence of osteoarthritis was confirmed radiographically. Each dog was fitted with an accelerometer (activity monitor) collar, previously validated, for 7 days prior to starting the supplement or placebo. In addition, owners completed a validated osteoarthritis questionnaire (Canine Brief Pain Inventory) before enrollment and at each reexamination. After 7 days of monitoring normal patient activity each patient was randomized to the treatment or placebo group. Patient activity was monitored every 60-seconds for the duration of the 49 day treatment period. Patient exams and owner questionnaires were completed on day 0, 28, and 49 of the study. Preliminary results (n=12/20) found a significant difference in patient activity in participants receiving the nutraceutical in comparison to the placebo treated dogs. Based on this preliminary information the use of this nutraceutical for the control of pain from osteoarthritis in dogs is supported.

SS-10

## Molecular Mechanisms that Drive Osteosarcoma Progression

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Osteosarcoma (OS) is the most common primary bone tumor of dogs and humans. In dogs, it is primarily a disease of large and giant breed dogs, whereas in humans, it primarily affects children and adolescents. The molecular characteristics of this disease are highly conserved between both species; previous research from our lab has shown that OS can be classified into two distinct molecular groups: a highly aggressive, rapidly progressing form and a less aggressive, more indolent form. Recent data suggest that the RB tumor suppressor gene is at least partly responsible for the gene expression signatures that differentiate these two groups. For this project, we are using genetic approaches in canine OS cell lines to establish the role of RB tumor suppressor protein in rapidly progressing OS. Specifically, we are characterizing endogenous RB-associated complexes in RB-intact OS cell lines, and we will compare these complexes to those formed when a CDK-Insensitive RB (super RB) construct is ectopically introduced to RB-deficient OS cell lines. We will purify complexes by immunoprecipitation of RB to detect the presence of E2F-1 and HDAC-1 by immunoblotting. Our results will allow us to establish mechanistic links between transcriptional control and chromatin organization mediated by RB and the biological behavior of canine and human osteosarcoma.

SS-11

## Serological evidence of pathogenic paramyxoviruses circulating in wild birds in the upper midwest

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Avian paramyxovirus serotype-1 (APMV-1) is the causative agent of virulent Newcastle disease (vND) and is known to infect over 240 species of birds. APMV-1 is associated with a severe neurologic disease in juvenile Double -crested Cormorants (*Phalacrocorax auritus*) and American White Pelicans (*Pelecanus erythrorhynchos*). Little is known about where the virus persists in the environment, how it is spread among wild bird species and the extent to which these strains may be introduced into poultry. Epizootics have occurred regularly in Minnesota waterfowl since the 1990's, most recently in 2008, 2010 and 2012. We hypothesize that maternal antibody inheritance one year after a major outbreak provides hatchlings with short-term protection against infection. To assess the prevalence of APMV-1, 380 waterfowl eggs were collected in May 2013 from 7 lakes in Minnesota and tested for antibodies using a commercially available ELISA kit. The antibody prevalence ranged from 54.8% to 100% among 7 cormorant populations. Overall, an average of 71.3% of cormorant eggs (n=328), 71.4% of pelican eggs (n=21) and 3.4% of Ring-billed Gull eggs (*Larus delawarensis*) (n=29) tested positive for antibodies to APMV-1. Although gulls share nesting habitat with cormorants and pelicans, they do not appear to be affected by vND during outbreaks. The high prevalence of antibodies in eggs one year after a major outbreak suggests that passive immunity may contribute to the biennial nature of APMV-1 outbreaks in Minnesota waterfowl.

SS-12

## Inhibition of defense against the cold during a forced swim as an indication of antidepressant activity

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Although depression is a common mental illness, its etiology is not fully understood, and it is clinically difficult to treat. Several weeks of treatment are necessary to produce an effect in patients undergoing drug therapy. The forced swim test is a common method of testing compounds for antidepressant activity. The amount of time rodents spend floating (i.e. immobility) is used as a measure of depressive behavior that is decreased by antidepressant compounds. A prior conditioning swim increases immobility, an effect that correlates with an increased ability to defend against cold. This can be prevented by the antidepressant compound, amitriptyline. Because immobility in the forced swim test correlates with behavioral thermoregulatory responses, and because both are sensitive to amitriptyline, we hypothesize that a variety of antidepressants influence both measurements, allowing body temperature to be used to screen for antidepressant activity. To test this, various classes of antidepressant drugs were compared in their effects on floating time and body temperature during two daily forced swims. Regardless of their effect on immobility, all classes tested (tricyclic, SSRI, atypical/aminoketone, atypical/tetracyclic) blocked the increase in body temperature during hypothermia of the second swim. This demonstrates that temperature may be used as a more objective and less time-consuming measure of antidepressant activity than immobility, which is not only more time consuming, but also subjective. Funding by NIH grant AR056092 from the National Institutes of Arthritis and Musculoskeletal and Skin Diseases. This project was supported by the NCRR and the ORIP of the NIH through Grant Number T35 OD011118.

SS-13

## Establishing methodologies for blood coagulation assays in red-tailed hawks (*Buteo jamaicensis*)

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Raptors that commonly prey on rodents are at risk of developing coagulopathies from secondary poisoning where anticoagulant rodenticides, such as brodifacoum, are widely used. This includes areas of agriculture, urban/suburban pest control, as well as island rodent eradication programs across the world. These rodenticides disrupt vitamin K recycling required for the production of vitamin K dependent coagulation factors, resulting in increased clotting times and impaired coagulation, leading to hemorrhage and anemia. Clotting times are therefore the key diagnostic tool used to assess rodenticide poisoning. Baseline values for blood coagulation assays have been established for poultry, but not in any raptor species. For this study, activated clotting time (ACT), whole blood clotting time (WBCT), and prothrombin time (PT) tests were evaluated in Red-tailed hawks (*Buteo jamaicensis*), one of the most common rodent eating raptor species in North America, to establish normal reference ranges. ACT uses an activator (kaolin) to initiate clotting and assesses the intrinsic pathway. WBCT evaluates the intrinsic pathway without the use of an activator. PT uses thromboplastin and a source of calcium in a point of care analyzer to assess the extrinsic pathway. We hypothesize that reference values for ACT, WBCT, and PT will be correlated with one another and yield a normal distribution that can be used for future evaluation of coagulation capacity in Red-tailed hawks. These baselines will aid in diagnosing and treating rodenticide poisoning in raptors clinically in rehabilitation and in conservation efforts where the effects on these predators may be monitored over a period of time.

SS-14

## Development of a panel of immune function assays for assessing subclinical immunotoxicity in raptor species

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Raptors are considered biological indicators of the health of their environments. In extreme cases, environmental contaminants such as lead, rodenticides, and organochlorine pesticides produce population effects that can present as failed reproduction, neurological signs, hemorrhage, and death. When these contaminants are present in sub-lethal amounts, as well as a host of others including pharmaceutical run-off and endocrine disrupting agents, they may trigger subclinical effects such as alterations of immune function; currently, little is known about these subclinical effects in raptors. Here, we examine the reference values for a panel of in-vivo tests of immune function in two raptor species with differing migratory behavior: red-tailed hawks (*Buteo jamaicensis*) and great horned owls (*Bubo virginianus*). The panel of tests includes the phytohemagglutinin (PHA) skin test, hemagglutination assay (HA) to sheep red blood cell (sRBC) immunization, fibrinogen levels, along with complete blood counts. The PHA skin test is thought to primarily be a measure of T-cell response while the HA to sRBC injection is a measure of humoral immunity. Fibrinogen is an acute phase protein that can be elevated during inflammation. While two of four great horned owls examined showed signs of concurrent infection, red-tailed hawks exhibited stimulation to PHA injections (n=5) and sRBC immunizations (n=6). Reference values obtained for the panel of immune function tests suggest that red-tailed hawks are amenable to future immunotoxicity studies; these values are essential to determining subclinical effects that contaminants have on immune function in this species.

SS-15

## Variation in lyme disease prevalence in *Ixodes scapularis* nymphs in four geographic regions of Minnesota

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Lyme disease is the most frequently reported vector borne disease in Minnesota. Reported Lyme cases have increased dramatically since the 1990's likely due to the expansion of tick range in Minnesota, increasing tick infection rates, and increasing awareness of physicians and the public. Lyme disease is a difficult disease to diagnose due to nondescript clinical signs; therefore a deeper understanding of the environmental factors that increase transmission rates to humans in various locations in the state will expedite identification and diagnosis of the disease. Black-legged tick (*Ixodes scapularis*) nymphs pose the greatest public health risk due to their small size and ability to remain attached without detection for the 24-36 hours required for transmission. We hypothesize that nymph densities and Lyme infection prevalence in nymphs will vary in different habitats throughout Minnesota, and further, that these risk factors will correlate with reported incidence of Lyme disease in humans. We used a tick drag sampling method to assess nymphal densities in four geographic regions of Minnesota with diverse forest habitats: northeastern, north central, central and southeastern. The highest nymph densities were found in the north central and central regions of the state, this correlates with the highest reported rates of Lyme disease in Minnesota. The nymphs will be analyzed to assess their infection rate with the Lyme agent, *Borrelia Burgdorferi*, to determine variation in infection rates among regions of the state. Land cover characteristics surrounding the sample locations will be analyzed to ascertain the role of microhabitats in determining nymph density and disease risk.

SS-16

## Role of CSF-1R$^{high}$ cells in hemangiosarcoma drug resistance

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Canine hemangiosarcoma (HSA) is an aggressive and highly metastatic tumor in dogs. Tumors are often chemoresistant to therapies such as doxorubicin. We identified a subpopulation of cells from HSA cell lines that express high levels of colony stimulating factor 1 receptor (CSF-1R$^{high}$) and are 3-7 times more resistant to doxorubicin than their CSF-1R$^{low}$ counterparts. CSF-1R$^{high}$ cells also appear to sequester doxorubicin intracellularly. Thus, we hypothesized that CSF-1R$^{high}$ cells evade doxorubicin activity by sequestering the drug within their lysosomes, and that disruption of lysosomal integrity might restore drug sensitivity. To test our hypothesis, we incubated unenriched (CSF-1R$^{high+low}$) HSA cells with doxorubicin followed by incubation with Lysotracker, a fluorescent dye that labels lysosomes. We also established a dose response curve with mefloquine, an antimalarial drug that disrupts lysosomal membranes. We noted co-localization of doxorubicin and Lysotracker, confirming doxorubicin sequestration. Mefloquine also reduced HSA cell viability. Studies are underway to determine if mefloquine disrupts doxorubicin and Lysotracker colocalization in lysosomes from CSF-1R$^{low}$ and CSF-1R$^{high}$ populations, and more specifically, if CSF-1R$^{high}$ cells preferentially sequester doxorubicin. Such results would emphasize the contribution of CSF-1R$^{high}$ cells in drug resistance and indicate disruption of lysosomes or lysosomal pathways as a method to restore drug sensitivity. Research Support: This work was supported by a Comparative Medicine Signature Program Grant through the University of Minnesota College of Veterinary Medicine. Student Support: Supported by the NCRR and the ORIP of the NIH, Grant Number T35 OD011118.

SS-17

## Effects of supplementing poor quality colostrum with colostrum replacer on passive transfer of IgG and calf health

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Colostrum is a key source of immunoglobulins (IgG) for newborn calves. Current recommendations suggest feeding calves 150 to 200 g of IgG in the first colostrum feeding within 2 hours of birth. However, maternal colostrum (MC) quality is variable and feeding poor quality colostrum (<50 g/L IgG) puts calves at risk of failure of passive transfer and disease. A potential solution to this problem would be to use a Brix refractometer to quantify the concentration of IgG and then supplement with powdered colostrum replacer (CR) to achieve a target of 200 grams of IgG. The objectives of this project are to describe if colostrum replacer powder can be added directly to poor quality MC to achieve significant improvement in passive transfer and serum IgG status of the calf without causing hyperosmotic diarrhea or D-lactic acidosis. The study is being conducted on a commercial Holstein dairy farm in Wisconsin. Eligible calves are enrolled at birth, weighed, and then randomly assigned to be fed one of four colostrum treatment groups by esophageal tube within 2 hours of birth: 1) 200 g IgG in 3.8 L good quality MC, 2) 100 g IgG in 3.3 L low quality MC plus 100 g IgG in 500 g non-reconstituted CR powder (total volume of 3.8 L), 3) 100 g IgG in 2.4 L low quality MC plus 100 g IgG in 1.4 L reconstituted CR, or 4) 200 g IgG in 3.8 L CR . Twenty mL of the colostrum treatment is collected and frozen for later IgG (g/L) measurement. Blood is collected from the calf at 0 and 24 hours for serum total protein (g/dL) and serum IgG (mg/ml) measurements. Blood is also collected for packed cell volume measurements at 0, 24, and 48 hours. Fecal pH is being measured at 24, 48, 72, and 96 hours. Fecal D-lactate is measured at 48 and 96 hours. Calf health scores are taken at 0, 24, 48, 72, and 96 hours. Calf enrollment will be completed by August, 2013 and data analysis will be conducted in the fall of 2013.

SS-18

## Canine brain tumor volumetrics: comparison of visual metric versus planimetry methods

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High-grade glioma tumors are one of the most prevalent primary brain tumors in dogs, yet they continue to have a poor prognosis. Median survival times range from 2 to 3 months, but all diagnosed cases expect a low survival time despite novel and aggressive treatments. The Ohlfest Brain Tumor Lab at the University of Minnesota is studying a cutting edge therapy aimed at minimizing to eradicating tumor recurrence after surgical removal for gliomas and other intracranial tumors. Lesion size quantification using magnetic resonance imaging (MRI) is commonly used as end point verification for research purposes. Historically, the product of the two or three largest orthogonal diameters has been used to calculate brain tumor size, but newer software has allowed for more precise measuring methods. We sought to compare the orthogonal diameter (visual metric) method against a manual tracing (planimetry) method. 30 MRI brain studies of client-owned dogs with histologically confirmed contrast enhancing gliomas were reviewed by two operators. Each operator calculated tumor volume by the visual metric and planimetry methods for each study. Variability was calculated for both inter- and intra-reader analysis. Our working hypothesis is that the manual tracing planimetry method will result in a decreased variability for both intra- and inter-operator calculations. Results have yet to be completed for all cases.

SS-19

# Development and validation of ELISA testing for serological monitoring and surveillance of porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDv) is a member of the *Coronaviridae* family and causes acute outbreaks of severe diarrhea and vomiting leading to significant mortality in suckling pigs. PEDv was first observed in Europe and is now a problem in Asia. Its emergence in the United States was first confirmed in May 2013 and has been identified in 15 states as of mid-July. Rapid, high-throughput diagnostic tests are needed in the US for serological monitoring and surveillance. We propose to create a PEDv ELISA assay that can be used in diagnostic labs to detect PEDv antibodies in pig serum, oral fluids, and feces. PEDv nucleocapsid (N) and spike protein domains 1 (S1) and 2 (S2) have been sequenced from multiple US PEDv isolates and are >99% similar, thus, recombinant proteins from any isolate should be representative of US isolates and should cross-react in an ELISA assay. In order to purify PEDv proteins, we have designed PCR primers to amplify N, S1, and S2. Amplified genes are then cloned into both pET-25b and pMAL-p5X vectors for bacterial expression. Both vectors will be used to increase the probability of obtaining large amounts of highly purified proteins. A 6X-his tag will be used for metal affinity protein purification (pET-25b), and maltose binding protein fusion for protein purification by amylose chromatography (pMAL-p5X). Once purified proteins are obtained, a direct ELISA will be developed for detection of anti-PEDv antibodies in pig serum. The protein or protein combination that gives the best ELISA results in serum will be further optimized for use with fecal and oral fluid samples.