CM-2

Maternal Dietary Folic Acid Deficiency Protects Against Medulloblastoma Formation in a Mouse Model of Nevoid Basal Cell Carcinoma Syndrome
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Nevoid basal cell carcinoma syndrome (NBCCS) is a hereditary condition caused by mutations in the PTCH1 gene that result in a wide variety of neoplasms. Approximately 5–10% of NBCCS patients develop medulloblastoma (MB), the most common malignant childhood brain tumor in the United States. Epidemiological studies have found an inverse association between maternal intake of prenatal vitamins, which contain folic acid (FA), and childhood brain tumors in offspring. We hypothesized that low maternal dietary FA levels during the peri-gestational period may decrease tumor incidence in mice genetically predisposed to tumor development. These findings may have implications for prenatal dietary FA intake recommendations for mothers in the presence of cancer syndromes.
Catecholamine Production by Vaginal Epithelial Cells: A Non-Neuronal Immunomodulatory Mechanism?

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We have previously reported (JNIP 5: S41, 2010) that epithelial cells in human vaginal tissue are immunoreactive for the norepinephrine transporter, which is normally expressed by neurons and is a site of cocaine action. We extended this initial finding by testing the hypothesis that vaginal epithelial cells produce and release catecholamines, and that these substances alter epithelial cell interactions with pathogens. Immunohistochemical experiments indicated that immunoreactivities for the catecholamine-synthesizing enzymes tyrosine hydroxylase and dopamine β-hydroxylase were present in two different human vaginal epithelial cell lines (ATCC CRL-2616 and a line generated at the University of Iowa). Furthermore, both epithelial cell lines were capable of synthesizing and secreting norepinephrine and dopamine in nanomolar concentrations. As previously observed (JNIP 6: S26, 2011), 10 μM norepinephrine, but not dopamine, enhanced interleukin-6 and interleukin-8 secretion in response to staphylococcal toxic shock syndrome toxin-1 (10-100 μg/ml). Norepinephrine also enhanced innate cytokine and chemokine responses to Staphylococcus aureus peptidoglycan (10 μg/ml), a component of the bacterial cell wall. Based on these findings, we hypothesize that vaginal epithelial cells may constitute a non-neuronal source of norepinephrine, which is capable of modulating immune responses to pathogenic microorganisms and their exotoxins.

Regulatory function of miR-708-5p on expression of CD38 in human airway smooth muscle cells (HASM)


Asthma is a common respiratory disorder characterized by chronic inflammation, reversible airway hyper responsiveness and remodeling of airway structure. The CD38, a transmembrane protein with multi enzymatic function, plays a role in the pathogenesis of asthma. Previous studies from our lab revealed that mice deprived of CD38, would show attenuated hyper-responsiveness compared to the wild type. HASM cells obtained from donors of fatal asthmatic patients showed increased expression of CD38 in the presence of TNF-alpha, when compared to healthy subjects. MicroRNAs fine tune the expression of gene by inhibiting the translation or by abolishing the transcripts. Since CD38 has a post transcriptional regulation via MAPK pathway, we determined to find out whether microRNAs also regulate the expression of CD38 post transcriptionally. The CD38 has many predicted microRNA target sites at its 3’UTR. We selected the microRNAs using more than two computational algorithms and if they expressed differentially in asthmatic HASM cells. MiR-708-5p is one of the two microRNAs we selected to study. Over expression of miR-708-5p significantly inhibited the expression of CD38 transcripts in a concentration dependent manner. Co-transfection of luc-reporter plasmid having CD38-3’UTR, with mimic of miR-708-5p, in NIH3T3 cell line significantly reduced the relative luciferase activity compared to the control. Mutation of the miR-708-5p target site, at 3’UTR of CD38, reversed the inhibitory effect of miR-708-5p on luciferase activity. This shows that miR-708-5p has target specificity at 3’UTR of CD38. When the antagonomer of miR-708-5p was transfected in HASM cells in the presence of TNF-alpha, they showed increased CD38 expression when compared to the mimic of miR-708-5p. This study shows that miR-708-5p can be used to control the expression of CD38 in HASM cells. MiR-708-5p may have a therapeutic potential to control the expression of CD38 in asthmatic patients.
**The Epidemiology of Shivers in Horses**

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Shivers is a poorly defined, chronic equine movement disorder. This study sought to characterize the epidemiology of Shivers. Data on signalment, onset, clinical signs, precipitating factors, effect of management, diet and treatments was obtained via a standardized web-based survey and a standardized video was also requested. Shivers was defined as hyper-flexion or -extension of the pelvic limbs during backwards walking and manual hoof lifting. Advanced Shivers cases additionally showed intermittent signs when forwards walking. Shivers horses were divided into 3 groups. 1) Confirmed (n=27): diagnosed by video submission or author examination, 2) Reported (n=67) and 3) Advanced (n=63): diagnosed by reported clinical signs ± video submission. The Control group consisted of 2 controls/Confirmed case that were normal, > 4 yrs old and in close proximity to the case. Groups were compared by Chi$^2$ or Fischers Exact tests with p<0.05.

The Confirmed group had significantly more WB, TB and Draft breeds, taller horses (>17hh) and males compared to the Control group (n=50). Clinical signs in the 3 affected groups frequently began at <10 yrs of age and consisted of farriery problems (Confirmed 96%:Reported 91%: Advanced 92%), muscle twitching (85%:85%:89%), muscle wasting (44%:34%:38%), weakness (33%:24%:43%) and exercise intolerance (30%:13%:24%). All 3 affected groups improved with reducing stress, increased turnout, exercise, increased fat/oil and vitamin E supplementation whereas NSAIDs, muscle relaxants, chiropractic and acupuncture therapy had minimal effect. Significantly more Confirmed cases showed progressively worsening signs over time (p<0.05) compared to Reported and Advanced cases (Confirmed 74%:Reported 43%:Advanced 63%).

In conclusion, Shivers often begins <10 yrs of age, is more common in male horses over 17hh, shows progressive signs in 43+% of cases and is best managed by increased activity, dietary fat/oil and vitamin E.

**Immunolocalization of VGF peptide processing in sensory neurons**

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VGF (non-acronymic), a neurosecretory protein of the chromogranin family, has been implicated in neuroplasticity associated with depression, learning and memory, and chronic pain. The 617 amino acid sequence of VGF contains over a dozen potential proteolytic sites, suggesting that a number of signaling peptides may be generated from the precursor. We have generated antisera that recognize different epitopes within the VGF C-terminal region (anti-TLQP21, anti-AQEE30, and anti-LQEQ19). The goal of these experiments was to characterize the patterns of immunostaining of these antisera in spinal cord and sensory neurons, which may reflect differential proteolytic processing of the C-terminal region of VGF. We compared labeling with anti-TLQP21, anti-AQEE30, and anti-LQEQ19 in L5 dorsal root ganglia from naïve rats and rats with nerve injury (spinal nerve ligation model) or sham surgery. Upregulation of VGF following nerve injury, as compared to sham and naïve animals, was confirmed, and there was consistent co-localization of labeling with the C-terminal antisera. However, the number of injured sensory neurons immunoreactive for the C-terminal antisera was much lower than prior results using antisera directed against the N-terminus of VGF. Further immunohistochemistry was performed on cultures of rat sensory neurons utilizing N-terminal antisera and combinations of C-terminal antisera. Approximately 80% of cultured neurons were immunoreactive to VGF antisera, and of these, there was co-localization of N-terminal antisera, anti-TLQP21, and anti-AQEE30 or and-LQEQ19 in approximately 75%. These studies suggest that, while VGF is upregulated in sensory neurons both in the nerve injury model and in culture, the levels of C-terminal peptides under these two conditions may be different, potentially reflecting differential proteolytic processing of VGF.
CM-7

**Candidate gene evaluation of equine neuroaxonal dystrophy based on genome-wide association analysis**

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During the first year of life, genetically-predisposed foals may develop the neurodegenerative disorder, neuroaxonal dystrophy (NAD). Currently, a definitive diagnosis of NAD requires post-mortem examination of the spinal cord and brainstem. NAD resembles ataxia with vitamin E deficiency in humans, an inherited disease caused by mutations in the α-tocopherol transfer protein (α-TTP) gene. Sequencing of α-TTP in affected and unaffected Quarter horses with NAD revealed no putative mutations. A genome-wide association analysis was performed with phenotyping based on clinical examination and exclusion of other neurologic disorders. 36 NAD affected and 58 unaffected Quarter Horses were genotyped using the Illumina 54KSNP platform. After 50,000 permutations, 21 significantly associated regions were identified (p<0.05). A large degree of population stratification was present (λ=2.69). A subset of the clinically phenotyped cases (n=23) and additional controls (n=63) were selected based on achieving minimal population stratification (λ=1.15) and genotyped on the Illumina 75K platform. Following permutations, there were no significantly associated regions identified (p<0.05). Therefore, after accounting for population dynamics, either enough power was not obtained to accurately map NAD or cases were potentially misphenotyped. To establish the most accurate phenotyping criteria possible, a final analysis was performed using 9 post-mortem confirmed cases of NAD and 65 controls. Following permutations, two regions of genome wide significance were identified (ECA8:62130705-62134644, p=0.029 and ECA28:5813320, p=0.02). In the region on ECA8, two candidate genes were present (RIT2 and PIK3C3) that are both involved in synaptic transmission. Sequencing of RIT2 revealed no putative mutations and sequencing of PIK3C3 has revealed two putative mutations (one exonic, one intronic) that require further evaluation.

CM-8

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

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Calcium oxalate (CaOx) urolithiasis (urinary stone disease) is a common and significant health problem. High heritability suggests a genetic component, but studies have failed to identify major susceptibility genes in humans. Dogs spontaneously form CaOx uroliths, and breed predispositions support genetic risk factors in the canine population as well. Research on metabolic and genetic determinants of canine CaOx uroliths will enhance the understanding of pathobiology in both species.

The first objective of this study was to compare urinary calcium and oxalate levels between breed-matched CaOx case and control dogs in three canine breeds: the Miniature Schnauzer, Bichon Frise, and Shih Tzu. We hypothesized that hypercalciuria 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.

Preliminary urinary data is available from 29 Miniature Schnauzer, 22 Bichon Frise, and 10 Shih Tzu dogs. Cases from all three breeds have significantly higher urinary calcium levels compared to the breed-matched controls. Urinary oxalate levels are not different.

The GWAS included 60 case and 37 control dogs. A strong signal was present on chromosome 37 (p_{raw} = 5x10^{-6}, p_{genome} = 0.004). Haplotype analysis identified a critical region that contains 18 protein-coding genes. Sequencing of positional candidate genes is currently underway. To date, two genes have been sequenced and one interesting variant in a solute carrier has been identified.
CM-9

Obesity suppresses allergen-induced airway inflammation but not airway hyperresponsiveness
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Objective: To evaluate the effects of diet-induced obesity (DIO) on allergic inflammation and lung function in allergic asthma. Methods: C57BL/6 mice maintained on standard chow (normal diet [ND], 18% calories from fat) or a high fat diet (HFD, 60% calories from fat) were sensitized and challenged with cockroach antigen (CRA). Control mice received saline at the same time points. 24 hours after the last allergen challenge, airway hyperresponsiveness (AHR) to methacholine was measured by invasive plethysmography and bronchoalveolar lavage fluid as well as lung tissue were collected to assess airway inflammation. Results: Relative to ND mice, HFD mice exhibited significantly decreased airway recruitment of inflammatory cells, specially eosinophils, reduced levels of lung Th2 cytokines (IL-5 and IL-13), LTC4, eotaxin-1 and MCP-1 but persistent AHR after CRA challenge. Interestingly, dynamic lung compliance (Cdyn), a measure of lung distensibility, in obese mice was significantly lower than in ND mice even in the absence of CRA challenge and remained unaltered by allergen exposure. Decreased Cdyn in these naïve obese mice was associated with increased lung collagen deposition and elevated levels of the fibrogenic factors TGF-β1 and hypoxia-inducible factor-1α relative to naïve ND mice. CRA-challenged obese mice exhibited elevated airway resistance (Rl) similar to ND mice despite minimal airway inflammation, absence of mucus hypersecretion and smooth muscle hypertrophy. These mice demonstrated significantly higher lung PAI-1 and airway epithelial arginase-1 expression as well as lower lung PGE2 levels compared to CRA-challenged ND mice which may explain the elevated Rl even in the absence of a strong inflammatory response. Conclusions: DIO results in reduced airway inflammation but persistent impairment of lung function after CRA challenge that is likely to be due to altered levels of PAI-1, arginase-1 and PGE2 as well as inherent fibrosis caused by obesity-induced increase in fibrogenic factors, thus contributing to the prevalence of asthma in the obese and overweight.

CM-10

CSF-1R Confers Differential Proliferative Capacity in a Subset of Hemangiosarcoma Cells Displaying Cancer Stem Cell Characteristics
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A population of cells within tumors with the exclusive ability to initiate and maintain disease has been recently identified and termed cancer stem cells (CSC). CSCs have been characterized in many cancers based on the expression of molecules associated with ‘stemness,’ ABC transport pumps, increased chemoresistance and the ability to exclude nuclear dyes in side population (SP) analyses. Identifying and characterizing CSCs may enable the design of therapies aimed at eliminating the cells responsible for tumorigenesis. We have previously described a subset of cells within the canine hemangiosarcoma (HSA) cell line SB-HSA possessing numerous properties of CSCs. Additional analyses revealed the presence of CD115 (colony-stimulating factor-1 receptor; CSF-1R), a molecule traditionally associated with cells of myeloid origin. Here we examine the function of CSF-1R in the pathogenesis of HSA by determining its role in maintaining the CSC phenotype of SB-HSA cells. We show that SB-HSA cells express progenitor molecules, CSF-1R and its cognate ligands CSF-1 and IL-34, ABC transporters, and contain a distinct dye-excluding SP. Functional analyses revealed that CSF1R+ cells divide more slowly than their CSF1R- counterparts. This suggests a putative CSF-1R+ CSC-like population for HSA that shares phenotypic, functional and proliferative properties reported for other CSCs. To define CSF-1R as a marker for a specific subset of HSA CSCs, further characterization is warranted including chemoresistance and in vivo tumor-initiation studies using both CSF-1R+ and CSF-1R- subsets. Further elucidating the properties of this CSF-1R+ CSC-like population may enable the design of novel therapeutic targeting strategies using receptor-specific inhibitors.
**CM-11**

**Vaccine Therapy for Spontaneous Canine Meningioma Extends Survival and Induces a Tumor-Reactive Antibody Response.**


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Most meningiomas are benign lesions managed with local therapies, but recurrent, inoperable, and malignant meningiomas lead to considerable morbidity and mortality. This study explored tumor lysate vaccination in 12 dogs with spontaneous meningioma as a model for more aggressive disease. Dogs were treated with surgical resection followed by vaccination with autologous tumor lysate plus immune adjuvant biweekly for 6 doses. Each dog had serial MRIs and blood collection for peripheral blood mononuclear cells and serum. Median survival for intra-institutional surgery monotherapy controls was 210 days, while median survival for vaccinated dogs (mean follow-up = 441 days) has not been reached. Eleven vaccinated dogs exhibited stable radiologic disease following surgery and all showed progressive increases in tumor-reactive antibodies. In contrast, one of 11 dogs tested had measureable expansion of tumor-reactive CD8+ T cells. Side-by-side histology comparing pre-treatment biopsies with post-treatment necropsies of 5 vaccinated dogs showed vaccine-associated increases in B and plasma cell infiltration. None had increased T cell infiltration adjacent to the resection site. Vaccine immunotherapy is a promising approach for inoperable and recurrent meningioma that likely acts through a polyclonal antibody response.

**CM-12**

**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 (catalog no. MPXPRCYTO40KPX23) for the simultaneous measurement of 23 cytokine concentrations was evaluated for use in a pool of serum collected from 2 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-13

**ORMDL3 regulates eosinophil trafficking, migration and activation during allergic inflammation**

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Using genome-wide association and microarray approaches, _orosomucoid 1-like 3 (ORMDL3)_ has been identified as a candidate gene for susceptibility to asthma, however, the function or mechanism by which this gene may contribute to asthma predisposition is not known. Our studies show that inflammatory cells recruited to the airways of allergen-challenged mice express ORMDL3. Further, we have established that ORMDL3 is expressed by murine lung and bone marrow eosinophils (Eos) wherein it is predominantly distributed in the endoplasmic reticulum. Since Eos are major pro-inflammatory cells contributing to the exacerbation of allergic asthma, we investigated the potential role of ORMDL3 in regulating their trafficking and activation. Exposure of Eos to mediators of allergic inflammation such as IL-3 and eotaxin-1, but not IL-5 or RANTES, induced ORMDL3 expression. Over-expression of _ORMDL3_ in Eos resulted in distinct cytoskeletal rearrangement, ERK phosphorylation and nuclear translocation of NFκB associated with increased rolling on VCAM-1 under conditions of flow. Knockdown of _ORMDL3_ with siRNA significantly inhibited activation-induced shape changes and adhesion to VCAM-1 and ICAM-1. Further, _ORMDL3_-silenced Eos demonstrated significantly reduced migration in response to eotaxin-1 along with reduced expression of CD49 (α4 integrin), CD18 (β2 integrin) and CD11b (Mac-1). ORMDL3 was also found to regulate expression of CD48, a cell surface molecule which plays a role in Eos activation (degranulation) upon ligation. _ORMDL3_-silenced Eos demonstrated decreased degranulation in response to activation with anti-CD48 antibodies. Overall, our studies for the first time demonstrate that allergen exposure induces recruitment of ORMDL3-positive inflammatory cells, including Eos, to the airways. Further, inflammatory mediators associated with allergic inflammation can induce expression of ORMDL3 by Eos which in turn can promote (i) trafficking and migration via regulation of cell surface adhesion molecule expression as well as (ii) activation/degranulation by regulating CD48 expression.

CM-14

**Characterizing the role of the alternative NF-kB pathway in diffuse large B-cell lymphoma**

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma in humans and dogs and has similar biology and clinical behavior in both species. Therefore, comparative approaches in understanding the disease may be beneficial to both species. The deregulation of nuclear factor kappa B (NF-kB) pathway, composed of classical and alternative pathways, is important in the pathogenesis of DLBCL. However, studies have largely focused on the classical pathway and the role of the alternative pathway is incompletely understood. In this study, we characterize the alternative NF-kB pathway in DLBCL to test its potential as a therapeutic target.

Methods: The activation of NF-kB pathways was analyzed by the expression, nuclear translocation, and binding to the NF-kB oligonucleotide probe, of classical and alternative NF-kB proteins in primary dog DLBCL cells using western blotting and electrophoretic mobility shift assay.

Results: We demonstrated that both classical and alternative NF-kB pathways are recurrently activated in primary dog DLBCL cells. The pattern of NF-kB protein expression was similar to that observed in human DLBCL cells.

Conclusions: We propose the alternative NF-kB pathway as a novel target for lymphoma therapies. We are currently analyzing the effect of small interfering RNAs targeting the alternative NF-kB pathway for cell proliferation/viability and changes in genome-wide gene expression using a RNA-sequencing technology. The results will provide new insights on the roles of the alternative NF-kB pathway to develop novel treatment strategies for human and dog DLBCL using comparative oncology approaches.
The Influence of Culture Medium Conditions on Canine Adipose Derived Stem Cells

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Adipose derived stem cells are a promising therapy for osteoarthritis. Allogeneic stem cells provide many advantages over autologous stem cells but require the selection of a medium that provides consistent, desirable cellular products. We hypothesize that medium type influences the phenotype of cells, allowing selection of a specified cellular product for clinical applications. Fat from six dogs were processed and cultured in these medium conditions: basic growth medium, keratinocyte n-acetylcysteine medium (KNAC), serum free medium, and multipotent adult progenitor cell medium. Cells from each treatment and the initial stem cell product were evaluated for pro-inflammatory and anti-inflammatory gene expression by qRT-PCR. Cell surface marker expression evaluating immunostimulatory, mesenchymal and hematopoietic stem cell markers were evaluated by flow cytometry. Cells from each medium condition were evaluated for multipotential differentiation into bone, fat and cartilage. The expression of pro-inflammatory and anti-inflammatory cytokines and immunostimulatory markers decreased in all medium conditions, with a greater anti-inflammatory potential in KNAC medium conditions. KNAC treated cells had the greatest expression of mesenchymal cell surface markers and most consistently differentiated into bone, fat and cartilage. Our results indicate culture of stem cells provides more predictable, consistent products compared to autologous preparations and allows specific selection of cell phenotype for cell therapy.

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**CM-17**

**Interleukin-8 Promotes Canine Hemangiosarcoma Growth by Regulating the Tumor Microenvironment**

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Canine hemangiosarcoma (HSA) is a common, aggressive and incurable cancer. Interleukin-8 (IL-8) gene expression is highly upregulated in canine HSA; however, its role in the pathogenesis of this disease is unknown. We investigated expression of IL-8 in canine HSA tissues and cell lines and the effects of IL-8 on canine HSA in vitro and in vivo using a mouse xenograft model. Constitutive expression of IL-8 gene and IL-8 protein, as well as those of IL-8 receptor, were variable among different tumor samples and cell lines, but they were stable in each cell line. Neither addition of exogenous IL-8, nor blockade of IL-8 using neutralizing antibodies affected HSA cell proliferation or survival in vitro. To explore the potential that cells with differential IL-8 expression had distinct and predictable patterns of biological behavior, we stratified samples into “IL-8 high” and “IL-8 low” groups. Genome-wide gene expression profiling showed that samples in the “IL-8 high” group had enrichment for genes associated with a “reactive microenvironment,” including activation of coagulation, inflammation, and fibrosis networks. Based on these findings, we hypothesized that IL-8 regulates interactions between the tumor and its microenvironment rather than affecting HSA cell growth and survival directly. This hypothesis was corroborated by in vivo experiments where survival of tumor cells in the initial stages of xenograft engraftment were significantly inhibited by administration of neutralizing anti-IL-8 antibodies. Together, our results suggest that IL-8 contributes to establish a permissive microenvironment during early stages of tumorigenesis in hemangiosarcoma.

**CM-18**

**Differential proliferation of neural stem/progenitor cells during Herpes Simplex encephalitis**

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Endogenous neural stem/progenitor cells (NSCs) respond to inflammatory cues in the brain to proliferate, migrate, and differentiate into new neurons or glial cells. However, little is known about NSC response to inflammation during herpes simplex encephalitis (HSE). Quantitative and phenotypic analysis of neurogenic regions in Herpes Simplex Virus (HSV)-1 infected brain showed significant increase in the number of proliferating Ki-67(+) endogenous brain cells at 3 d.p.i. On further analysis, it was found that increase in numbers of nestin(+) stem cells were delayed until 6 d.p.i., indicative of cell-type specific proliferative responses in infected brains. Interestingly, at 15 d.p.i. proliferation of brain cells was abrogated and both Ki-67(+) proliferating cells and Nestin(+) stem cells decreased significantly compared to uninfected animals (8.5 ± 2.9% vs. 43.0 ± 4.9% and 7.70 ± 4.2% vs. 18.8 ± 2.0% respectively). These data suggest temporal, cell type specific modulation of proliferative responses during HSE. The specific cellular phenotypes involved in the proliferative response are currently under investigation. To determine if modulation of NSC proliferation is associated with expression of neurogenic factors, gene expression analysis was performed. Using a PCR array, expression of several neurogenic factors was found to be down-regulated at 15 d.p.i. On further analysis of fibroblast growth factor-2 expression kinetics during HSE, it was found that this gene was significantly up-regulated at 3 d.p.i. and subsequently down-regulated at 15 d.p.i, suggesting an association between observed inhibition of brain cell proliferation and changes in growth factor expression. Studies are underway to identify mechanisms by which FGF-2 alters neurogenesis during HSE. These studies will help identify novel points of intervention to develop therapies for neurological deficits ensuing viral encephalitis.
CM-19

**Hemangiosarcoma and its Cancer Stem Cell Sub-Population are Effectively Killed by a Toxin Targeted through Epidermal Growth Factor and Urokinase Receptors**

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Targeted toxins have the potential to overcome intrinsic or acquired resistance of cancer cells to conventional cytotoxic agents. Here, we hypothesized that EGFuPA-toxin, a bispecific ligand-targeted toxin (BLT) consisting of a deimmunized *Pseudomonas* exotoxin (PE) conjugated to epidermal growth factor and urokinase, would efficiently target and kill cells derived from canine hemangiosarcoma (HSA), a highly chemotherapy resistant tumor, as well as cultured hemangiospheres, used as a surrogate for cancer stem cells (CSC). EGFuPA-toxin showed cytotoxicity in four HSA cell lines (Emma, Frog, DD-1, and SB) at a concentration of £100 nM, and the cytotoxicity was dependent on specific ligand-receptor interactions. Monospecific targeted toxins also killed these chemoresistant cells; in this case, a “threshold” level of EGFR expression appeared to be required to make cells sensitive to the monospecific EGF-toxin, but not to the monospecific uPA-toxin. The IC50 of CSCs was higher by approximately two orders of magnitude compared to non-CSCs, but these cells were still sensitive to EGFuPA-toxin at nanomolar (i.e., pharmacologically relevant) concentrations, and when targeted by EGFuPA-toxin, resulted in death of the entire cell population. Taken together, our results support the use of these toxins to treat chemoresistant tumors such as sarcomas, including those that conform to the cancer stem cell model. Our results also support the use of companion animals with cancer for further translational development of these cytotoxic molecules.

CM-20

**Risk Factors for Equine Metabolic Syndrome (EMS) and Laminitis**

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Laminitis, a devastating and painful condition of the equine hoof, has been observed more frequently in horses that display a metabolic phenotype now termed equine metabolic syndrome (EMS). Generalized obesity or regional adiposity and insulin resistance have been reported as defining features of EMS but descriptions have varied and more research is needed to further characterize the EMS phenotype.

Methods: Epidemiologic, phenotypic and environmental data has been collected from a cohort of 311 suspected EMS cases and 324 mostly breed-matched controls.

Objective: Better define the EMS phenotype and identify possible breed differences in EMS phenotypic measurements.

Results and Conclusions: Blood concentration of triglyceride, insulin, and the insulin response to an oral sugar challenge are all positively correlated. Horses with elevations in blood triglyceride and insulin concentration and exaggerated insulin responses to oral sugar challenge are more likely to have experienced laminitis in the past. Morphometric measurements of adiposity are significantly higher in horses with a history of laminitis and positively correlated with blood insulin and triglyceride concentration. However significant breed differences in these measurements inhibit their utility as a diagnostic measurement. Hypertriglyceridemia and dysinsulinemia are consistent features of the EMS phenotype. Identification of the key underlying metabolic derangements in horses with EMS will hopefully lead to a better understanding of EMS pathophysiology and allow for detection of these abnormalities prior to the onset of laminitis.
The effect of porcine reproductive and respiratory syndrome virus (PRRSV) infection on the porcine antibody repertoire

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Antibody responses are critical to effective immunity to viral infections. Thus, extensive efforts have been directed to characterize antibody responses, including neutralizing antibodies, to PRRSV infection, in the expectation of elucidating key insights into protective and cross-protective immunity. Despite these efforts, the role of antibody responses in PRRSV immune protection remains poorly understood due, in part, to the lack of specific information about porcine immunoglobulin structures. To address this limitation, we characterized the expressed heavy and light chain immunoglobulin repertoires in healthy and PRRSV-infected pigs using amplicon-based 454 high-throughput sequencing. Bioinformatic analysis of approximately 450,000 heavy chain reads revealed differential heavy chain variable gene (IGHV) usage specific to PRRSV infection, including several exceptionally common immunoglobulin sequences representing clonally expanded B cell populations. The total richness of the heavy chain repertoire was approximately 3.5x10^5, equivalent to that reported in humans, suggesting that the swine antigen-binding repertoire is similarly complex, despite lack of diversity in the porcine IGHV framework regions. Analysis of approximately 500,000 light chain reads confirms that swine have a highly restricted light chain repertoire. Additionally, pigs infected with PRRSV possess several highly abundant clonal populations of light chain sequences, suggesting their possible role in PRRSV immunity.

Loss of RB Function is a determinant of a prognostically significant gene expression signature in osteosarcoma

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We achieved stratification of canine osteosarcoma patients into prognostically relevant subgroups based on tumor gene expression profiles. There were two distinct groups, one comprised of tumors from patients with “worse outcome” (survival shorter than the median with standard of care), and another comprised of tumors from patients with “better outcome” (survival longer than the median with standard of care), with the latter resembling non-malignant bone cells. This signature unmasked orthologous molecular subtypes in five independent data sets from human patients with osteosarcoma. We hypothesized that this gene signature, which includes coordinated over- or under expression of approximately 300 genes was consequence of abnormal function in one or a few regulatory factors. We used bioinformatic tools to identify transcription factors responsible for our gene signature and validated transcription factor activity using dual luciferase reporter assays and qRT-PCR. Using the MEME Suite for de novo motif discovery the most significant motifs in the promoter sequences of genes in our gene signature were variants of RB-E2F-DP-1 binding sites. Using the Ingenuity Pathway Analysis Suite we found transcriptional regulators in the RB pathway were significantly associated with our gene signature. Therefore, we assessed the effect of ectopic expression of RB on the activity of a reporter controlled by the promoter of AURKB, a differentially expressed gene from our signature. The extent of suppression of AURKB activity by ectopic RB was greater in tumor cells having the worse prognostic signature. qRT-PCR analysis showed that introduction of RB led to 55-100% reduction in expression of multiple cell cycle genes that are over expressed in tumors from worst outcome dogs. Together, our results suggest that RB activity may uniquely determine or be a significant contributor of tumor behavior, clinical progression, and outcome in patients with osteosarcoma.
Infection of EcoHIV, a novel murine model of HIV, and Morphine as comorbidities reduce gut barrier function

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Recent attention has focused on the role of gut homeostasis as the source of inflammation in HIV progression to AIDS. As a comorbidity to HIV, opiates have been observed to enhance pathogenesis of HIV, but these interactions have primarily been studied in the CNS. Studies in humans or primates imply compromised gut barrier function by both HIV/SIV and morphine; however these models have drastic limitations for studying gut that do not allow the underlying mechanisms to be directly examined. EcoHIV was developed as a way to simulate HIV pathogenesis by genetically altering HIV to infect mouse cells by substituting gp80 for gp120. Our results show that chronic morphine in EcoHIV treated mice additively enhances bacterial translocation and disrupts tight junction organization measured by immunofluorescence of tight junction protein occludin. We observed an upregulation of the HIV receptor CCR5 in both blood and lamina propria macrophages, suggesting an upregulation of inflammatory markers in response to EcoHIV infection and morphine. To understand whether these macrophages could have an effect on gut barrier function, we cultured J774 cells with morphine and/or EcoHIV in the presence or absence of LPS to simulate the microenvironment of the gut. The supernatant was then applied to IEC4 (mouse ileal epithelial) cells and trans-epithelial resistance was measured using an Electric cell impedance sensing (ECIS) system. Interestingly, barrier function was modulated solely by the presence of LPS and not EcoHIV or morphine; however, epithelial repair was drastically reduced in the presence of EcoHIV. Based on these observations, we conclude that EcoHIV and morphine act as comorbidities to contribute to gut barrier dysfunction, likely through a deficiency in gut repair mechanisms and bacterial clearance. This effect likely contributes to the enhanced inflammation and pathogenesis observed in opiate-abusing HIV patients.

Role of coat color genotypes in risk and severity of melanoma in gray Quarter Horses

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Background: Both graying and melanoma formation in horses have recently been linked to a duplication in the STX17 gene. This duplication, as well as a mutation in the ASIP gene that increases MC1R pathway signaling, affect melanoma risk and severity in gray horses.

Hypothesis: We hypothesized that melanoma susceptibility in gray Quarter Horses (QH) is lower than in other gray breeds due to decreased MC1R signaling resulting from a high incidence of the MC1R chestnut coat color allele in the QH population.

Animals: 335 gray QH with and without dermal melanomas.

Methods: Candidate gene association study was performed. Blood and hair samples with intact roots were collected from all horses for DNA extraction and PCR for the STX17, ASIP and MC1R genotypes. Age, gender and the presence and appearance of external melanomas was recorded for all horses. The effect of age and genotype on melanoma presence and severity was evaluated.

Results: Melanoma prevalence and grade was lower in this cohort of QH than previously described in other breeds. Age was significantly associated with melanoma prevalence and severity. No statistically significant effect of MC1R, ASIP or STX17 genotypes on melanoma prevalence or severity was found in any of the analyses performed. Conclusion: Melanoma prevalence and severity appears to be lower in gray QH than in other breeds. This could be due to infrequent STX17 homozygosity, a mitigating effect of the MC1R mutation on ASIP potentiation of melanoma, other genes in the MC1R signaling pathway, or differences in genetic background between breeds.
Cartilage canals are minute channels present in the epiphyseal cartilage of growing humans and animals. Failure of vessels contained within these canals has been shown to play a key role in the pathogenesis of osteochondrosis (OC) in animals; however, there is no available imaging modality that has the demonstrated ability to image these vessels. The purpose of this study was to develop and validate novel MRI sequences capable of identifying cartilage canals in unperfused cadaveric porcine specimens. The left pelvic and thoracic limbs of six piglets, aged 1 to 6 weeks, were perfused with a radiographic contrast agent. After euthanasia, the humeral trochelea and femoral condyles (OC predilection sites) were harvested and imaged using μCT followed by clearing using the modified Spalteholz technique to allow gross visualization of the vessels. The contralateral humeral trochelea and femoral condyles were harvested and imaged using sensitivity weighted imaging (SWI) in a 9.4T magnet. These samples were then routinely prepared for histology and serial sections were cut in a plane matching that of the MRI technique. Sections stained with hematoxylin and eosin were compared to the matching MRI slices. Three-dimensional reconstructions of vessels identified using MRI were compared to 3D reconstructions of the μCT images and cleared specimens of the contralateral limb. Vessels contained in the cartilage canals were positively identified using MRI; their sizes and locations matched those observed in the histological sections. The location and course of the vessels detected in μCT images and in the cleared specimen corresponded with the MRI findings, however the MRI was able to resolve further detail by identifying vessels not apparent in the μCT images or in the cleared specimens. Visualization of vessels contained in cartilage canals within the epiphyseal cartilage was successfully completed using sensitivity weighted MRI sequences. Future studies of cartilage canals using SWI are planned in live pigs. If successful, the results would easily be translatable to human beings.

The role of estrogen in the modification of DRG neuron mRNA expression in cancer pain.

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It has been demonstrated that women experience greater cancer pain severity than men, yet the potential role of estrogen in regulating tumor-induced nociception remains unclear. A major nociceptive mechanism associated with the development of cancer pain is the transient receptor potential vanilloid-1 (TRPV1) channel. Both estrogen (ER) and TRPV1 receptors are expressed on dorsal root ganglion (DRG) neurons of the peripheral nervous system. Manipulation of 17β-estradiol concentrations in both in vivo and in vitro models of pain have shown modulation in TRPV1 signaling at the level of the DRG neuron. 17β-estradiol binds to both ER-α and ER-β with the same affinity. This raises the question of whether one or both ERs can affect TRPV1 receptors to modulate tumor-induced pain. Thus, we evaluated the effects of estrogen on fibrosarcoma-induced mechanical allodynia in male and female C3H mice in a model of bone cancer pain. Tumor nociception in both males and females was associated with a significantly greater TRPV1, ER-α, and ER-β receptor mRNA levels compared to their saline-injected animals. Female tumor mice had significantly higher increases in TRPV1 and ER-β mRNA compared to males. Although there were significant differences in mRNA levels, male and female tumor mice do not show differences in mechanical allodynia scores. When tumor pain scores were evaluated based on female estrus cycle, diestrus females showed significantly less mechanical allodynia than proestrus or estrus, or males. This decrease in mechanical allodynia in diestrus was associated with a significant decrease in the expression of ER-β receptor mRNA. To delineate potential changes that estrogens have on mechanical pain and DRG receptor expression, we next manipulated the concentrations of sex hormones by gonadectomizing both females (OVX) and males (ORCH) and, in half of the gonadectomized animals, replaced estradiol (E2) with a silastic implant (s.c.). In E2 replaced tumor animals (OVX+E, ORCH+E), both TRPV1 and ER-β receptor mRNA expression in DRG neurons were significantly higher than in counterparts. Mechanical pain of OVX was significantly lower than OVX+E and comparable to females in diestrus. Conversely, ORCH+E display significantly less mechanical allodynia compared to ORCH and intact males. These results indicate that increases in ER-β receptor mRNA levels correlate with increases in female mechanical pain and suggest this receptor may play a role in estrogen’s ability to modulate pain in females. Alternatively, ER-β expression in males also significantly increased with E2 replacement, but this had the opposite effect and decreased tumor-induced pain.
Identification of Three Genomic Regions Associated with Idiopathic Epilepsy in Australian Shepherd Dogs.

Idiopathic epilepsy (IE) is a seizure disorder for which no underlying medical cause can be identified, and implies a likely genetic predisposition. The prevalence of canine IE is between 0.5% and 5.7%. It has been reported in nearly every dog breed, with a particularly high prevalence in Australian Shepherds (AS). The large variability in phenotypes and inheritance patterns between breeds suggests its genetic complexity, and multiple loci have been hypothesized to be involved. We genotyped 44 IE affected and 44 unaffected AS dogs using the high density 170K Illumina SNP chip. Data were analyzed by a genome wide association study (GWAS) with a chi-square association test and correction for multiple testing with 10,000 phenotype label-swapping permutations. Although none reached genome-wide significance, we observed strong peaks on CFA19 and CFA26. In CFA26, the peak was formed by a single marker (P_{raw}=9.94x10^{-6}). In CFA19, we observed a trail of 63 SNPs (ranging from P_{raw}=3.64x10^{-5} to 9.78x10^{-4}). We further performed haplotype analysis in this ~9Mb region and found 11 haplotype blocks associated with IE. One of these haplotypes was present in 18.2% of the cases versus 1.1% of controls. To ensure minimal population stratification, we graphically represented our samples using multi-dimensional scaling. We identified and removed 5 outliers which left 42 cases and 41 controls for a new GWAS analysis. With a genomic inflation factor of 1.12, we identified, in addition to the CFA19 and CFA26 hits, a new single SNP peak in CFA16 (P_{raw}=1.02x10^{-5}) that also did not quite reach genome-wide significance. Using GWAS and haplotype analysis, we have identified three chromosomes with regions associated with IE in AS dogs. In CFA19, the region falls over the SPATA5 gene, and next to NUTD and FGF2 genes. The latter has been reported to be overexpressed in spontaneous recurrent seizures and epilepsy in people. The SNP in CFA16 is close to CSMD1 (associated with autism and epilepsy in humans), and the one in CFA26 falls between the PRKG1 (associated with schizophrenia) and ACF genes. Therefore, we have identified three candidate gene loci potentially contributing to IE in AS dogs.

Emerging and Zoonotic Diseases Signature Program: 213 Pomeroy Center

EJD-1

Indirect transmission of influenza A virus in two different biosecurity settings
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While it is known that direct transmission of influenza is an important means of transmission in pigs, indirect routes (e.g. fomites) have not been studied in detail. The objective of this study was to evaluate the role of fomites in influenza virus transmission between pig populations separated by two different biosecurity settings. Thirty-five influenza virus negative pigs were assigned to one of four experimental groups. Ten pigs were assigned to the infected group (I); 10 pigs (2 replicates of 5 pigs) were assigned to the low biosecurity sentinel group (LB); 10 pigs (2 replicates of 5 pigs) were assigned to the high biosecurity sentinel group (HB); and 5 pigs were assigned to the negative control group (NC). Thirty-six hours following inoculation of pigs in the infected group, personnel movement events took place in order to move potentially infectious clothing and personal protective equipment (PPE) to sentinel pig rooms. Nine movement events from infected pigs to sentinel pigs in each group occurred over a 5 day period. Influenza virus infection status of pigs was determined daily via nasal swabs tested by RRT-PCR. Fomites were also swabbed and tested via RRT-PCR. All pigs in the infected group (10/10) were influenza virus positive and during the 5 days in which the 9 movement events took place, 5, 8, 9, 10, and 10 pigs were influenza virus positive. Of the 144 fomite samples collected following contact with infected pigs, 11 (8%) were low level positives (Ct value >35 and <40) via RRT-PCR. One replicate of each sentinel groups LB and HB were infected with influenza virus. All pigs in the negative control and the remaining replicates of sentinel groups LB and HB remained negative. This study provides evidence of indirect transmission of influenza A virus from an infected population of pigs to sentinel pigs. The biosecurity procedures practiced in the HB group were not able to fully prevent transmission. Further work is needed to help identify the most likely gap which led to transmission in the HB group.
Development and validation of a loop-mediated isothermal amplification assay for rapid detection of *Streptococcus suis*  
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We report on the development and validation of a loop-mediated isothermal amplification (LAMP) assay for the rapid and sensitive detection of *S. suis*, an economically important swine pathogen. Primers were designed targeting conserved region of the capsular polysaccharide (*cps2J*) gene of *S. suis* serotype 2 and 1/2. The LAMP assay produced reliable amplification in 60 min at isothermal conditions. The assay was found to be specific; it did not detect other swine pathogens including *Actinobacillus suis*, *A. pleuropneumoniae*, *Haemophilus parasuis*, *Pasteurella multocida*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella spp*, β-haemolytic *Escherichia coli*, non-haemolytic *E. coli* and *Mycoplasma hyopneumoniae*. Based on testing serial dilutions of genomic DNA of *S. suis*, the LAMP assay was found to be 100-fold more sensitive than the currently used conventional *S. suis* PCR assay indicating that LAMP could be a better choice for rapid and sensitive detection of *S. suis* especially in resource poor laboratories.

Ecology of Anthrax in Queen Elizabeth Protected Area Ecosystem in Uganda: a case study of outbreak management and monitoring  
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Anthrax is an ancient disease of warm-blooded animals mostly affecting herbivores, caused by the ubiquitous gram-positive aerobic endospore forming bacteria, *Bacillus anthracis*. While some animal species appear more resistant, devastating outbreaks have been reported around the world in wild and domestic herbivores, humans and non-human primates. Anthrax infectious cycles vary from years to centuries depending upon the regional ecology of the disease. Changes in environmental factors like soil calcium content, pH, mean annual temperatures, precipitation, elevation, are amongst key trigger factors. This study is taking place in Queen Elizabeth Protected Area (QEPA), in the southwestern part of Uganda. Repeated anthrax outbreaks have occurred in this ecosystem over the last 50 years. Outbreaks seem to increase in frequency and intensity. Two major recent outbreaks occurred in 2004/5 and 2010 and minor outbreaks happened in 2007, 2009 and 2011. In these outbreaks, 440 hippos, 78 buffalo, 14 warthog, 12 Uganda Kob, 5 waterbuck, and at least 10 people died.

This study describes park management efforts to contain the outbreaks by burning and burying carcasses in mass graves and coordinating concerted community outreach programs; outbreak monitoring strategies were employed. It also highlights knowledge gaps existing and proposes further research questions to understand the ecology of the disease for better outbreak and biodiversity management around the ecosystem.
Isolation and characterization of a turkey arthritis reovirus

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During the Spring and Summer of 2011, the Minnesota Veterinary Diagnostic Laboratory (MVDL) received 14 submissions of 15- to 18-week-old tom turkeys that were recumbent with wing tip bruises (“wing walkers”) and uni- or bilateral swelling of the hock (tibiotarsal) joints. Gastrocnemius or digital flexor tendons were occasionally ruptured. A total of five turkey arthritis reoviruses (TARV-MN1 through TARV-MN5) were isolated in specific-pathogen-free embryonated chicken eggs and QT-35 cells. The identity of the isolates was confirmed by electron microscopy, reverse transcription-polymerase chain reaction (RT-PCR) and gene sequence analysis. Blast analysis on the basis of 880bp nucleotide sequence of S4 gene confirmed all isolates as reovirus. Phylogenetic analysis divided the five isolates into two subgroups: subgroup I containing TARV-MN 1, 2, 3 and 5 and the other subgroup containing TARV-MN4. Isolates in subgroup I had a similarity of 97% to 100% with each other while subgroup II (TARV-MN4) had a similarity of only 89.2% with subgroup I viruses. This isolate showed 90% to 93% similarity with turkey enteric reoviruses in the US while subgroup I isolates had 89% to 97.6% similarity. These results indicate divergence within TARVs as well as from enteric viruses, which needs to be confirmed by complete genome sequence analysis. Molecular characterization on basis of complete S and M gene is on progress to obtain a clear picture of viral phylogeny.

Host Response to Mycobacterium avium subsp. paratuberculosis Infection Depends upon Epithelium Processing

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The intestinal epithelium serves as a gatekeeper to allow for nutrient absorption yet prevent pathogen invasion. Mycobacterium avium subsp. paratuberculosis (MAP), the causative agent of Johne’s disease in ruminants traverses this hostile intestinal epithelial barrier prior to residing in its niche, the macrophage. The initial interaction between intestinal pathogens and the host epithelium sets the course for the ensuing infection. Studies from our laboratory, using a bovine epithelium/monocyte derived macrophage (MDM) co-culture system, show that MAP takes advantage of acute host responses at the epithelial interface to recruit macrophages to the site of infection. In order to understand the dynamics of host-MAP cross-talk during early infection, we profiled both host (epithelium and macrophage) and MAP transcriptomes under separate and co-culture conditions using RNA-seq. We identified upregulation of pathways involved in cell motility and restructuring, glycolysis, fibronectin and complement receptors, and calcium binding in MAP infected epithelial cells co-cultured with MDMs in comparison to infected epithelial cells alone. MAP passage through epithelial cells and subsequent invasion into MDMs in the basolateral chamber of the co-culture model resulted in upregulation of cathespin genes, while interferon genes were downregulated in contrast to infected MDMs cultured alone. These results indicate that regulation of fibronectin and complement receptors as well as restructuring of the cell via actin promotes MAP’s invasion into the epithelium. Upon entrance, MAP upregulates calcium binding proteins and cell motility pathways in order orchestrate its escape into MDMs. Post-epithelial processing of MAP results in enhanced regulation of cathespin genes involved in lysosome function but downregulates interferon responses in order to avoid immune clearance by the host. This is the first study to show that pathways involved during early stages of MAP infection are influenced by pathogen processing by the epithelium and cell to cell cross-talk. These results depict an active and poignant role of the epithelium in establishment of MAP infection, which augments our knowledge of MAP pathogenesis as well as elucidates pathways for disruption in novel therapeutic and vaccine designs.
Transcriptome mapping of pAR060302, a bla\textsubscript{cmy-2} positive, IncA/C broad host range plasmid.

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

PRRSv half-life in cell culture medium and manure

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PRRSv is an economically important swine pathogen which can be disseminated from infected pig herds via movement of contaminated manure. The process of manure handling and inadequate cleaning of transport vehicles are commonly implicated as sources of PRRSv transmission. Stability of PRRSv in pig manure at different temperatures is unknown. The objective of this study was to determine PRRSv-infectivity half-life in manure and in a cell culture medium at 4, 20, 60 and 80 °C. To assure sample consistency across the study, all samples were prepared from common homogenized solutions (MEM and manure) and frozen at -20 °C. Samples were thawed, transferred to a water bath set at a specific temperature, inoculated with 100 ul of PRRSv at designated time points and then tested for virus infectivity.

Regression models were created to estimate PRRSv half-life based on incubation temperature. There was an exponential decrease in PRRSv infectivity with increasing temperature. At every temperature tested, PRRSv had shorter half-life when incubated in manure compared to MEM. PRRSv half-life in MEM and manure was estimated at 112.6 and 120.5h at 4°C, 14.6 and 24.5h at 20°C, 1.6 and 1.7h at 40°C, 2.9 and 8.5 min at 60 °C, and 0.36–0.59 min at 80 °C, respectively.

Results of this study can be used as basis for developing strategies to inactivate PRRSv present in manure-contaminated environments using heating treatments. For example, these data suggest that submitting transport trailers to temperature of 50 °C for 8 h would decrease PRRSv from 10\textsuperscript{6} TCID\textsubscript{50}/ml to less than 10\textsuperscript{-1} TCID\textsubscript{50}/ml.
Phylogenetic analysis of swine group C rotavirus G genotypes from the United States and Canada

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Group C rotavirus (RVC) is a major cause of gastroenteritis in swine. Initially named pararotavirus, group C rotavirus (RVC) was discovered in a 27-day old nursery pig from an Ohio, United States, displaying clinical signs of diarrhea. While swine RVC infections can appear as both clinical and subclinical, recent discoveries have identified RVC from a substantial number of swineherds in North America with clinical signs of diarrhea. While recent data on the genetic diversity of RVC G genotypes in North America is unavailable, we investigated the molecular diversity by sequencing the VP7 open reading frame (ORF) of 70 porcine RVC samples with clinical signs (as measure by diarrhea and weight loss) from 11 states (USA) and one Canadian providence between 2009-2011. Using published RVC VP7 sequences and the novel sequence data generated in this study, the previously proposed RVC VP7 89% amino acid genotypes classification cut-off value was modified to include an 85% nucleotide cut-off value based on phylogenetic analysis, yielding 9 proposed genotypes, G1 to G9. While a commercially RVC vaccine is unavailable for pigs in North America, these preliminary characterizations of RVC G genotypes may help guide future vaccine development.

Description of two microsporidian parasites, *Heterosporis sutherlandae* n. sp. and *H. superiorae* n. sp., infecting fish in Minnesota, USA

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Three angler caught fish were submitted to the Minnesota Veterinary Diagnostic Laboratory with moderate to severe lesions of the skeletal muscle tissue. The muscle tissue was white-opaque in appearance and liquefied. Mature spores of a microsporidian parasite, consistent with the genus *Heterosporis*, were observed by light and electron microscopy. Molecular analysis was performed to determine the species. Following DNA extraction, a PCR was performed with general microsporidian primers, and the entire 16s rRNA gene of the product was sequenced. Two new species were identified from this investigation. *H. sutherlandae* (formerly *H. sp.*) was identified from yellow perch, *Perca flavescens*, and walleye, *Sander vitreus*, from lakes in North Central Minnesota. *H. superiorae* was collected from a cisco, *Coregonus artedi*, in Lake Superior. There was 87.8% homology between the two species, and both were unique species in the genus *Heterosporis*. Improved diagnostics and future surveillance efforts will determine the distribution of these important parasites in the region.
Influence of pig age on the neutralizing antibody response to PRRSV

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There are inconsistencies in our understanding of the role of neutralizing antibodies in immune protection to Porcine reproductive and respiratory syndrome virus (PRRSV). After initial exposure to the virus, neutralizing antibodies are produced, but not until after peak viremia has abated. This suggests they are not the primary immunologic mechanism for viral clearance from the circulation, however the role these neutralizing antibodies play in protection against subsequent exposure to PRRSV is unclear.

Age-dependent resistance to PRRSV has been observed, whereby younger pigs exhibit higher levels and longer duration of viremia compared to older pigs despite similar timing and magnitude of antibody production against the virus. Most research investigating neutralizing antibodies to PRRSV has been conducted in young pigs, however in this study, we observed that older sows show high neutralizing antibody titers. Using a high-throughput ELISA-based assay to examine the neutralizing antibody response to PRRSV, we sought to distinguish whether these high titers are a function of age at which pigs are first infected with PRRSV or a response to cumulative exposure to multiple PRRS viruses over time. The findings suggest that immune responses to PRRSV may be calibrated by age. Though the underlying factors responsible are not known, the importance of high neutralizing titers for protection against diverse PRRS viruses in older pigs can now be investigated.

Detection and molecular characterization of parvoviruses in turkeys

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Poult enteritis syndrome (PES) is characterized by enteritis and decreased body weight gain in growing turkey poults. Another syndrome called the light turkey syndrome (LTS) causes a decrease in body weight of adult tom turkeys leading to huge economic losses. Reovirus, rotavirus, and astrovirus are considered to be involved in LTS and PES flocks in Minnesota. In order to determine if a DNA virus might be involved in these cases, we tested 216 fecal sample pools collected from LTS and PES flocks for the presence of parvovirus. The samples were tested by PCR using primers for the non-structural 1 (NS1) gene. From 100 cases of LTS, 40 were positive for parvovirus. The prevalence of parvovirus in PES was relatively low; only five of 116 PES pools were positive. Partial gene sequence of NS1 gene suggested that the virus detected in our study was closely related to previously described parvoviruses from turkeys and chickens. Further studies are in progress to understand the role of parvoviruses in turkey enteritis.
Attenuation of virulence and loss of prophage-like elements in *Lawsonia intracellularis* after serial passages *in vitro*

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*L. intracellularis* is an obligate intracellular bacterium and causative agent of proliferative enteropathy. Clinical disease has been reproduced using pure cultures after up to 13 passages in cell culture. The present study evaluated the susceptibility of pigs to *L. intracellularis* after 10, 20 and 40 passages *in vitro*. Further, the whole genome sequence of a pathogenic isolate (passage 10) was compared with the homologous non-pathogenic isolate (passage 60).

Twenty-four 3-week-old pigs were divided into four groups. Three groups were infected with a pure culture of *L. intracellularis* at passage 10, 20 or 40 and one group with placebo. The animals were monitored for clinical signs, fecal shedding and serological responses during 28 days post-inoculation. The levels of infection were graded by immunohistochemistry based on the amount of positive labeled *Lawsonia*-antigen in the intestinal epithelium. The whole genome of this isolate was sequenced using Illumina® platform. Only animals infected with passages 10 and 20 demonstrated proliferative lesions associated with the presence of *Lawsonia*-specific antigen in the intestinal epithelium. A significant (p<0.05) lower amount and shorter period of *Lawsonia* shedding was identified in the passage 40-infected pigs. Additionally, IgG responses were observed in passages 10 and 20 but not in passage 40-infected animals. The comparative genome analysis showed a deletion of 18,088 bp in the non-pathogenic homologous *Lawsonia* isolate passed 60 times *in vitro*. This region comprises 15 protein-encoded genes including prophage DLP12 integrase. This prophage-associated genomic island has been conserved between various porcine isolates cultivated in monolayer at low passage and has been lost at high passages. However, the only equine isolate available does not harbor this prophage-like element. In conclusion, this genomic island appears to be porcine strain specific and its loss was coincident with the spontaneous attenuation of the virulence *in vitro*. The exact role in the ecology and pathogenesis of *L. intracellularis* is still under investigation.

Identification and molecular characterization of porcine kobuvirus in U. S. Swine farms

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The genus Kobuvirus belongs to the family *Picornaviridae* and consists of viruses that are non-enveloped and contain single-stranded, positive-sense RNA genome. Porcine kobuvirus has been associated with piglet diarrhea in Hungary, China, Thailand, Japan, and Korea, but there are no reports of its presence in U.S. swine farms. We screened intestinal contents from 114 diarrheic pigs submitted to the Minnesota Veterinary Diagnostic Laboratory for the presence of kobuvirus by reverse transcription-polymerase chain reaction (RT-PCR) using 3D (RNA polymerase) gene primers (amplicon size 216 bp). Twenty five (22%) of the 114 samples were positive for porcine kobuvirus. Of these, only five samples had kobuvirus exclusively while the other 20 had mixed infection with transmissible gastroenteritis virus and/or rotavirus (groups A, B, or C). Phylogenetic analysis revealed that all 25 porcine kobuvirus strains had 93.1%-96.5% nucleotide identity with NLD45 strain from the Netherlands and BRA24 strain from Brazil. Pigs less than four weeks of age showed higher prevalence of kobuvirus than older pigs. The results of this preliminary study indicate that porcine kobuvirus is present in diarrheic pigs in the U.S. either alone or in the combination with other enteric viruses such as TGEV and/or rotavirus (groups A, B, and C). Further studies are needed to determine the role of this virus in gastrointestinal infections of pigs and the strain diversity of porcine kobuviruses circulating in U.S. swine.
Economic evaluation of air filtration in large sow herds in North America

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Air filtration systems implemented in large sow herds have been demonstrated to decrease the probability of having a PRRSV outbreak. However, an economic study based on real production data from both filtered and non-filtered farms has never been completed. Therefore, the objectives of this study were: 1) to assess and model productivity in filtered and non-filtered sow farms; 2) to assess the profitability of the filtration system investment in modeled farms based on a partial budget analysis.

A cohort of filtered and control herds were enrolled from a contemporaneous PRRSV study. Repeated measures of quarterly production data, together with independent variables as weather, PRRSV outbreak, air filtration status and pig number of pig sites within 3 miles, were the variables analyzed in the longitudinal mixed model. The timeline of the study was Oct 2004 to June 2011.

For the cost analysis, three scenarios were compared in a spreadsheet model of weaned pig cost on a representative 3,000-sow non-filtered farm: 1) control, 2) filtered conventional attic, and 3) filtered tunnel ventilation. Scenario 1 was based on the data from control and pre-filtration periods of the future filtered farms. Scenarios 2 and 3 were identical except that the initial filtering equipment cost $150/sow for the conventional versus $200/sow for the tunnel. Filtration was assumed to change pigs weaned/sow/year, farrowing rate, female replacement rate, female death rate, veterinary expenses, and the annualized cost of replacing pre-filters every six months and replacing filters every three years.

Filtered farm produced 5,927 more piglets than non-filter farm and the payback period for the investment was estimated in the model as 5.35 years for scenario 2 and 7.13 years for scenario 3. However, this could be considered a conservative estimation because no value penalty in selling PRRSV positive piglets was accounted for. Much shorter payback periods of the order of 2.1 to 2.8 years were calculated when downstream benefits of weaning PRRS negative pigs were incorporated.

Randomized Non-Inferiority Clinical Trial Evaluating Three Commercial Dry Cow Mastitis Preparations

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The objective of this study was to evaluate the efficacy of 3 different dry cow therapy products by comparing overall risk for presence of IMI after calving, ability to cure existing intramammary infections (IMI) present at dry off, ability to prevent new IMI during the dry period and risk for experiencing a clinical mastitis case from calving to 100 days in milk (DIM). The study included 1,091 cows (4,364 quarters) and was conducted in 6 commercial dairy farms in 4 different states. The formulations tested were penicillin-dihydrostreptomycin, ceftiofur hydrochloride and cephapirin benzathine. Random assignment to treatment was done at the cow level. Quarter milk samples were collected prior to dry off, between 0-6 DIM and between 7-13 DIM. Samples underwent culture for determination of bacterial pathogens according to NMC Guidelines. Data analysis was conducted at the quarter level using SAS version 9.2. Cox Proportional Hazards regression was used to examine the effect of treatment on the risk for clinical mastitis from calving to 100 DIM and multivariate logistic regression (Proc GLIMMIX) was used for all the other outcomes. Overall, the prevalence of IMI at calving was 14.7%, the cure rate was 88.9%, the new IMI rate between dry off and 0-6 DIM was 13.3% and 4.4% of quarters experienced a clinical mastitis case between calving and 100 DIM. There was no effect of treatment on risk for presence of an IMI at 0 to 6 DIM ($P = 0.34$), risk for presence of an IMI at 7 to 13 DIM ($P = 0.37$) risk for a quarter to experience a cure ($P = 0.79$), risk for a quarter to develop a new IMI between dry off and 0 to 6 DIM ($P = 0.27$), risk for a quarter to develop a new IMI between dry off and 7 to 13 DIM ($P = 0.60$) or risk for a quarter to experience a clinical mastitis event ($P = 0.27$). In conclusion, there was no difference in efficacy between the three products evaluated when assessing the mentioned quarter level outcomes.
Issues in Agricultural Occupational Health – A Review of Needle-stick Injuries in Livestock Workers and Suggestions for an On-Farm Agricultural Sharps Injury Prevention Program

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Introduction: Veterinary medicine and agriculture have historically lacked interest in NSI research, education, and mitigation due to the relative absence of zoonotic blood-borne pathogens and the “perceived” benign nature of the injury. However depending on the procedure and/or pharmaceutical used, these injuries may include mild to severe bacterial or fungal infections, severe lacerations from large bore needles, severe local inflammation, vaccine or antibiotic injection reactions, vaccine adjuvant reactions, amputation, miscarriage, and potentially death. Our objective was to identify human needle-stick exposures to veterinary biologics and review guidance documents and recommendations that exist to prevent NSI in agricultural workers and veterinarians.

Methods: A classic Participants, Exposure, Outcome (PEO), and type of studies review study format was used. Both PubMed and CABI search engines were explored using several keyword and Mesh terms.

Results: Forty-eight articles were identified, reviewed, and data abstracted. Literature consisted mostly of case reports and survey articles. Within the case reports, thirty individuals were identified with adverse effects from veterinary biologic exposures. Biologic exposures included vaccines, antibiotics, and hormones used in animal health. Survey articles identified focused on veterinarian/veterinarian technician NSI exposure and indicated significant frequency of NSI. Biologics were categorized and risks were assessed. Guidance documents and recommendations that existed to prevent needle-stick injuries in agricultural workers/veterinarians were obtained.

Conclusion: NSI in agricultural workers/veterinarians can result in significant injury and work time-loss. A comprehensive on-farm agricultural sharps injury prevention program has the potential decrease these risks. Guidance documents and prevention recommendations were scarce in the body of literature. Only two articles were identified, indicating a need further prevention material development.

Alternative Risk Management Strategies for High Consequence Animal Disease Outbreaks in the United States

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High consequence animal diseases (HCADs) such as foot-and-mouth disease (FMD) and classical swine fever (CSF) could have devastating economic and social impacts within the United States (US).

The most common HCAD financial risk management strategy used by the US government has been indemnity paid to animal owners for direct losses associated with animal disease outbreaks. US livestock producers may employ other risk management strategies to deal with the routine financial risks of raising livestock. However, none of these strategies directly address HCADs.

This paper explores alternative financial risk management strategies used globally, primarily examining public-private partnerships and insurance products that have application for HCADs in the US livestock sector.
Dynamics of infection of *Mycoplasma hyorhinis* in two commercial swine herds

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*M. hyorhinis* is an important cause of post-weaning mortality. There is currently very little information regarding the dynamics of infection of *M. hyorhinis* in modern swine production systems. The objectives of this study were: a) To describe the dynamics of colonization of *M. hyorhinis* in pigs from birth to finish; b) To evaluate the effect of parity on piglet *M. hyorhinis* colonization. In each herd, A and B, a longitudinal sampling of pigs at different ages was performed. Fifty young sows (p1 and p2) and fifty older sows (p3 and older) were randomly selected and tested for *M. hyorhinis* by nasal swab qPCR and *M. hyorhinis* antibodies in serum by ELISA. One piglet per litter was randomly selected from each sow. A nasal swab and a serum sample was collected from each pig at birth, weaning and 10 days post-weaning. Two final samplings were performed in the nursery and finishing stage during the peak of polyserositis/arthritis/pneumonia. A total of twelve pigs were euthanized and necropsied (10 clinically diseased and 2 clinically healthy) during these two sampling points. Oral fluids were also collected at each post-weaning sampling point. Most pigs became colonized between 4-6 weeks of age and the prevalence of *M. hyorhinis* colonization in sows was low (<5%), confirming results from our previous study. No correlation was found between sow parity and piglet nasal colonization at birth. However, a higher proportion of colonized piglets from young sows was observed at 29 days of age in one of the two herds. The use of oral fluids for the detection of *M. hyorhinis* appears to be useful for surveillance, however more validation is required. ELISA results showed a decay in maternal antibodies around 3 weeks of age. The role of *Mycoplasma hyorhinis* in polyserositis and arthritis could be demonstrated in these two herds. Knowledge of the dynamics of infection within the herd will allow implementation of better control strategies in affected herds.

Economically Motivated Adulteration of Honey: Does Quality Control Exist in the International Honey Market?

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Economically-motivated adulteration (EMA) is the adulteration of food for financial advantage. The high value of honey puts it at risk for EMA due to strong economic incentives. The types of EMA typically seen in honey include using less expensive syrups to extend honey, intensive supplemental feeding of honey bees to increase honey production, unapproved use of chemicals and antibiotics, and masking the true country of origin. The honey market is a truly global market, with over 60% of honey used in the U.S. coming from imports. Additionally, there is no U.S. federal standard of identity for honey. This makes it difficult to ensure the safety and quality of honey being consumed in the U.S. Despite efforts to force change, including temporary bans on honey imports, anti-dumping regulations, and implementation of stiff import tariffs on honey from certain countries, regulatory agencies and trade organizations have not been able to ensure safe, high quality honey on the international market. This lack of quality control has led to a large quantity of inexpensive and potentially fraudulent honey in the international market. The lack of quality control in the honey industry has allowed potentially adulterated products to reach the consumer, leading to possible public health impacts and loss of consumer confidence.
Impact of Emergency Vaccination in a Foot-and-Mouth Disease (FMD) outbreak in Minnesota

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OBJECTIVES: The objective of this study was to evaluate emergency vaccination control strategies for a simulated FMD outbreak in Minnesota (MN).

METHODS: The North American Animal Disease Spread Model (NAADSM) was used to simulate FMD outbreaks (1,000 model iterations) that begin in and are limited to MN. Vaccine related variables explored included time to deliver vaccine (7, 14 and 21 days), time to develop immunity from vaccine (4 and 7 days), and number of herds vaccinated per day (two levels: 50 herds per day (assumption with federal/state veterinarian applied vaccination) and 1,500 herds per day (assumption with industry vaccinators under the supervision of accredited veterinarians)).

RESULTS: Our results suggest that vaccination has important implications in a MN outbreak and is associated with large differences in disease and outbreak duration and number of animals/herds infected. These results are more striking for scenarios in which disease begins in a dairy. Assuming a dairy herd is initially infected, the mean number of animals infected ranged from 30,000 to 88,000 with 50 herds per day vaccinated and varying delivery and immunity time. However, when vaccination capacity was increased to 1,500 herds per day and other conditions held constant, the mean number of animals infected was consistently below 20,000. Variability around means was also decreased with vaccination. When disease control did not include vaccination and only stamping out was used, the mean number of herds infected in the dairy index herd scenario was 62,000.

CONCLUSIONS: The application of a large scale, rapidly administered emergency vaccination program greatly diminished the duration and severity of a simulated FMD outbreak, assuming a Dairy Index herd.

Effect of pre-farrow administration of tulathromycin injectable solution on Mycoplasma hyopneumoniae prevalence in suckling pigs at birth and weaning

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Mycoplasma hyopneumoniae (Mhp) is one of the most prevalent and economically significant respiratory pathogens in the swine. Vertical transmission of Mhp has been proposed as the most important means of transmission in segregated production systems. Additionally, Mhp prevalence at weaning has been shown to be an important factor in determining clinical presentation of disease downstream. Therefore, the objective of the study was to determine if tulathromycin administered pre-farrow to sows and gilts reduces the prevalence of Mhp in suckling pigs at birth and at weaning. Forty-eight females within one, 5000-sow farm were randomly assigned to either the treatment or control group. Treatment females received one intramuscular (IM) injection of tulathromycin (2.5mg/kg) at day 112 of gestation. Nasal and tonsil swabs were collected from all females at day 112 of gestation, farrowing, and weaning. Treatment and control groups were housed in separate farrowing rooms. All viable piglets from females were enrolled in the study. Individual nasal and tonsil swabs were collected from piglets at birth and weaning. Piglet swab samples were tested in pools of 5 by litter. All swab samples were submitted for Mhp detection by real-time PCR (VetMax™). Tonsil swabs were used to verify “suspect” nasal swabs, when applicable. Proportions of positive samples were compared using Fisher’s Exact test using Statistix 9.0 software. Treatment was associated with significant reductions in Mhp prevalence in sows at farrowing (p=0.008) and weaning (p=0.001) and in piglet pools at birth (p=0.01) and weaning (p=0.042). Results from this study indicate that it is possible to reduce the Mhp shedding in sows and gilts by administering tulathromycin at day 112 of gestation. Subsequently, this reduced shedding in sows and gilts led to a reduced number of Mhp positive piglets at birth and at weaning. As prevalence at weaning has been associated with disease severity in downstream pigs, further studies are indicated to define the potential benefits of this approach in reducing respiratory disease and improving performance in growing pigs.
Using discriminant analysis to estimate sow culling-decision error rate and its posterior probability based on lameness and productivity

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Sow culling may not only depend on biological factors but also on human factors. Questions of the current study are 1) whether culling decision can be accurately classified from variables of interest and lameness status recorded on farms, 2) which of these variables are most informative in this classification (culled vs retained). The objectives of current study were 1) to determine the culling-decision misclassification rate, 2) to estimate the posterior probability error rate of the culling decision, and 3) to determine which variables are most informative the culling decision. A 744-sow dataset were extracted from PigCHAMP record-keeping system. Discriminant analysis was employed for this classification including three models (linear, quadratic and Epanechinikov Kernel density estimation models). Groups for classification were “culled” and “not culled” sows. From stepwise sub-selection model results, the predicted variables that were most informative for considering culling sows for all three models were lameness, born alive, wean to first service interval, and parity. Test of homogeneity of two within-covariance matrices for culled and not culled groups was significantly heterogeneous ($\chi^2$ 1503.86, df=45, p=0.000). For initial models, the decision error rate calculated for culling decision for linear, quadratic and Epanechnikov models were 0.4680, 0.4661 and 0.4991 respectively, and error rates for their posterior probability were 0.3867, 0.1563, and 0.3725 respectively. For predicted final models, the decision error rates calculated for culling decision for linear, quadratic and Epanechnikov models were 0.4315, 0.4356 and 0.4424 respectively, and error rates for their posterior probability were 0.3985, 0.3009, and 0.4030 respectively. Therefore, even though farmers receive good information in their hands for culling sows, the rate of misclassified culled sows was still high.

Network analysis of cattle movements in relation to bovine tuberculosis transmission risk in MN

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Bovine tuberculosis (BTb) was first diagnosed in cattle through slaughter surveillance in northwestern Minnesota (MN) in 2005. By the end of 2008, 12 cattle herds had found to be infected with BTb, and one of the causes for infection was determined to be the movement of infected animals between herds. USDA granted split-state status to MN in 2008, upgrading most of the state to modified-accredited advanced (MAA) and only a smaller area of 6,915 km2 in northwestern Minnesota as modified accredited (MA). The state has now been declared BTb free; however, since January 2008 all cattle movements within the MA were recorded electronically. The objective of this study is to characterize cattle movements in a high risk area for BTb in MN and also identify which herds might have a higher risk to become infected and to infect other herds. The data used in this analysis includes the years 2008 through 2011. During this period, 3,762 movements were recorded with 57460 cattle being moved, corresponding to permits issued to 682 premises, mostly representing private farms, sale yards, slaughter facilities and county or state fairs. Although, sale yards represented less than 2% of the nodes (premises), 60% of the movements were to or from a sale yard. Less than 2% of movements, both into and out of the MA zone involved locations outside MN (other states and Canada). Movements occurring between herds in the MA zone corresponded to 24% off the total number of recorded movements. Preliminary network analysis was performed on 35% of the movement data (complete analysis will be presented at the conference). The network showed a density of 1%, a fragmentation of 88% and a clustering coefficient of 56%. The betweenness centralization index was 6.52%. The degree distribution showed that 25% of nodes performed 81% of movements. This analysis provides novel description about the contact structure of cattle movements in a high risk area for BTb, essential to support future surveillance decisions.
Effects of sample site on detection of *Staphylococcus aureus* in pigs

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The emergence of “Livestock Associated” (ST398) MRSA in many countries has raised concerns about food animals as sources of these zoonotic organisms. However, there is a virtual absence of research of *S. aureus* ecology in pigs, which is essential for understanding the epidemiology of these organisms in this species. To address this, we sought to obtain elemental information about *S. aureus* in swine herds necessary to inform broader epidemiological studies in MRSA and MSSA in the future. Our specific objective was to compare the effect of sample site (nose, skin, tonsils, feces, and vagina) on detection of *S. aureus* in pigs of various ages. A longitudinal study was conducted in 2 independent pig production systems in Minnesota. In each system, samples were conveniently collected from lactating sows (n=12) together with one piglet from each respective litter (n=12). Subsequent sampling of the same birth cohorts of pigs occurred 4 and 20 weeks later “nursery” (n=12) and “finishing” (n=12) phases of pig production, respectively. Two cohorts of animals were sampled in each flow. Samples were collected from the nose, tonsil, feces, and skin (axilla) of each pigs (plus vagina in sows). All samples were cultured in parallel by two culture procedures, described as selective (specific for culture of MRSA) and non-selective enrichment techniques (MSSA). Suspect isolates were confirmed as *S. aureus* using the tube coagulase test (Difco, Detroit, MI) and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad). Methicillin resistance was evaluated using an MRSA latex agglutination test (Oxoid Ltd., Hants, UK). No MRSA were detected, but *S. aureus* was isolated at high prevalence (59 – 66%) from nasal, tonsil, and skin samples, and at lower prevalence in feces (43%) and vaginal samples from sows (27%). Spa typing is not yet complete but spa types are very diverse, but only a small a minority of isolates belong to the ST398 lineage.

Experimental Model for *Haemophilus parasuis* Colonization in Swine

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Veterinary Population Medicine

Early colonization of pigs with virulent *Haemophilus parasuis* (HPS) reduces mortality after weaning. Nevertheless, little is known about the immune response that is generated during colonization rather than systemic infection. Therefore the objective of this study was to establish a HPS colonization model in conventional pigs in order to study the early events of HPS infection and immune response. At day 0 of the study, groups 1 and 2 (n=6 each) and 3 (n=4) received $10^6$ or $10^4$ CFU/ml of highly virulent HPS, strain Nagasaki, or saline, intranasally, respectively. Clinical evaluation and nasal swabs (for bacterial culture) were collected before and every day after inoculation (dpi) during 7 days. Blood samples were collected on 1, 3 and 4 dpi for bacterial isolation. At 2 time points (4 and 7 dpi), half of the pigs were euthanized and assayed for the presence of HPS in the respiratory tract and systemic sites. ERIC-PCR genotyping demonstrated that the HPS strains isolated before inoculation were identical, and frequently isolated from the nose of all the pigs throughout the study. The Nagasaki strain, also identified by ERIC-PCR, was recovered from the nose of 5 pigs after inoculation. Moreover, tracheal swabs collected at necropsy yielded only Nagasaki isolates in 10 pigs. Overall the Nagasaki strain was isolated at least once from all 12 inoculated pigs, but was never recovered from systemic sites of experimentally inoculated pigs, control pigs or before inoculation. There were no differences in isolation of the Nagasaki strain based on inoculation doses. The absence of fever, clinical signs, lesions and bacteremia demonstrates that there was no systemic infection, even though the Nagasaki strain can be highly virulent. Trachea represents a less competitive niche for HPS colonization, which may explain why tracheal swabs yielded higher number of Nagasaki isolates compared to nasal swabs. In summary, we established an HPS colonization model in conventional pigs using a highly virulent strain which will help in the study of the early events of HPS infection and immune response in conventional pigs infected with HPS.
Utilizing spleen transcriptome analysis to identify responses to aflatoxin B₁ in the domestic turkey

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Domestic turkeys (Meleagris gallopavo) are extremely susceptible to aflatoxicosis, caused by consumption of aflatoxin B₁ (AFB₁). AFB₁ causes liver damage, hepatocellular carcinoma, and immunosuppression. Mycotoxin contamination of corn and grains is a worldwide food safety issue and adverse effects of AFB₁ lead to over $140M in losses annually for the poultry industry. Feed additives such as Lactobacillus (LGG) have been investigated for their potential to mitigate AFB₁ toxicity. Impacts of AFB₁ and LGG on the turkey can be characterized through changes in gene expression after exposure. To obtain genome-wide effects in an immune context, RNA-sequencing (RNA-seq) of the spleen transcriptome was performed on the Illumina GA Ix. 12 libraries (3 spleen samples per challenge group: control, AFB₁, LGG, and LGG + AFB₁) were sequenced to an average depth of 8.8M reads. RNA-seq datasets (7.2 Gb total sequence) were assembled two ways, short-read alignment (TopHat and Cufflinks) and de novo (Velvet and Oases), to produce predicted transcripts. The number of reads mapping to each transcript were compared to identify uniquely and significantly differentially expressed transcripts and to determine the effects of AFB₁ and LGG on expression. Spleen transcriptome analysis provides gene targets to increase resistance in the domestic turkey and improve health and production.

Effect of influenza vaccination on influenza bioaerosol generation

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Influenza vaccination is commonly used in pigs as a strategy to decrease clinical and economic impact. Influenza vaccination can also have an effect on shedding and as a result reduce transmission. However, its effect on the generation of aerosols is not fully understood. The aim of this study was to evaluate influenza vaccination in pigs as a means to reduce aerosolization of virus and subsequent airborne spread. Thirty-six, three-week old weaned pigs were obtained from an influenza seronegative herd and allotted to four different treatment groups Pigs were vaccinated at 3 and 5 wks of age using 4 different treatments: a) an autogenous vaccine (HOM), b) a commercially monovalent vaccine (HETE-MON), c) a commercially multivalent vaccine (HETE-MULTI) and d) saline solution (CTRL). The commercial vaccines were considered heterologous to the challenge virus based on HA sequencing. The autogenous vaccine was prepared using the challenge virus A/Sw/IA/00239/04 H1N1 strain. Pigs were inoculated intratracheally and intranasally two weeks after the second vaccination was completed. Pigs were tested daily using nasal swabs and assayed by qRT-PCR. Oral fluids and air samples were also collected three times a day and tested by qRT-PCR and virus isolation. Room temperature and relative humidity were collected using automated data loggers. Individual nasal PCR results indicated that all pigs in the control group and all pigs but one in the HETE-MULTI group were influenza positive. In the HETE-MON-group 5 out of 9 pigs became positive and only one pig was positive in the HOM group. No positive air samples were detected in the vaccinated groups. However, in the CTRL group, positive air samples were detected at DPI 2, 3 and 4 by qPCR. However, virus was not isolated from these samples. Average temperature and relative humidity among rooms was 28°C (24°-33°) and 53% (36%-75%), respectively.

In this study, influenza virus detection in pigs and air samples was reduced through vaccination. However, virus detection in air samples in the non-vaccinated pigs was limited. This may be explained by the increased room temperature and humidity observed. Nevertheless, influenza virus was detected in aerosols generated in hot and humid conditions. More studies are needed to further assess vaccination as a means to decrease the risk of influenza airborne transmission under field conditions.
Genomic Signatures of Selection in the Horse

We have used genome-wide SNP data from more than 800 horses representing 33 breeds to identify putative genomic regions under selection in the horse. Such loci were identified using the $F_{ST}$-based statistic ($d_i$) calculated in sliding, 500 kb windows. This statistic detects locus specific deviation in allele frequencies for each breed relative to the genome-wide average of pair-wise $F_{ST}$ summed across breeds. Numerous potential targets of selection were identified, and analysis of breeds fixed for the chestnut coat color mutation demonstrated the utility and shortcomings of this method. One striking feature of these genome scans was a 6 Mb region on ECA18 with a highly significant $d_i$ value in the Quarter Horse. Further analysis of the region revealed a ~1 Mb conserved haplotype surrounding the $MSTN$ gene that is present in 92.8% of QH and 50% of Thoroughbreds, but rare (<1%) in all other breeds. $MSTN$ variants including a promoter SINE insertion and an intronic SNP are significantly associated with the conserved haplotype. Histological data from 79 horses shows a significant association of muscle fiber type proportions with the $MSTN$ polymorphisms. Another striking result was that the gaited breeds, including the Standardbred, Icelandic, Peruvian Paso, and others, share a highly conserved haplotype on ECA23 under a strong signal of selection that contains a polymorphism demonstrated to be important in the ability to gait. Further, conserved haplotypes underlying signals of selection on ECA11 in the Belgian, Percheron, Shire, Clydesdale, and Miniature horse suggest the presence of a locus or loci important in the determination of size. Numerous other loci await a detailed evaluation. Mapping signatures of selection in the modern horse is the first step in the identification of genes important in the domestication and specialization of modern horse breeds.

Investigating the genetic variability of Mycoplasma hyopneumoniae strains circulating within a swine production system
Kalie Pettit, BA; Maria Pieters, DVM, PhD; Rick Tubbs, DVM; Lucina Galina-Pantoja, DVM, PhD. University of Minnesota, Tubbs Contract Research Organization, Pfizer Animal Health.

Introduction Enzootic pneumonia, caused by Mycoplasma hyopneumoniae, is of great economic concern in the swine industry worldwide. Common practices for controlling $M. hyopneumoniae$, such as antimycoplasmal antibiotics and vaccines, do not always provide the expected control. It was our hypothesis that $M. hyopneumoniae$ genetic variability exists within the system and influences the clinical signs seen within the swine herd. Therefore, the objective of this study was to characterize the diversity of the $M. hyopneumoniae$ strains within a swine production system and to compare that diversity to clinical signs.

Materials and Methods Tracheal aspirates and bronchial swabs were obtained from 130 four to five month old pigs on three different nursery to finish sites. At two time-points during sample collection, an unbiased observer recorded health and cough status of each room. Samples were quantified at the UMN VDL using RT-PCR, and then typed in our lab for genetic diversity at four different gene locations using Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) by capillary electrophoresis. MLVA results were further processed using Bionumerics®, and a neighbor-joining tree was created to understand the relationships of the $M. hyopneumoniae$ strains within the production system.

Results and Discussion Ninety four samples were processed by MLVA and analyzed using Bionumerics®. Two unique $M. hyopneumoniae$ strains were identified, with five samples containing both strains. Strain 1 was more prevalent, being present in 69 pigs in 95% of the positively-testing rooms, while Strain 2 was present in 29 pigs in 37% of the rooms. No correlation was found between sow farm source, cough status of the room, and strain. Our data suggests that the genetic variation of $M. hyopneumoniae$ did not appear to influence the different clinical presentation.
Categorizing food supply chains according to complexity: A tool for investigating foodborne illness outbreaks

Timothy Snider
Center for Animal Health and Food Safety

The speed at which foodborne illness outbreak investigations occur is critical in the successful effort to minimize their disease impact. At the onset of an investigation, traceback investigators collect historical information from cases in order to build an epidemiological association with exposures to specific foods and/or food ingredients. With these associations, they begin their investigation into the food supply chain in order to evaluate each of the potentially causative exposures. A successful investigation will follow the food supply chain pathways of the various cases being considered to a common point of convergence.

Understanding the nature of the pathways that constitute different food supply chains is an important characteristic of an effective investigation. Being able to determine whether or not a specific food supply chain pathway constitutes a plausible explanation for how an exposure might have occurred will help direct the investigation efficiently.

The goal of this study was to create a set of food supply chain categories that could be characterized according to the elements that contribute to how these investigations should take place for each supply chain type. This task was completed by categorizing a group of over 137 exemplar foods and food ingredients according to the complexity of their supply chains in order to expedite the outbreak investigations through an improved understanding of the minimum amount of 1) data needed (i.e. critical tracking events (CTE) and relevant key data elements (KDE)) and 2) subject matter expertise input from private sector firms in order to perform more time efficient preliminary investigations.

A pilot study to describe the epidemiology of PRRS virus in the field from a large cohort of US sow herds

Tousignant, Steve; Morrison, Bob
Department of Veterinary Population Medicine

Porcine reproductive and respiratory syndrome (PRRS) virus continues to devastate the U.S. swine industry and was recently reported to cause $644 million in losses annually. This situation exists despite the best efforts of the scientific community to advance the knowledge of vaccines and immunology, epidemiology and ecology, host genetics, regional control projects and education. It seems clear that there is still much to be learned about the disease and how best to control it.

We designed a project to study the epidemiology of PRRS virus in a sample of US sow herds. Veterinarians from 12 systems representing approximately 270 farms, 820,000 sows across 12 states have agreed to participate and report weekly PRRS status for each farm dating back to July 2009. Several systems are sharing geographic locations as well. From this data we monitor incidence and prevalence at the aggregated and system specific level. Additionally, we use a statistical method known as Exponentially Weighted Moving Average (EWMA) to monitor incidence of new infection as a means of detecting the onset of the PRRS epidemic.

While this sample of farms is not representative of all US sow herds, the results are strikingly similar among participating systems and across the last three years of data. Since 2009, 30-40% of all farms in the database report at least one infection per year. Using the EWMA we note the PRRS epidemic begins in mid-October and subsides around April. Interestingly, these data suggest a mini epidemic in May lasting only a few weeks. Finally, this data will allow temporal and the first ever spatial analysis of the epidemics across these systems and during the past three years.
The Development of two non-invasive screening assays for the detection of tuberculosis infection in non-human primates

Wolf, Tiffany, Singer, Randall, Sreevatsan, Srinand
Veterinary Biomedical Sciences and Population Medicine

The introduction of novel infectious diseases has become a major threat to endangered primate populations. This is particularly true for habituated great ape populations, conditioned for close encounters with human observers, and there is ample evidence that exposure of primates to human pathogens, particularly those of respiratory origin, readily occurs. In an effort to sustain the health of habituated great ape populations, continued health monitoring of these populations is recommended. Unfortunately, health monitoring for some diseases is hampered by a paucity of sensitive, non-invasive diagnostic assays.

Tuberculosis, a disease of high prevalence among humans in many African regions, is an example and poses a significant health risk for habituated great ape populations. The goal of this project was to validate fecal and urine biomarkers for the detection of Mycobacterium tuberculosis (M.tb) infection in non-human primates. An ELISA was developed to detect in urine lipoarabinomannan, a cell wall lipoglycan specific for pathogenic mycobacteria of the M.tb complex (MTC). A second set of ELISAs was also developed to detect fecal antibodies to highly antigenic proteins specific to members of the MTC: ESAT-6, Cfp10, and Ag85. Validation would be carried out with the testing of known M.tb positive and negative macaques (Macaca spp.).

CVM Summer Scholars: Animal Science/Veterinary Medicine Building Lobby

SS-1

The Role of IL-8 in Hemangiosarcoma Growth and Differentiation
Katie L. Anderson, Jong Hyuk Kim, Ashley Graef, Erica Merrill, Brandi Gorden, Milcah Scott, Erin Dickerson, Jaime Modiano
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Hemangiosarcoma (HSA) is a spontaneous, fatal disease that affects dogs of all breeds. Recent data suggest that HSA develops from and is maintained by cancer stem cells. These cells are resistant, adaptable, and able to differentiate along several lineages. It also has been shown that HSA relies on interactions with its microenvironment for survival. Several proinflammatory cytokines, such as IL-8, are enriched in HSA cells, indicating that these factors may be important for tumor survival. The goal of my project was to investigate the role of IL-8 in tumorigenicity and differentiation of HSA. I hypothesized that IL-8 plays a vital role in the self-renewal and multipotency of HSA. The in vitro phase of my project involved transfecting HSA cell lines to create RFP-expressing IL-8 knockdown cells. After successful transfection, these cells were placed in a sphere forming assay. The IL-8 knockdown cells formed fewer spheres in vitro, suggesting that IL-8 may be required for self-renewal. HSA cells also were exposed to varying levels of IL-8, and their growth capabilities were tested using MTS assays. The results show little difference between groups, suggesting that IL-8 does not play a role in the growth of monolayer cells. In vivo, four groups of mice were inoculated subcutaneously with either HSA monolayer or sphere cells. Two of the groups received anti-IL-8 antibodies. Cell proliferation was followed for four weeks using bioluminescence. Necropsies are underway, and histology and IHC will be used to determine tumor type and composition. Based on the bioluminescence data, the groups receiving anti-IL-8 antibodies appear to have smaller tumors than the control groups. This suggests that IL-8 may be important for HSA growth in vivo. The sum of my experiments will show the role of IL-8 in tumor proliferation and multipotency. If IL-8 proves to be crucial for the development and maintenance of HSA, disrupting this factor may enhance the sensitivity of HSA cells to conventional cancer therapies.
The influence of inflammatory cells on neural stem cells following herpes simplex encephalitis.

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Herpes simplex encephalitis (HSE) is a rare but serious complication from infection with herpes simplex virus (HSV)-1. This encephalitis leads to an acute inflammatory state that can result in chronic issues such as neural and behavioral deficits. Although it is known that inflammatory cells enter the brain during HSE, little is known about how these cells influence neural stem cells (NSCs). This project aims to explore how inflammatory cells influence NSC proliferation during HSE. The first step is to isolate inflammatory cells from the brains of Balb/c mice following HSV-1 infection. These cells will be quantified and phenotyped in order to show when certain inflammatory cells enter the brain following HSV infection. Then, purified inflammatory cell populations such as T regulatory cells, CD8+ T cells and monocytes will be co-cultured with NSCs. At the end of these in vivo experiments the number of living NSCs will be quantified. The overall goal is to show that certain inflammatory cell populations either inhibit or stimulate NSC proliferation. From what is known about the action of CD8+ cells in the brain, the expectation is that these cells will inhibit the proliferation of NSCs. It is additionally expected that T regulatory cells will increase NSC proliferation and ameliorate the impact of CD8+ cells on NSC proliferation. It is also hypothesized that monocytes will inhibit NSC proliferation. These experiments are important because they may identify points of medical intervention that could help alleviate some of the ensuing problems of HSE.

A Novel Reporter System to Identify the Osteogenic Progenitor Cells from Embryonic Stem Cells.

Bellrichard, Mitch Zou, Li Kaufman, Dan
College of Medicine and The Stem Cell Institute

The goal of our project is to identify osteogenic progenitor cells derived from embryonic stem cells. The RUNX2- Yellow Fluorescent Protein (YFP) reporter system was developed in human embryonic stem cells (hESCs) and tested in different culture conditions in vitro. RUNX2-YFP reporter system, tagged with luciferase, was introduced into the hESCs by nucleoinfection. By selecting luciferase positive cells, the RUNX2-YFP tagged hESCs were purified and subcultured on mouse embryonic fibroblasts (MEF) feeder cells. To evaluate the expression level of YFP in these cells, the hESCs expressing RUNX2-YFP were cultured in different extracellular matrix and with various differentiation media. The YFP expression in the RUNX2-YFP hESCs was evaluated by flow cytometry quantitatively or under fluorescent microscope at different time points. The RUNX2-YFP cells were successfully selected by luciferase positive signal in the cells after co-cultured with luciferin, and the YFP expression level was consistently higher when the cells were cultured in the presence of gelatin or matrigel, rather than fibronectin or the uncoated surface. The fluorescent microscope showed similar results. Other than that, gelatin does not only promote YFP expression in the cells, it also induces differentiation of hESCs to mesenchymal stem cells, based on the expression profile of the cell surface markers revealed by flow cytometry. It’s easier to select the target cells using the RUNX2-YFP reporter system tagged with luciferase. Gelatin and matrigel appear to promote hESCs differentiation and RUNX-2 expression. However, further study on the RUNX2-YFP labeled hESCs is needed to verify the YFP expression level is in parallel with the RUNX2 expression level evaluated by PCR, and the bone formation of these YFP positive cells in vitro and in vivo.
**Staphylococcus aureus enterotoxins in swine and retail pork**  
Botting, Danielle; Davies, Peter; Linhares, Leticia; Sreevatsan, Srinand; Yang, My  
Department of Veterinary Population Medicine, University of Minnesota

The emergence of “livestock associated MRSA,” a potential threat to public health, has focused research efforts to look for MRSA among livestock species. There has been negligible research of Staphylococcus aureus epidemiology in pigs, on farms and in harvesting/processing facilities in the United States. More investigation is required to characterize the ecology of Staphylococcus aureus in modern swine production systems to quantify the public health risk, and to improve surveillance, control and prevention measures. The Centers for Disease Control and Prevention has reported that Staphylococcus aureus is the most common agent of foodborne disease outbreaks linked to pork products in the United States. This important foodborne pathogen causes gastroenteritis in humans, and is the result of toxins produced by the staphylococcal bacteria. Therefore, the risk of foodborne illness is determined by the ability of Staphylococcus aureus to produce toxins via expression of enterotoxin genes.

My project focuses on identifying and characterizing Staphylococcus aureus isolates collected from pigs and retail pork products. The specific aims of my research project are:

a) To characterize the isolates using PCR with respect to methicillin resistance (presence of mecA gene), spa typing, and the presence of enterotoxin genes, and

b) To use PCR to test for enterotoxin genes in Staphylococcus aureus isolates previously collected (by Drs. Davies, Sreevatsan and Linhares) from swine farms, market hogs at slaughter, and retail pork.

**Survival and growth patterns of antibiotic resistant versus pansusceptible non-STEC E. coli strains in different environments**  
Ashley Chirco¹, Mastura Akhtar², Francisco Diez-Gonzalez², and Fernando Sampedro³  
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**Background:** The purpose of this study is to determine the survival, persistence and growth characteristics of antibiotic resistant vs. susceptible E. coli strains at varying temperatures and pH levels, as well as finding effective methods of control.

**Methods:** E. coli strains were grown overnight, washed, diluted and inoculated (10³ CFU/mL) into tryptic soy broth (TSB) at different pH levels (4.0, 5.5 and 7.0) and incubated at different temperatures (4, 10 and 23° C) for 48 h. Samples were collected at 0, 1, 2, 4, 6, 24 and 48 h and bacterial counts were determined. To evaluate the effects of different control methods, lactic acid was tested using 2.5 and 5% and samples were collected at 0, 1, 2.5, 5, 7.5 and 10 min and bacterial counts were determined.

**Results:** At 4° C, both E. coli strains showed similar survival characteristics and bacterial concentrations remained unchanged compared to inoculation levels. At an abuse refrigeration temperature (10° C), E. coli counts were constant at pH 4.0, while at pH 5.5 and 7.0, the multidrug-resistant and susceptible strains increased ~1 log CFU/mL, reaching 10⁴ CFU/mL by 48 h. At 23°C growth kinetics increased reaching 10⁸ CFU/mL after 24 h. When treated with lactic acid (2.5-5%), 3-4 log reductions of E. coli cells was achieved being the different strain susceptibility dependent on the lactic acid concentration.

**Conclusion:** E. coli strains were found to be tolerant to low pH and various temperatures; however, no direct link was observed between antibiotic resistance and survival capacity. Lactic acid was found to be an effective method to control E. coli strains.
Inflammatory Bowel Disease (IBD) is believed to be an autoimmune condition resulting from aberrant response to antigens found in the bowel lumen, but the etiology of this response is not fully understood. Recent studies have shown that patients (human and canine) with IBD have reduced intestinal bacterial biodiversity when compared with healthy individuals, with loss of normal anaerobes and an increase in the frequency of pathogens. Our study seeks to define the population dynamics of bacterial microflora in dogs diagnosed with IBD as compared to healthy dogs and the relationship between microbiome changes, T-cell responses, and active disease in dogs with IBD.

Using duodenal biopsies from VMC IBD patients and healthy lab beagles, our study will evaluate the microbial population using T-RFLP analysis and define host response by evaluating the expression of cytokines and transcription factors associated with specific T-cell responses.

Microbiome sequencing results are only available for our first 5 VMC patient participants, but they indicate a canine IBD microbiome largely dominated by members of the proteobacteria and firmicutes phyla. The protocol for quantifying host response is still being optimized. We intend to continue this investigation as more patients become available.

Anticoagulant rodenticides are used worldwide for small mammal pest control; they are increasingly being used in island ecosystems for rodent eradication efforts. These eradication efforts pose risk of secondary non-target poisoning to raptors and other birds. Rodenticides act by inhibiting the recycling of vitamin K, thereby interrupting the production of essential coagulation factors. In the Galapagos Islands, the current mitigation strategy is to manage endemic hawks in captivity during eradication programs. This process is resource intensive and stressful for wild hawks. Daily vitamin K supplementation is recognized as an effective treatment for rodenticide poisoning, but is not a reasonable mitigation strategy for wildlife. A sustained-release polymer system has been successfully used to deliver therapeutic agents and sustain drug concentration in target tissues. Thus, we reasoned that a novel, sustained-release formulation of vitamin K would be a valuable tool for conservation medicine. We hypothesized that a single dose injection of sustained-release vitamin K will produce comparable plasma vitamin K levels to daily oral dosing of vitamin K in two species of birds. We evaluated two sustained-release vitamin K test products in chickens, and compared resulting plasma vitamin K levels with the oral formula. Over two weeks of sample collection, sustained levels of increased plasma vitamin K were achieved, but did not appear to match the levels of oral dosing. Further work needs to be done to optimize the sustained release formula. This drug model has the potential to become a novel tool in avian medicine, which can be applied in conservation efforts, as well as clinical wildlife and zoo medicine.
Myocardial infarction and ischemia due to coronary artery disease is the primary cause of chronic heart failure, a leading cause of mortality in the United States. By using the rat as a model for chronic heart disease in humans, we can better understand the pathophysiological mechanisms of heart failure and their regulation. Increased activity of the renin angiotensin system (RAS) in the brain is known to drive sympathetic nervous system activity and much of the pathophysiology of heart failure. Oxidative stress has been a proposed mechanism that may mediate actions of an increase in RAS in the brain. It has been shown that overexpression of the free radical scavenging enzyme, superoxide dismutase (SOD), in one of the circumventricular organs of the brain improves cardiac function in a mouse model of heart failure. A downstream hypothalamic site, the median preoptic nucleus (MnPO), may act as a relay center for reactive oxygen species to act as mediators in the pathophysiology of heart failure. In order to test the hypothesis that elevated reactive oxygen species in the MnPO are related to the pathophysiology of heart failure and decreased cardiac function, we injected adenovirus vectors capable of overexpressing SOD in the MnPO of normal rats. Subsequently, rats were subjected to coronary artery ligation to create a myocardial infarct and eventual heart failure. Cardiac function was monitored at two week intervals over six weeks via echocardiography. At the end of the experiments, rats were perfused with paraformaldehyde and brains and hearts were removed for purposes of immunofluorescence to detect SOD in the brain and histopathological analyses of infarct size and apoptosis, respectively. The myocardial infarcted (MI) rats demonstrated marked ventricular fibrosis and area of infarct, as well as a decrease in ventricular function when compared with non-myocardial infarcted (non-MI) rats. The myocardial infarcted rats with superoxide dismutase (MI/SOD) in the MnPO tended to have less impairment in ventricular function when compared to MI rats without the superoxide dismutase, although this finding is not statistically significant. Therefore, we cannot conclude at this time that there is any improvement in cardiac function in the MI/SOD rats compared to MI rats.

Evaluation of sampling strategies to diagnose influenza virus in pre-weaned pigs.

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Influenza virus (IAV) is endemic in pigs and despite its widespread appearance it may be difficult to diagnose. Specifically, IAV is difficult to reliably diagnose in pre-weaned pigs due to low infection prevalence, the short course of clinical signs and infection, and variable levels of maternal antibodies. Both nasal swabs and tissues are used for diagnosis, but it is unclear whether one method is superior to the other when diagnosing IAV in pre-weaned pigs. The objective of this study was to compare two sampling strategies, nasal swabs and tissues from dead pigs, in their ability to diagnose IAV in pre-weaned pigs and consider a farm infected with IAV. Two farms in Southern Minnesota, known to be IAV positive before the study, were selected and monitored over a 4 week period. Sampling method 1 included nasal swabs were collected 4 weeks apart from pre-weaned pigs at 21 days of age, and sampling method 2, necropsies were performed weekly on dead piglets 7 to 21 days of age. Swabs were collected from the upper respiratory tract (nasal cavity) and lower respiratory tract (trachea, and main stem bronchi). All samples were tested by a real time RT-PCR targeting the IAV matrix gene, and positive swabs from RT-PCR were confirmed positive using virus isolation. Prevalence was 8.9% (7/79) and 13.9% (21/151) based on samples from dead or live pigs, respectively. Seven nasal swabs and 4 lung swabs tested RT-PCR positive from samples within a dead pig, and 5 of these swabs were positive for IAV using virus isolation. In this study, prevalence levels were higher in samples collected from live pigs compared to natural mortality, and live pigs were older than the naturally occurring dead pigs, which may explain why tissue swabs did not offer an advantage over nasal swabs from live pigs. Overall, this study showed the difficulty of diagnosing IAV in pre-weaned pigs due to low prevalence levels. In conclusion, ongoing diagnostic submissions are necessary in order to characterize IAV infections in breeding herds.
The ecology of eastern equine encephalitis virus in wildlife and mosquitoes in Minnesota
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Eastern equine encephalitis virus (EEEV) is a mosquito-borne zoonotic virus that is prevalent in North America. The primary transmission cycle involves mosquitoes and wild birds as reservoir hosts and infection has been documented in horses, humans and wildlife species. The aim of this correlative study was to examine vectors of EEEV in relation to high antibody titers found in moose and elk that were sampled as part of the Minnesota Department of Natural Resources’ wildlife health surveillance efforts. Adult mosquitoes were sampled weekly during the summer of 2012 from 6 regions in northern Minnesota. In each region, one trapping site was in an area with high moose or elk seroprevalence, while another was in a matching low seroprevalence area. Specimens were identified to species by light microscopy and taxonomic key.

Preliminary results indicate that both Aedes sticticus and Aedes vexans were more abundant in high seroprevalence trapping locations. Culiseta melanura, the enzootic amplifying vector has not been obtained in any of our 6 trapping regions.

Utilizing these findings along with previous studies, we can prioritize which species to test for EEEV by RT-PCR. After testing, if infection prevalence is sufficient, regression methods will be employed to determine which factor, or combination of factors: mosquito species, region, or time is the most predictive of wildlife exposure status. When this project is complete a more effective approach to targeting mosquito populations that transmit the virus can be developed to protect the spread of disease to susceptible populations.

Effect of using a Novel Method to Heat-Treat Small Volumes of Bovine Colostrum
Andrew A. Kryzer, Sandra M. Godden
Veterinary Population Medicine

The Perfect Udder System is designed to allow producers on smaller dairies to heat-treat colostrum one gallon at a time. However, this system requires validation. The objective of this study is to describe the effect of the Perfect Udder heat-treatment system on colostrum characteristics and on passive transfer of IgG in neonatal calves, compared to a negative control group (fresh colostrum) and a positive control group (batch heat-treated colostrum).

We expect to observe:
1. Significant reduction in bacteria counts but no significant change in colostrum IgG concentration in the Perfect Udder prepared colostrum compared to fresh colostrum.
2. No significant difference in either bacteria counts or IgG concentrations in the Perfect Udder prepared colostrum compared to batch heat-treated colostrum.
3. Significant improvement in apparent efficiency of absorption of IgG in neonatal calves fed Perfect Udder prepared colostrum compared to calves fed fresh colostrum.

First milking colostrum will be pooled to achieve a unique, consistent starting batch. The colostrum will be divided four ways with 4 quarts of each treatment group: 1. Fresh refrigerated in Perfect Udder bag (negative control); 2. Fresh frozen in Perfect Udder bag (negative control); 3. Heat-treat in Perfect Udder bag and freeze; 4. Batch heat-treat, freeze in Perfect Udder bag (positive control). Colostrum will be sampled for testing for IgG concentrations and bacterial culture. Newborn heifer calves (n=120) will be randomly assigned to be fed one of the four colostrum treatment groups with 30 calves in each group. Blood will be sampled at age 0-1 hour (pre-feeding) and at age 24 hours (post-feeding) for measurement of serum IgG levels. If successful, the Perfect Udder system could be an excellent approach for small to medium sized dairies that have only 1 or 2 calves a day and do not meet the 2 to 4 gallon minimum of batch pasteurizers.
Genetic Basis of the Flaxen Phenotype in Horses

Tali McNeil, Shea Anderson, Jessica Petersen, James Mickelson
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The goal of this study was to investigate the endothelin 3 gene (EDN3) as a candidate coat color gene in horses for containing a mutation that results in a “flaxen” phenotype. The flaxen phenotype describes horses that have a light cream or white colored mane and tail. A genome-wide association study of single nucleotide polymorphism (SNP) markers in flaxen and non-flaxen horses identified a region on equine chromosome 22 as containing the flaxen gene locus. The EDN3 gene lies in this locus and the protein product of EDN3 plays a major role in melanocyte differentiation and migration, and thus makes a good candidate gene for the flaxen phenotype. Identification of the gene responsible for this phenotype would allow the creation of a genetic test for breeders to determine the outcome of matings as well as provide new insights into coat color biology. EDN3 gene segments were amplified with PCR from DNA samples obtained from flaxen and non-flaxen (control) horses. The PCR products were sequenced, and the flaxen and non-flaxen sequences were compared using Sequencher software to identify any potential causative mutations. DNA samples from 12 horses were obtained, three flaxen and three non-flaxen Franches Montagnes horses, and three flaxen and three non-flaxen Black Forest horses. EDN3 is composed of a 5’ UTR, five exons, four introns and a 3’ UTR. All regions except the second intron, a portion of intron 4 and the 3’ UTR were successfully amplified. Approximately 6,000 base pair were analyzed and five SNPs were identified. All of the SNPs are intronic and none show a strong association with the flaxen phenotype. A larger sample size and completing the sequencing of EDN3 may reveal additional SNPs within EDN3, however it likely that the flaxen phenotype is either caused by a more complex genetic mechanism than was analyzed here, or that the causative mutation is not within EDN3 but instead lies in a nearby gene on chromosome 22.

Mechanisms of Cancer Targeting Through the Epidermal Growth Factor Receptor

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Epidermal growth factor receptor (EGFR) has been implicated in the proliferation of cancer cells through mutations leading to over-expression. Many anti-cancer agents have been developed that interfere with EGFR activity. A bispecific ligand-targeted (BLT) toxin, containing epidermal growth factor (EGF), urokinase (uPA), and a pseudomonas exotoxin was developed that effectively kills sarcoma cells in vitro and is entering the first phase in clinical trials for dogs with hemangiosarcoma. We showed that internalization of the toxin requires binding to EGFR and urokinase receptor (uPAR); however, sarcoma cells have much lower levels of EGFR expression than carcinomas, so the role of EGFR in this process is not well understood. We sought to define if the relationship between EGFR expression and BLT toxin internalization fits a linear model or a threshold model. For this purpose, we used constitutive and inducible systems to overexpress EGFR in canine hemangiosarcoma and osteosarcoma cells. The data show that transfection of hemangiosarcoma cells was inefficient, whereas osteosarcoma cells were readily receptive to transfection and expressed ectopic EGFR, albeit only at low levels and expression was not easily tunable. Expression was episomal, as it was lost in the absence of selection. Nevertheless, preliminary data suggest that the osteosarcoma cells with ectopic EGFR proliferated faster and were sensitive to similar doses of BLT toxin. Generation of stable transfectants with the tunable EGFR construct is needed to fully assess how this receptor enhances cell growth and to precisely define the relationship between EGFR expression and sensitivity to the BLT toxin.
Assessing Changes in Stress During Rehabilitation in Six Species of Wild Raptors Using Heterophil to Lymphocyte Ratios.

Kathleen L Neshek, Patrick T Redig

From the College of Veterinary Medicine, University of Minnesota, & The Raptor Center, Saint Paul, MN
Supported by the University of Minnesota, College of Veterinary Medicine Summer Scholars Program

The goal of this study was to assess the changes in stress levels of successfully released raptors during their rehabilitation using heterophil-to-lymphocyte ratios (H:L) as a parameter of evaluation. We tested the hypothesis that the H:L ratio would decrease from the time of a bird’s admission to its release owing to its habituation to captivity and recovery from morbidity, and that these changes may vary with species. Chronic stress can have serious negative effects during rehabilitation, such as, predisposing a bird to infections (e.g., A. fumigatus) and decreasing its ability to heal. While direct measurement of circulating glucocorticoids is an accurate assessment of acutely stressful events, H:L ratios, which have been shown to vary inversely with levels of stress, are more indicative of the variety of perturbations experienced by an individual bird (Siegel and Gross 2000). In addition, this ratio is not responsive to acute changes in stress associated with handling for blood sample collection. In this study H:L ratios were evaluated in relation to species, duration of rehabilitation, and the category of major problems upon admission (e.g., starvation, trauma, trauma with fracture, and lead toxicity). Species included in this study were birds admitted for traumatic injuries to The Raptor Center during the years 2009-2011 and included Red-Tailed Hawks (49), Bald Eagles (45), Great Horned Owls (34), Great Grey Owls (11), Coopers Hawks (15) and Barred Owls (18). The birds were subjected to a variety of procedures including anesthesia, radiology, orthopedic surgery, injections, blood draws, oral medications, oral gavage, handling and exercise. The length of time spent at the Raptor Center ranged from 5 to 409 days. PCV, TP, and total and differential WBC counts from quick stained slides were recorded upon admission and within 5 days of release and comprised the data compiled for this study. The data collected has been tabulated and analysis is underway.

Effect of oral Vitamin D supplementation on antibody response to PRRS virus

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One of the functions of Vitamin D₃ in the body is to stimulate leukocyte expression and response to foreign agents. Vitamin D₃ is primarily produced by exposure to UVB light from the sun. We have confirmed that swine housed indoors have low levels of this crucial vitamin, potentially leaving them more susceptible to disease. This study will seek to understand how oral vitamin D supplementation influences the production of Porcine Reproductive and Respiratory Syndrome (PRRS) antibodies in infected pigs and to determine if supplementation is of value.

This study used a 2x2 design. 40 barrows were be sourced from a high health sow herd tested negative for PRRS. On day 2 post farrowing, select piglets were treated orally with 40,000 IU vitamin D. The barrows were split into 4 weight-matched groups. The other two groups were inoculated with PRRS. Groups that were to be supplemented with Vitamin D received a second dose of vitamin D on day 0 of the study. Blood samples were collected from each pig on Day 0, 8 and 14. PRRS ELISA was performed and Serum 25-Hydroxyvitamin D (25-OHD) assays were performed at Heartland Assays of Ames, Iowa. Results of this study showed that 25-OHD were increased in pigs that were supplemented. The ELISA tests also showed that at both 8 and 14 days the levels of antibodies for PRRS virus were increased in pigs that were supplemented. It would appear that having a higher level of Vitamin D in the body has an effect on the time required to produce PRRS antibodies as well as how strong the response is.

This study demonstrated the role of Vitamin D in the immune response to PRRS virus resulting in an enhanced humoral response in an experimental challenge model. The increased level of PRRS antibodies in supplemented pigs suggests a faster and more robust immune response. If this change in immune response were to decrease recovery time in infected pigs, then vitamin D supplementation may prove to be a valuable tool for producers.
Understanding the Evolution of Multidrug Resistance-Encoding Plasmids
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The broad-host-range plasmid IncA/C contains multiple loci encoding for antibiotic resistance and has caused the emergence of many multidrug resistant strains of bacteria. Little is known about the basic mechanisms of persistence and dissemination of this plasmid type despite its posed dangers to human and animal health. The goal of this study was to determine if adaptive evolution plays a role in its ability to persist and spread in bacterial populations. Two different species of bacteria harboring the IncA/C plasmid pAR060302, Salmonella enterica and Escherichia coli, were experimentally evolved for 1,190 generation in vitro. Competition experiments were performed to determine if experimental evolution altered the bacterial host’s fitness cost for carrying the IncA/C plasmid. The cost for carrying pAR060302 was decreased for both the S. enterica and E. coli evolved strains. The evolved plasmid’s stability was also examined. Maintenance of the three accessory regions of pAR060302, each encoding for florfenicol, ampicillin and streptomycin resistance, was measured by calculating the proportion of drug susceptible clones that arose during the experimental evolution. The only region lost over the experimental evolution was pAR060302’s ampicillin resistance region in S. enterica. These results suggest that IncA/C plasmids co-adapt with their bacterial hosts to become more efficient to the host, and that IncA/C plasmid stability is host and locus specific.

Comparison of immunomodulatory potential of canine stromal vascular fraction and cultured adipose derived stem cells
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Canine adipose-derived stem cells (ADSCs) are multipotent cells of mesenchymal origin. These cells have shown promise in the treatment of osteoarthritis in dogs through autologous therapy using the stromal vascular fraction (SVF), which is the product of digested adipose tissue. Previous studies have shown that cultured ADSCs exhibit immunomodulatory properties in vitro, and this is suspected to be a major mechanism of action in effectiveness of treating osteoarthritis. However, the immunomodulatory properties of the SVF have not been compared to the cultured cells. This is significant because the SVF contains additional cells such as erythrocytes and leukocytes, which could potentially affect immunomodulation in vivo. Currently, the clinical use of ADSCs for treatment of osteoarthritis in dogs has been limited to the SVF. However, cultured allogeneic ADSCs could provide many potential advantages over the SVF, including the generation of multiple treatments and a more predictable and available therapeutic product. We hypothesized that the stromal vascular fraction exhibits similar immunomodulatory properties when compared to cultured cells. To test this, we co-cultured canine ADSCs and SVF with mitogen activated allogeneic peripheral blood mononuclear cells (PBMCs) and measured the proliferation of the PBMCs. PBMC proliferation was assessed with flow cytometry using carboxyfluorescein succinimidyl ester as a marker of proliferation. The preliminary data suggests that canine ADSCs decrease mitogen stimulated PBMC proliferation. There is a trend for cultured ADSCs to have a greater effect on suppressing PBMC proliferation. There is some variability between stem cell donor cell populations and the effect they have on allogeneic PBMCs. This assay requires further investigation with additional allogeneic PBMC donors and ADSC donors to verify repeatability.
Serological analysis of Newcastle virus exposure in raptors and waterfowl

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Newcastle disease virus (NDV) is associated with morbidity and mortality in over 240 species of birds, and causes particularly severe neurologic disease in juvenile Double-crested Cormorants and American White Pelicans. Epizootics have occurred regularly in waterfowl since the 1990’s, most recently in Minnesota in 2008, 2010 and 2012. NDV has also been isolated in sub-clinically infected Bald Eagles and Great Horned Owls, but the prevalence among raptors is unknown. Since the potential for future outbreaks is high, it is critical to understand the ecology of NDV and identify host and environmental factors affecting its transmission and pathogenicity. We assessed the prevalence of NDV in target wild bird species by testing waterfowl eggs collected in 2011 at the Lac Qui Parle Wildlife Refuge and plasma samples from raptors archived from 2008-2012 at the Raptor Center. Among predators and scavengers of wetland birds, 5.2% of Bald Eagles and 5% of Great Horned Owls were positive for antibodies to NDV. Peregrine Falcons and Red-tailed Hawks did not show evidence of exposure. The occurrence of antibodies in raptors does not correlate with reported NDV outbreaks in waterfowl. Thus, NDV appears to persist in the environment outside of reported outbreaks. Great Horned Owls and Bald Eagles may be more susceptible to exposure based on diet and habitat preferences. Among waterfowl, 10% of Double-crested Cormorant eggs and 6.7% of American White Pelican eggs collected in 2011 were positive for antibodies to NDV. The presence of antibodies in eggs one year after a major outbreak suggests that short-term maternal antibody protection may be responsible for the biennial nature of outbreaks in Minnesota waterfowl.

Reduced osteoarthritis severity in aged MIF knockout mice

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Osteoarthritis (OA) is an important age-related disease, but the mechanisms for its development are not fully understood. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine produced by chondrocytes. The absence of MIF has recently been associated with increased lifespan in mice and with a significant reduction in OA severity in adult mice. The purpose of the present study was to determine if MIF-KO mice developed OA in advanced age or continued to remain relatively disease free during aging. Stifle joints from 2-year-old wild type (WT, n=15) and MIF-KO (n=16) mice were fixed in formalin, decalcified in 10% EDTA, and embedded in paraffin for sectioning and staining of a representative mid-coronal section with hematoxylin and eosin (H&E). The medial tibial plateau and medial meniscus were semiquantitatively evaluated for articular cartilage (AC) structural damage using a previously established murine grading scheme, and measurements were made of thickness and area of AC and subchondral bone (SCB) using an Osteomeasure Histomorphometry system. The number of viable chondrocytes, area occupied by dead chondrocytes in the AC, and weight-bearing meniscal area were also measured. Compared to WT mice, the SCB thickness (p<0.05) and area (p<0.01) were significantly decreased in MIF-KO mice, while AC thickness (p<0.001) and area (p<0.001) were significantly increased. In addition, AC structure scores were significantly lower (p<0.01) in MIF-KO mice. Thus, aged MIF-KO mice had significantly reduced osteoarthritis severity compared with WT controls, suggesting that MIF may be a key factor in the development and progression of age-related OA.
Diabetes mellitus is a disease that affects 25.8 million people of all ages; and according to the CDC, 1 in 3 adults will be diagnosed with diabetes by 2050. Diabetes is also a very costly disease; with an estimated cost in the United States in 2007 due to diabetes of approximately 174 billion dollars. In both type 1 (T1DM) and type 2 diabetes (T2DM) there is a loss of glucose regulation and a loss of the insulin secreting beta cells. T2DM patients initially develop a resistance to the action of insulin, and a compensating proliferation of beta cells. If the disease is left uncontrolled however, this will lead to beta cell death and insulin dependence. The mechanisms that trigger beta cell death are still not totally understood, but it is known that oxidative stress does play a role. The exact signaling pathways that lead to the apoptosis of these cells however are not as clearly understood. The goal of this study is to examine the apoptotic pathways that play a role in death of beta cells in order to identify targets to treat advanced T2DM. Initially, we investigated p53, a tumor suppressor, by inducing T2DM in mice that are genetically deficient in p53 activity. Mice were fed a high-fat diet, and monitored for weight gain, as well as blood glucose and modified hemoglobin species (HbA1c) to indicate the onset of T2DM and beta cell loss. Pancreas samples were collected, sectioned and stained with a TUNEL assay, allowing us to see DNA damage that occurs during apoptosis. Western blot analysis then can be utilized in order to determine the amount of phosphorylated or activated p53 that is present in our mouse beta cells. This will help us determine whether p53 does indeed play a role in the apoptosis of beta cells during T2DM. Histology will be also done on the pancreas samples to allow us to measure beta cell mass and apoptosis. This project was supported by the NCRR and the Office of Research Infrastructure Programs of the NIH through Grant Number T35 OD011118.

Environmental change and degradation and the subsequent increase in human interactions with wildlife have been implicated in emergence of new zoonotic diseases and the spread of new and existing vector-borne diseases. Long-term ecological research sites (LTER) have contributed to a greater understanding of complex ecological systems, including those of diseases. The purpose of this study is to better understand the ecological relationship between disease, wildlife, and the environment by utilizing a well-established LTER site. More specifically, this study will determine how small mammal biodiversity and intestinal parasite biodiversity are affected by vegetative succession following abandonment from agriculture. Small mammals were collected along a successional gradient of fields. The six fields studied are part of established LTER at Cedar Creek Ecosystem Science Reserve in central Minnesota and represent early (26 years), mid (35-44 years), and late (at least 60 years) stages in succession. Trapping was conducted for 5 nights using large Sherman live traps set in 6 by 6 square grids with traps 15 meters apart. Feces, ear biopsies, ticks, and blood samples were collected. Feces will be used to calculate intestinal parasite biodiversity using the Shannon biodiversity index. A small mammal biodiversity index will also be established for each field, allowing for comparison of small mammal biodiversity along the successional gradient. Additionally, samples will be used for further disease studies, including viral biodiversity, metagenomics, and tick-borne disease. This study investigating the influence of vegetative succession on small mammal parasite biodiversity as a marker of disease risk will set the stage for future studies on microbial ecology and disease risk.