

**Department of Allied Health Sciences
2104 Graduate Student Research Forum
Poster Presentation Abstracts**

Prevalence and risk factors of self-reported chemosensory disorders and preliminary associations with adiposity in United States adults: Results from 2011-2012 National Health and Nutrition Examination Survey (NHANES)

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A taste and smell questionnaire was administered to a nationally-representative sample of 3603 adults as part of the NHANES 2011-2012 home interview to provide data on perceived chemosensory-related problems and comorbidities. Participants who were 40 years and older were asked to report their taste and smell function, presence of chemosensory disorders, and history of symptoms, risk factors and medical treatment for these disorders. Six percent of the population reported phantosmia and 19% reported diminished/absent smell; overall prevalence of smell dysfunction was 23%. Five percent reported dysgeusia, and including 18% who reported diminished/absent taste or flavor, overall prevalence of taste/flavor dysfunction was 19%. Among those who reported chemosensory problems, 12.5% sought medical attention, 3.5% tried any recommended treatment, and 3% said that the dysfunction interfered with their daily life. Factors independently associated with report of any chemosensory dysfunction were identified with multiple logistic regression and included less education, persistent dry mouth, reported poor health, nasal/sinus problems and history of head/face injury. The strongest risk factors were persistent dry mouth (OR= 2.65; 95% CI: 1.97, 3.56) for reported taste dysfunction and nasal/sinus problems (OR= 1.97; 95% CI: 1.53, 2.55) for reported smell dysfunction. In preliminary analyses, self-reported chemosensory dysfunction was associated with higher body mass index after controlling for many other predictors of adiposity, including education, smoking status and physical activity. These findings suggest that perceived chemosensory disorders are prevalent in US adults and that perceived disorders may explain some of the variability in body mass index.

**Department of Animal Science
2014 Graduate Student Research Forum
Poster Presentation Abstracts**

Effects of Maternal Nutrient Restriction on Muscle Satellite Cell Activity in Lambs

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Poor maternal nutrition during gestation affects postnatal growth of the muscle. Satellite cells are myogenic precursor cells that contribute to postnatal muscle growth, and their activity is evaluated by the expression of transcription factors. Pax7 is expressed in quiescent and active satellite cells, MyoD in proliferating satellite cells and myogenin in terminally differentiating myoblasts. We hypothesized that poor maternal nutrition during gestation alters the temporal expression of Pax7, MyoD and myogenin in satellite cells. Ewes ($n = 23$) were fed 100% or 60% NRC requirements from d 31 ± 1.3 of gestation. Lambs from control-fed (CON) or restricted-fed (RES) ewes were euthanized within 24 h of birth (d 1; $n = 12$) or were fed a control diet until 3 mo ($n = 11$). Satellite cells isolated from the semitendinosus muscle were cultured for 24, 48 or 72 h before immunostaining for Pax7, MyoD, myogenin and Hoescht dye. After 24 h of culture, the percent of cells expressing MyoD was greater in RES lambs at birth ($58.40 \pm 12.08\%$) than in CON lambs ($11.68 \pm 17.08\%$; $P = 0.03$). After 48 h of culture, there was a greater percentage of cells expressing myogenin in RES lambs at birth ($63.25 \pm 14.00\%$) compared with cells from CON lambs ($17.57 \pm 17.08\%$; $P = 0.04$). After 72 h of culture the percent of satellite cells expressing myogenin in RES lambs at birth ($40.07 \pm 14.00\%$) was lesser than cells from CON lambs ($83.98 \pm 17.08\%$; $P = 0.05$). There were no differences in the percent of Pax7 immunopositive cells at birth, or any factors in cells from lambs at 3 mo of age ($P > 0.05$). In conclusion, restricted nutrition during gestation alters the temporal expression of myogenic regulatory factors in the offspring.

Complete Transcriptional Profiles of Bovine In Vivo Pre-implantation Development

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During mammalian pre-implantation development dramatic and orchestrated changes in transcriptomes occur. Here we report the first complete transcriptome dynamics of single in vivo matured bovine oocytes and all stages of in vivo pre-implantation embryos by RNA-seq. Surprisingly, nearly half of the bovine genome, 11,488 to 12,729 genes of more than 100 pathways, is expressed. A total of 2,845 genes were differentially expressed among different stages, of which the largest change was observed between the 4- and 8-cell stages, demonstrating unambiguously the bovine embryonic genome activation. Additionally, 774 genes were identified as only expressed/highly enriched in particular stages of development. Using weighted gene co-expression network analysis, we found 12 stage-specific modules of co-expressed genes. Furthermore, we identified conserved key members (hub genes) of the bovine expressed gene networks. Their vast association with other embryonic genes suggests that they may have important regulatory roles in embryogenesis; yet, the majority of the hub genes are relatively unknown/understudied. We also conducted the first embryonic expression profile comparison across three mammalian species, human, mouse and bovine, for which RNA-seq data are available. We found that the three species share more maternally deposited genes than embryonic genome activated genes and there are more similarities in embryonic transcriptomes between bovine and humans than between humans and mice, demonstrating that bovine embryos are better models for human embryonic development. In summary, this study provides the first complete “gold” standards and a timeless database for gene activities in bovine embryos and identified little-known potential master regulators of pre-implantation development.

**2014 Graduate Student Research Forum
Department of Nutritional Sciences
Poster Presentation Abstracts**

Metabolic syndrome parameters and hypercholesterolemia are correlated with elevated liver enzymes

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Thirty-eight individuals (13 men and 25 women) aged 40-70 years with metabolic syndrome (MetS) were recruited to evaluate whether serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), markers of liver function would be elevated in this population and whether these enzymes could be associated with specific parameters of MetS. The mean values for this population were within normal ranges for both enzymes: ALT, 32 ± 15 U/L and AST, 31.5 ± 10.7 U/L, respectively. However, 8 individuals presented AST concentrations > 35 U/L, the reference value. In addition to having MetS, 28 volunteers had plasma LDL Cholesterol (LDL-C) >120 mg/dL. Strong positive correlations were found between liver enzymes and LDL-C (ALT: $r = 0.403$ and AST: $r = 0.540$, $P < 0.01$). All individuals in the highest tertile for ALT were also characterized by having 2 distinct parameters of MetS: high fasting glucose and high waist circumference. These preliminary results suggest that individuals having high LDL-C and MetS characteristics associated with central adiposity and hyperglycemia may be at risk for liver dysfunction.

Does nutrition education influence preschooler's snack choice?

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Poor diet and lack of physical activity have been associated with increased risk of obesity in young children. Studies have shown that early nutrition and physical activity education are effective in teaching preschool children about healthy snack food choices in an effort to prevent childhood obesity. The main objective of this study was to determine the impact of a nutrition education program on preschoolers' actual snack food choices. To test this objective, children ages 3-5 years old (n=49) were recruited to receive a 9-lesson nutrition education intervention from the *All 4 Kids* program's "Eat Smart" unit which focused on healthy snacks and staying active. Pre/post assessments included a validated snack identification and preference questionnaire, as well as an in-class snack food selection trial in which each child was offered a choice between two snacks (grapes or cookies). The results of the study revealed that the nutrition education program did not significantly ($p>0.05$) change preschool children's snack choices. However, there was a significant improvement in the children's reported preference of healthier snacks ($p=0.025$) and the ability to distinguish them ($p=0.013$) from other snacks. Other potential factors that may influence actual child snack decisions such as child demographics and body weight will be explored. These results indicate that other factors such as taste and potentially the home environment may have a stronger influence on preschoolers' snack choices. Future research should determine if additional nutrition education and what education targets are needed to significantly change preschool children's snack decisions.

Evaluating differences in the perceptions of the Dietary Guidelines consumer messages between nutrition educators and their low-income clients

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The 2010 Dietary Guidelines for Americans (DGA) contain seven key consumer messages intended to communicate the DGA to the public. However, these messages have not been extensively tested for feasibility or efficacy. The objective of this research was to determine the parallelism between community nutrition educators and their low-income clients' perceptions of the DGA messages' clarity, feasibility, and ease. One-on-one interviews were conducted with nutrition educators (n = 9) and with low-income clients (n = 40) to assess current dietary intake, food related behaviors, and barriers related to the DGA messages. The low-income client participants were primarily women (98%), from diverse ethnic/racial backgrounds (28% Hispanic, 50% black), with at least one child between the ages of 3 and 10 years old. Interviews were audio-taped, transcribed verbatim, and analyzed using the classical analysis approach to examine differences between the nutrition educators and their clients. The analysis found similarities between educators and clients in perceptions of messages related to increasing fruit and vegetable intake, drinking water, and reducing portion sizes, but differences in perceptions of increasing whole grains, drinking lower fat milk, and reducing sodium intake. Thus, nutrition educators are addressing clients' perceived barriers related to certain DGA messages, but do not have complete understanding of the reasons for clients not following the DGA related to whole grains, low fat milk and sodium. Further research is warranted to determine ways to close this gap such as through new communication tools or other strategies that complement the Dietary Guidelines.

What nutrition and physical activity policies and practices are most lacking in early childhood centers within rural communities?

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While some researchers have examined nutrition and physical activity policies within early childhood programs, little is known about the needs of rural communities. Early childhood centers serving preschool children located within low-income rural communities (n=31) from seven states (IN, KS, MI, ND, OH, SD, WI) were assessed to determine current nutrition and physical activity practices and policies. As part of a large scale childhood obesity prevention project, the Community Healthy Living Index previously validated Early Childhood Program Assessment Tool was used to collect data. Descriptive statistical analysis was conducted to identify high priority areas. Weaknesses in nutrition related policies or practices included a lack of: a research based healthy eating curriculum, farm to school initiatives, vegetable variety, whole grains, and limited saturated fat intake within meals, healthy celebration guidelines, policies prohibiting food as a punishment/reward, child mealtime involvement, and referrals to nutrition assistance programs. Programs also had limited structured physical activity opportunities. Overall, programs lacked parental outreach, staff training, and funding/resources to support nutrition and physical activity. These results provide insight into where early childhood programs within low-income, rural communities may need assistance to help prevent childhood obesity.

Black currant extract attenuates ovariectomy-induced bone loss in mice

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Bone remodeling is orchestrated by osteoblasts and osteoclasts in response to changes in redox status, inflammatory cytokines, and hormones. Several animal and cell studies indicate that black currant (BC) exerts antioxidative and anti-inflammatory properties, which could potentially improve bone mass. This study was aimed to evaluate the effect of BC on bone mass and structure in a mouse model of ovarian hormone deficiency. 14-wk old C57BL/6J mice were ovariectomized (OVX) or sham operated. The OVX mice were divided into two groups; OVX fed a basal diet (control) and OVX fed the same diet but with 1% BC extract (provided by the **Just The Berries LTD**), and sacrificed at 4, 8, and 12 wk. Femoral bone mineral density (BMD) and trabecular bone volume (TBV) by DXA and micro-CT and serum bone markers were determined. OVX significantly reduced BMD and TBV ($p < 0.05$) at all time points. At 8 and 12 wk, BC supplementation attenuated OVX-induced BMD and TBV. OVX and BC treatment did not alter serum biomarkers of bone formation and resorption. Additionally, we tested if BC supplementation affects osteoclast like cell (OCL) formation in bone marrow (BM) cell cultures. BM cells from OVX significantly induced OCL formation compared to cells from sham controls ($P < 0.05$). BC treatment decreased the number of TRAP(+) OCL compared with OVX at 8 and 12 wk ($P < 0.05$). Furthermore, BC supplementation reduced bone resorption activity when measured by resorption pit assay, compared with OVX group ($P < 0.05$). These results show that BC is effective in mitigating osteoclast-induced bone loss accelerated by ovariectomy.

Funding Source: this study was supported by the University of Connecticut USDA Hatch Grant.

Acknowledgement: black currant extracts were gifts from the Just The Berries LTD (Palmerston North, New Zealand)

**Department of Plant Science and Landscape Architecture
2014 Graduate Student Research Forum
Poster Presentation Abstracts**

Effect of pruning heights on growth and visual appeal of under-utilized native shrubs

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The utilization of native plants for landscaping is increasing due to restrictions on invasive ornamentals and interest in sustainable landscaping. Nursery growers must expand their native product offerings to meet increasing demand from the consumers. A good nursery system doesn't just require best growing conditions but also a correct pruning. Pruning reduces apical dominance and promotes side branches growth resulting on a fuller and more appealing plant. Therefore, the objective of this study was to evaluate the growth and visual appearance of three different native species in response to different pruning heights. For this, *Cornus rugosa* plants were subjected to three different pruning heights: 25 cm, 50 cm (from the base of the plant) and control with no pruning. They were evaluated for their growth habit and visual appeal. Control had significantly taller plants in comparison to both pruning heights at $\alpha=0.05$. 50 cm pruned plants were significantly denser and wider with more number of shoots in comparison to control at $\alpha=0.05$. Similar study was conducted with *Viburnum acerifolium* and *Viburnum cassinoides* with two pruning heights: 14 cm (from the base of the plant) and control with no pruning. With *V. acerifolium*, control plants were significantly taller at $\alpha=0.05$. With all the three native shrubs, pruned plants were visually more appealing as compared to control at $\alpha=0.05$.

A Novel gene RED1 plays a vital role in maturation of Arabidopsis seeds

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In higher plants, the seeds development is comprised of two stages: embryogenesis and maturation. The term embryogenesis is the process of transformation from a single cell to a multicellular entity which is a functionally specialized organization. During the maturation stage, the embryo and seed lose water and begin to accumulate oil, carbohydrates, proteins and other storage compounds. In *Arabidopsis thaliana*, there are many well-studied important transcription factors control the process of seed development, especially in seed maturation. According to previous studies, four major transcription factors (ABSCISIC ACID INSENSITIVE3[ABI3], FUSCA3[FUS3], LEAFY COTYLEDON1[LEC1] and LEC2) play key roles in accumulation of storage compounds, cotyledon identity, acquisition of desiccation tolerance and dormancy. These four regulators form a local and redundant network to regulate their sustained expression levels in embryo. Here we report the characterization of a novel gene named RED1, which encodes a plant specific protein and is localized in nucleus. The mutation of this gene causes retarded embryo development (RED) and seed abortion, which can block the embryogenesis in late heart stage or bent-cotyledon stage. Overexpression of RED1 gene in *Arabidopsis* wild type results in increased expression levels of the four master regulators and elevated oil content in mature seeds. These results suggest that RED1 gene may function upstream of the four regulators and play a vital role in late stage of embryogenesis and seed maturation.

Induced autotetraploidy results in reduced fertility in Japanese Barberry

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Japanese barberry (*Berberis thunbergii* DC.) is a desirable landscape shrub due to its ease of cultivation, deer resistance, and high quality foliage in a variety of colors. However, it is identified as invasive in more than 30 eastern and central U.S. states and Canada. Sterile or near-sterile cultivars of Japanese barberry are desirable to replace invasive types. Data generated previously by this lab have shown that fertility is highly variable among diploid (2n) and induced autotetraploid (4n) genotypes, with some 4n exhibiting near or total sterility. Multi-year evaluations of fertility in putative sterile or near-sterile 4n individuals are necessary for the development of cultivar introductions. This study was undertaken to evaluate both male and female fertility of 2n and 4n Japanese barberry 'Crimson Pygmy' [CP], one of the most popular cultivars. Male fertility was assessed by evaluating pollen viability and vitality via microscopy utilizing acetocarmine staining and in vitro pollen germination procedures, respectively. Female fertility was assessed with field grown individuals. Reduced-fertility 4n individuals were selected from a larger population before field planting. For two consecutive years, plants were allowed to open-pollinate, all fruit were harvested from each individual, and numbers of fruit and seeds were recorded for each. Male fertility was highly variable within and between 2n and 4n CP populations, with 4n pollen showing both reduced and increased viability and vitality as compared to 2n controls. This suggests that additional selection for reduced male fertility is possible. Female fertility evaluation showed that 4n fruit- and seed-set was significantly reduced compared to 2n controls. However, some 4n individuals which did not fruit in the first year did fruit in the second year, which underscores the need for multi-year evaluation. This study shows that induced autotetraploidy in Japanese barberry 'Crimson Pygmy', coupled with fertility evaluation and selection, has resulted in putative sterile or near-sterile individuals that may be suitable for commercial production.

Evaluation of short-growth mutants of perennial ryegrass for their shade tolerance

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Shade tolerance is an important trait for perennial ryegrass (PRG; *Lolium perenne* L.). Under shade environment, typical symptoms of PRG include thin, narrow and elongated leaf blades, reduced leaf appearance and tiller number, poor wearing ability, and weak root system. We have used mutagenesis techniques to breed short-growth (dwarf) mutant PRG varieties. A number of M2 generation plant lines were selected based on height, tiller number and leaf width. One line, named EMS18, displayed lower leaf extension rate (shorter leaf blade), better leaf appearance (greener color) and longer root when compared to the wild-type controls under greenhouse conditions. FN4, another line, also showed reduced elongation in leaf blade and internodes length when grown in the field.

We have further evaluated their responses to shade under field conditions. EMS18 and FN4 were planted in woods and mowed at a height of 3 inches regularly. After 1.5 months of totally shaded (no direct sunlight) environments, these two mutant lines were green in color and healthy in general, while the wild-type controls became yellow and some leaves were dying. The root systems, particularly the root lengths of both EMS18 and FN4, were similar to that of the wild-type controls. After two months, the wild-type controls totally died but EMS18 survived and appeared to be healthy.

**Department of Pathobiology and Veterinary Science
2014 Graduate Research Forum
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**Replication-Inducible Vaccinia Virus Vectors Based on the Conditional Expression of the
A3 or A6 Virion Core Proteins**

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Vaccinia virus (VACV) is the prototypical member of the family *Poxviridae*, genus *Orthopoxvirus*. Although VACV is an excellent candidate for vaccine, therapeutic, and oncolytic vectors, an increasing segment of the population is susceptible to adverse reactions associated with vaccination. The goal of this study is to develop a built-in safety mechanism using elements of the *tetracycline* (*tet*) operon to control the expression of genes that are likely essential for VACV replication, thereby restricting virus growth to the presence of tetracyclines. Four VACV genes (F17R, E8R, A3L, and A6L) that encode virion core proteins or proteins involved in virion morphogenesis were selected and placed under the control of the well-characterized late VACV promoter P₁₁ with a *tet*O₂ operator sequence (P₁₁O₂). The VACV expressing F17R under the control of the inducible P₁₁O₂ promoter replicated only in the presence of tetracyclines, though plaques were significantly smaller than the wild-type virus. Surprisingly, the VACV with E8R under P₁₁O₂ replicated even in the absence of tetracyclines, albeit at low levels. VACVs inducibly expressing A3L or A6L formed plaques only in the presence of tetracyclines and replicated indistinguishably from wild-type virus in the presence of doxycycline. Furthermore, in the absence of tetracyclines, these recombinant VACVs do not form plaques, even when cells were incubated for multiple days. Thus, replication-inducible VACVs based on the conditional expression of the A3 or A6 proteins could be used as safer vectors for the development of live recombinant vaccines, oncolytic therapies, and even next-generation smallpox vaccines. Specifically, in the event an individual experiences an adverse reaction after vaccination due to uncontrolled viral replication, the simple cessation of tetracycline treatment should inhibit further viral replication and prevent progression of the complication.

Immunogenicity Studies on Nanoparticle Vaccine constructs for Avian Influenza Virus

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Avian influenza (AI) outbreaks pose a significant risk to poultry production in the U.S. and in other countries, human health as well. Type A influenza viruses typically infecting avian hosts. Control of AI in poultry varies by country and may be based on vaccination and/or surveillance/eradication programs. Limitations exist with the use of live, recombinant, or whole virus inactivated vaccines. Vaccine designing, however, is challenging due to the highly mutable genome of influenza virus. Peptide nanoparticle technology has been demonstrated as powerful platform for the delivery of epitopes to elicit strong immune response, providing promise in the development of universal influenza vaccines. Particularly, in this study, four self-assembled nanoparticle peptides (SAPNs) were designed to organize AI M2e peptide as a tetramer in addition to include conserved helices A, C and the cleavage peptide of hemagglutinin as well as chicken specific T cell epitope (HTL), respectively in each construct. Groups of specific pathogen free (SPF) chickens were immunized intramuscularly with these four constructs and boosted two times with two weeks apart. ELISA results suggesting that tetra-M2e-HTL and tetra-M2e-Helices A construct induce strong antibody titer in serum of vaccinated chicken comparing to control group. Immunogenicity studies are in progress on virus neutralization and further T cell mediated immunity induced by these constructs will be evaluated by lymphocyte proliferation and flow cytometry will also be presented. The most promising nanoparticle vaccines will then be evaluated in challenge of immunity studies using a highly pathogenic AIV. These studies will advance this innovative vaccine platform by developing an AI vaccine based on B and T cell epitopes from hemagglutinin and M2e.

Pilot Studies in Swine of a Novel T cell Epitope Mosaic DNA Vaccine Expressing ORF5 Protein of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Porcine reproductive and respiratory syndrome (PRRS) causes significant economic loss due to reproductive failures in sows and respiratory disease in piglets. In the US alone, it costs more than \$600 million annually to the pig industry. PRRS is caused by a highly mutagenic RNA virus named PRRSV. Current vaccines only target homologous PRRSV strains resulting in limited protection which calls for new generation vaccines with broad-spectrum protection. Unfortunately, vaccine improvement is very slow because of the great genetic and antigenic diversity displayed by the virus. There are two major genotypes of PRRSV which share only 60% similarity in nucleotide sequence and genetic divergence within the same genotype is up to 20%. To address this problem, a Mosaic Vaccine Tool Suite originally developed to design HIV vaccines, was utilized to design mosaic T cell vaccines for PRRSV. Two mosaic DNA vaccine candidates based on nearly 800 genotype II ORF5 sequences were constructed. The mosaic DNA vaccines were tested in pigs in two pilot vaccination/challenge trials. In these trials vaccinated pigs were challenged with a homologous virus strain. Gene gun and electroporation were utilized for delivery in the first and second trials respectively. Lymphocyte proliferative responses, expression of interferon- γ mRNA in virus-treated PBMCs, antibody responses and viral loads in blood and porcine alveolar macrophages are being tested. Lesions in lungs are being evaluated macroscopically and microscopically.

A potential role for PRRSV non-structural protein 3 (NSP3) in modulation of apoptosis during infection.

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PRRSV is the etiologic agent of porcine reproductive and respiratory syndrome (PRRS), a complex disease of swine of great economic significance for pig production in the US and worldwide. PRRSV genetic determinants involved in the pathogenesis of this disease and virus persistence in pigs are still unknown. It is thought that at the molecular level, specific interactions between proteins of the host primary target cell, the macrophage, and viral proteins drive the pathogenesis of PRRS. Using a GAL4-based yeast two-hybrid system we have screened a swine macrophage expression library searching for interactions between PRRSV and host cell proteins. We have observed that cellular FK506-binding protein 38 (FKBP38), an immunophilin in the mTOR kinase pathway central to a wide array of cellular processes, such as cell survival, proliferation, and metabolism, interacts with PRRSV non-structural protein 3 (NSP3). One of the functions of activated FKBP38 is that it participates in apoptosis signaling by inhibiting the anti-apoptotic protein Bcl-2. Apoptotic deregulation is usually related to persistent viral infections, persistence being a hallmark of PRRSV infection in swine. The NSP3-FKBP38 interaction seems to be specific, mapping to residues ₁₈₁PYDIHHY₁₉₇ in NSP3. This interaction is maintained among PRRSV isolates as it is also observed with NSP3 of highly diverging isolates, differing more than 50% at the amino acid level. Here we are studying the effect of the interaction between NSP3-FKBP38 on apoptosis in PRRSV infected MARC 145 cells.

Transcriptomic analysis of *Mycoplasma gallisepticum* in vivo and in vitro

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Mycoplasma gallisepticum is the primary etiologic agent of Chronic Respiratory Disease (CRD) in poultry. This is a disease affecting the respiratory and reproductive tracts of chickens, causing significant monetary loss to poultry industries around the world. The currently available vaccines do not offer adequate levels of protection. The development of a more efficacious vaccine will require further information on *M. gallisepticum*'s molecular mechanisms of pathogenesis. We are undertaking detailed transcriptomic analysis to generate a more in-depth understanding of the mechanisms of virulence employed by this pathogen. This information is critical for the development of improved disease control strategies and rationally designed vaccines. Currently, we are conducting transcriptomic analysis of the virulent *M. gallisepticum* strain R_{low} using Illumina RNA sequencing to compare the gene expression profiles of broth grown organisms vs organisms exposed to chicken trachea. This will aid in the identification of genes of interest (GOI) that are differentially expressed during biologically relevant interactions with host cells. The roles of the proteins encoded by those GOIs in pathogenesis will be analyzed by creating isogenic mutants and assessing them in the natural host. The data obtained will provide key insights into the processes vital for infection, pathogenesis, and transmission of *M. gallisepticum* and will be instrumental in next generation vaccine designs.

Direct Sub-Lethal Effects of Corexit® 9500 and Oil on the Eastern Oyster (*Crassostrea virginica*)

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Following the explosion of BP's Deepwater Horizon exploration platform on April 20, 2010, 4.9 million barrels of crude oil were released into the Gulf of Mexico. BP used an unprecedented 1.84 million gallons of chemical dispersant, predominantly Corexit® 9500, to help control the spill. The direct toxicity of the oil dispersant Corexit® 9500 on marine organisms is not well studied. Oysters are an economically and ecologically important marine species in the Gulf of Mexico. Oyster filter-feeding behavior results in significant interaction with particulate matter in water, indicating that oysters may serve as sentinel species for oil and dispersant exposure. The objectives of this study were to evaluate the toxic effects of Corexit® 9500, crude oil, and a dispersant/oil mixture on immune functions, clearance rates, tissue concentrations and histopathology of Eastern oysters (*Crassostrea virginica*) following acute exposure. Hemolymph was collected from the adductor muscle sinus for the evaluation of two innate immune functions: the ability of hemocytes to engulf fluorescent beads (phagocytosis) and produce H₂O₂ (respiratory burst). Corexit® did not affect phagocytosis, but significantly reduced respiratory burst at concentrations ≥ 20 ppm following a 3 day exposure. Corexit® concentrations ≥ 20 ppm also significantly reduced oyster clearance rates. Tissue concentrations revealed that oysters could quickly and efficiently take up Corexit® from the water. One week exposure to oil high-energy water-accommodated fraction (heWAF) did not affect immune functions, though 1 and 3 day exposures remain to be tested. This continuing study will assess toxicity of sweet Louisiana crude oil and a Corexit®/oil mixture to achieve a more accurate risk assessment concerning the impact of oil and Corexit® on the health of oysters and aid in making scientifically sound decisions regarding dispersant use in future spills.

THE EFFECT OF CHICKEN PARVOVIRUS ON SPECIFIC PATHOGEN FREE WHITE LEGHORN CHICKENS

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The major viral enteric disease effecting broiler chickens is Runting-Stunting Syndrome (RSS). Upon necropsy, thin-walled intestines filled with a watery content are seen. Previous studies in broilers show Chicken Parvovirus (ChPV) may be a major factor in the cause of RSS. More studies are needed in understanding the viral protein roles of ChPV in RSS which can lead to the development of a proper vaccine. Because most of the specific pathogen free (SPF) broilers have been exposed to ChPV, a proper study model needs to be established. The SPF white leghorn chickens provided by Charles River are ChPV negative. Although they are layer chickens, viral shedding and immunohistochemistry can be compared to previous studies using broilers infected with ChPV.

To measure virus shedding, cloacal swabs were collected from 6-week old female SPF White Leghorn chickens that were infected with ChPV. Each bird was inoculated via oral route and cloacal swabs were collected over several days post inoculation. Each swab was DNA extracted and amplified by ChPV PCR.

To see whether the virus can be detected in the duodenum and jejunum sections by immunohistochemistry, 6-week old SPF female chickens were inoculated with ChPV, orally. Over several days, the intestinal sections were removed and screened for the presence of virus using antibodies against the viral protein VP2.

When comparing viral shedding between ChPV infected broilers and SPF white leghorn chickens, 100% of the white leghorns were shedding at day 10 post inoculation. During harvest of the duodenum and jejunum sections, intestinal walls appeared thin and filled with excessive amounts of fluid. Therefore, when not factoring in differences in bird weight, SPF white leghorn chickens can be used in place of SPF broilers when studying the roles of ChPV viral proteins in the cause of RSS.