2010 WSU College of Veterinary Medicine

Student Research Symposium

Sponsored by Pfizer Animal Health

The Pfizer Animal Health Award for Research Excellence recognizes faculty members for outstanding research efforts and productivity. Established in 1985, the national award promotes innovative research that advances the science of veterinary medicine.

These abstracts are the work of students who were engaged in research in the College of Veterinary Medicine in 2010. Posters detailing their work were presented at the session held on October 26. The Student Research Symposium is sponsored by Pfizer Animal Health. This symposium is designed to recognize the efforts of students, residents and interns who contribute to the research mission of the college and profession.

Table of Contents

Page	Author	Title
4	Salma Adwani	Passive immunotherapy using egg-yolk antibodies to reduce *Campylobacter jejuni* from the intestinal tract of broiler chicken at time of harvest
5	Sebastian Aguilar	Comparative genomics of vector borne transmission: resolving trait-gene relationships in *Anaplasma marginale*
6	Valerie Akin	CCK-induced activation of glutamatergic myenteric ganglion neurons: A potential source of glutamate for control of food intake by abdominal vagal NMDA-receptors.
7	Abdalhamid Alkar	Effect of GnRH administration 7 days prior to resynchronization with Ovsynch on AI pregnancy in lactating dairy cows
8	Not Available	
9	Jonathan Bliggenstorfer	Antibiotic resistant breakouts of Salmonella enterica tolerate high concentrations of the antibiotic florfenicol
10	Katie Boland	Response to Mucosal Application of Intimin at the Recto-anal Junction in Cattle
11	Sukolrat Boonyayatra	Detection of *Mycoplasma bovis* from Milk Samples by real-time polymerase chain reaction
12	Amanda Brenna	Characterization of creatine kinase isoenzymes in canine cerebrospinal fluid

Page	Author	Title
13	Angela Brooking	Development of a Molecular Assay for Detection and Quantitation of Alcelaphine Herpesvirus 2
14	Julie Caldwell	Obtaining a prevalence estimate for and identifying management practices associated with failure of passive transfer in dairy calves on Washington dairies
15	Lee Cyr	Generation of Peripheral Vagal Afferents and Disparities in Cell Morphology of the Nodose Ganglia Following Axotomy and Capsaicin Treatment in Adult Rats.
16	Lauren Eberhart	Defining the Mechanism of Proximity-Dependent Inhibition in *Escherichia coli*
17	Dan Erwin	Identification of the Host-cell Contact-Dependent Induction Mechanism of *Vibrio parahaemolyticus* Type III Secretion System 1
18	Zach Gallaher	Neural proliferation and restoration of neurochemical phenotypes and compromised functions following capsaicin-induced neuronal damage in the nodose ganglion of the adult rat
19	Heather Gardner	Canine Oral Fibrosarcomas: 65 cases
20	Karol Gliniewicz	The role of *rpoB* gene in the rifampicin attenuation of *Flavobacterium psychrophilum*
21	Erin Jeffress	Detection of integron and gene cassettes of Arcanobacterium pyogenes in uterus of dairy cows
22	Katherine Kilzer	Molecular Epidemiological Strain Typing of *Mycoplasma ovipneumoniae* to Investigate the Etiology of Bighorn Sheep Pneumonia
23	Pei-Shin Ku	Evolution of *Anaplsma marginale* msp2 pseudogene repertoire
24	Seth Nydam	Identifying Signal Motifs for Transport through the Type III Secretion Systems of *Vibrio parahaemolyticus*
25	Jesse Olsen	Adiponectin levels in postpartum dairy cows: association with body condition score and uterine disease
26	Marjolein Oostrom	Lesion of sap injection in arcuate nucleus alters circadian feeding pattern
27	Lisa Pearson	Adiponectin and related gene expression in placenta and uterus in tocopherol supplemented late pregnant ewes
28	Lisa Pearson	Safety of stallion testicular biopsy performed by novice operators

Page	Author	Title
29	Casey Peterson	Comparison of 1 vs. 2 doses of Prostaglandin F2a administration at CIDR removal in a 5-day CIDR CO-Synch protocol on AI Pregnancy Rate in Angus Cross Beef Heifers
30	Yessenia	Effect of the testicular size of the sire group on the pregnancy rate in alpacas (*Vicugna pacos*)
31	Yessenia	Effect of Recipient Lactation Status on Pregnancy Rate Following Embryo Transfer in Alpacas
32	Brandon Roberts	Understanding how brain glucose and neuronal pathways in the brainstem control appetite.
33	Jacob Rodriguez	Cryopreservation and fertility of Bighorn (*Ovis canadensis c.*) cauda epididymis semen
34	Devandra Shah	Highly pathogenic strains of *Salmonella* Enteritidis show enhanced tolerance to acid, oxidative stress and better survival in egg albumen.
35	Deevandra Shah	Transposon mutagenesis in a highly invasive isolate of *Salmonella* Enteritidis revealsa number of genes with potential roles in cell invasion.
36	Murugan Subbiah	Not all antibiotics retain their biological activity in soil
37	Rachael Wood	The potential cardioprotective role of adiponectin in hibernating grizzly bears
38	Jason Wright	Selective antagonism of hindbrain NMDA-type glutamate receptors reverses reduction of food intake by cholecystokinin.
39	Carlyn Zylstra	DPYD as a candidate gene for 5-fluorouracil toxicity in dogs

Passive immunotherapy using egg-yolk antibodies to reduce *Campylobacter jejuni* from the intestinal tract of broiler chicken at time of harvest

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Campylobacter jejuni is one of the most important causes of food-borne gastroenteritis in humans in the USA, with contaminated chicken meat being the main source of human infection. *C. jejuni* is a commensal bacterium in chickens and colonizes gastro-intestinal (GI) tract without causing any disease. Flagellar (FlaA and FlaB) and membrane associated proteins including CadF, Peb1a, JlpA, MOMP, FlpA, CmeC and Cj1349c play a role in colonization of *C. jejuni* in chicken. *We hypothesize that passive immunotherapy using egg yolk derived IgY antibodies against the colonization-associated proteins (CAPs) will reduce the rate of colonization of C. jejuni in chicken*. To test this hypothesis we have cloned nine genes encoding above proteins into a prokaryotic expression system and produced recombinant CAPs. This was followed by hyper-immunization of egg laying hens with individual recombinant proteins to produce egg derived IgY antibodies. The rapid and economical production and simple delivery methods makes egg-yolk derived IgY an attractive molecule for passive immunotherapy. Efficacy of IgY antibodies to reduce the rate of *C. jejuni* colonization *in vivo* will be tested by oral administration of feed coated with each antibody to the *C. jejuni* infected broiler chickens. It is expected that the binding and neutralizing effects of these antibodies will significantly reduce and/or clear *C. jejuni* colonization in chickens.

Comparative genomics of vector borne transmission: resolving trait-gene relationships in *Anaplasma marginale*

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The Order *Rickettsiales* accounts for a number of important tick-borne pathogens, including the emerging human infectious agents *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*, and *Anaplasma marginale,* the most globally prevalent vector-borne pathogen of cattle. The evolution of fitness needed to cause significant health, production and livelihood hazards is surprising in the context of vector transmission for organisms with small genomes, little opportunity for genetic exchange, and the need to evade protective mechanisms in two different hosts. Although pathogens in this Order have similar infection biology, little is known about the microbial determinants of transmission. *A. marginale* provides unique tools to study the genetic determinants of vector transmission, with multiple strain sequences available that display distinct and reproducible transmission phenotypes. The complete gene content conservation found between *A. marginale* strains suggests that any phenotypic differences must be attributed to single nucleotide polymorphisms (SNPs). We used a comparative genomics approach to identify the genetic basis for tick transmissibility. Our strategy compared the genomes of five strains with contrasting tick transmission status and developed a list of candidate SNPs that segregate with phenotype. Candidate SNPs included coding region polymorphisms that resulted in amino acid changes and putative promoter SNPs. The high level of variation found among *A. marginale* strains allowed for segregation of SNPs with phenotype and generation of a list of eight candidate genes with at least one nonsynonymous SNP and 11 promoter regions that can potentially affect 13 genes. Expression analysis of the candidates confirmed transcription of all candidates. Furthermore, two genes downstream from promoter SNPs were significantly differentially transcribed in strains with contrasting phenotypes. These genes are being tested for their potential to affect transmission.

CCK-induced activation of glutamatergic myenteric ganglion neurons: A potential source of glutamate for control of food intake by abdominal vagal NMDA-receptors.

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Anatomical and electrophysiological experiments suggest that most vagal afferent neurons express NMDA-type glutamate receptors (NMDAr) (Czaja et al., 2006) on both central and peripheral axon endings (Aicher et al.1999; Slattery et al. 2006). While a precise contribution of NMDA receptors to vagal afferent function remains uncertain, our published work indicates that they participate in control of food intake by gastrointestinal signals, such as cholecystokinin (CCK). Specifically, systemic administration of NMDA receptor antagonists reverses reduction of food intake by CCK (Covasa et al., 2004) and attenuates CCK-induced Fos expression in the hindbrain nucleus of the solitary tract (Guard et al. 2009). Because peripheral NMDA receptors modulate electrical activity of gastrointestinal vagal afferents (Slattery et al. 2006), we hypothesized that CCK-induced release of glutamate from a gastrointestinal source may participate in control food intake. Here we report that administration of an NMDAr antagonist, via the celiac arterial supply to the GI tract, delays satiation during a palatable sucrose meal, at a dose that was ineffective when administered intravenously. In addition, we characterized a sub-population of intestinal myenteric ganglion neurons that are immunoreactive to vesicular glutamate transporter 2 (VGLUT2), a marker for glutamatergic neurotransmission. Finally, using Fos immunohistochemistry, we found that cholecystokinin triggers increased Fos immunoreactivity in VGLUT2 immunoreactive myenteric neurons. Collectively, these results suggest that glutamate released from myenteric neurons may participate in the control of food intake by vagally mediated satiation signals, such as CCK. Supported by NIH grants DK052849 and NS 020561 to R.C. Ritter.

Effect of GnRH administration 7 days prior to resynchronization with Ovsynch on AI pregnancy in lactating dairy cows

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The objective is to determine the effect of GnRH administration 7 days prior to Ovsynch resynchronization on conception rates. All parity lactating Holstein cows (N=2713) from 3 dairy farms from Idaho were enrolled in this study. Cows that were not detected in estrus by 25 to 32 d post insemination were assigned by odd and even ID number to receive either no GnRH (Control) or GnRH (100 µg, im). Cows not detected in estrus in the next 7 d were presented for pregnancy diagnosis (32 to 39 d post-AI) and open cows in the Control and GnRH groups were submitted for resynchronization with Ovsynch protocol (briefly cows received 100 µg GnRH on day 0, 25 mg PGF2α on day 7, 100 µg GnRH 48 h later on day 9 and were inseminated at a fixed-time AI 16 h later on day 10). Cows were submitted for pregnancy diagnosis at 32 to 39 d post AI. Open cows were re-enrolled in the study or subjected to other reproductive management program of the farm. GLM procedure of SAS was used to examine the effect of treatments on resynchronization conception rate. The variables included in the model were treatment (GnRH vs. no GnRH), location (1 to 3), lactation (1, 2 and 2+), days in milk at resynchronization initiation (<100 and ≥100), resynchronization for first or more services, season (winter, spring, summer, fall) and appropriate interaction. The P value at 0.05 was considered significant. For model reduction, the P value was set at < 0.1 for inclusion and > 0.1 for exclusion until the model contained only significant main and interaction effects. Treatment was retained during model reduction and in the final model. There was no difference in the conception rate between GnRH and Control groups (42.4% vs. 42.3%; P>0.1). In conclusion, GnRH treatment 7 days prior to resynchronization with Ovsynch did not improve conception rate in lactating dairy cows.

Antibiotic resistant breakouts of *Salmonella enterica* tolerate high concentrations of the antibiotic florfenicol

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Salmonella enterica can be found in the digestive tracts of both animals and people. In people, *S. enterica* attacks the host gastrointestinal system and induces a diarrheal illness called salmonellosis. *S. enterica* infections in humans often originate from contaminated animal food products and farmers treat livestock with various antibiotics including florfenicol. Therefore, antibiotic resistance is problematic for veterinary and human medicine. Genes on the bacterial chromosome or on horizontally transmissible plasmids typically encode resistance. Strain AM04528, a *Salmonella enterica* serovar Newport, harbors a 160 kbp multidrug resistance plasmid. Among the genes encoded by this plasmid is *floR*, which encodes an efflux pump that confers resistance to florfenicol. Initial studies of growth inhibition demonstrated that strain AM04528 will not grow in inhibitory concentrations of florfenicol (200 ug/ml). However, when incubations are extended, subpopulations will begin to grow within 48 hr. These “breakouts” are capable of growing with very high concentrations of florfenicol (up to 1200 ug/ml), a phenotype we call “super resistant.” We hypothesize that breakouts arise from alterations in the expression of *floR,* because passaging the “breakouts” without the presence of florfenicol results in a loss of the “super resistant” phenotype and sequencing of the coding region shows no mutations in the gene itself. We are examining the expression of the *floR* gene by real-time PCR and if super resistance is correlated with *floR* expression, further sequencing will be employed to find alterations in the promoter region and to examine the level of *FloR* protein expression.

Response to Mucosal Application of Intimin at the Recto-anal Junction in Cattle

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Escherichia coli O157:H7 is an important zoonotic pathogen that colonizes animals and causes disease in humans, ranging from self-limiting to severe bloody diarrhea. There are also life-threatening sequelae, including hemolytic-uremic syndrome, the number one cause of acute renal failure in children. Human disease caused by *E. coli* O157:H7 infection most frequently results from consumption of contaminated food. Cattle are the major reservoir with no associated disease and healthy cattle can passively shed the bacteria in their feces. Decreasing the numbers of organisms shed by cattle is vital to decreasing the prevalence of food-borne illness in humans by decreasing beef and environmental contamination. The recto-anal junction (RAJ) is the primary site of *E. coli* O157:H7 colonization in cattle. Intimin is an outer membrane protein of *E. coli* O157:H7 essential for RAJ colonization. Mucosal or subcutaneous immunization of mice with intimin induces long-lived humoral immunity and decreases intestinal colonization of murine pathogens similar to *E. coli* O157:H7. Induction of local antibody production at the RAJ mucosa, may present an effective way of decreasing colonization and shedding of *E. coli* O157:H7 in cattle, leading to the question: Will immunization of naïve cattle with *Escherichia coli* O157:H7 derived intimin induce an adaptive immune response and will this adaptive immune response be stronger at the site of colonization (the RAJ) with mucosal immunization compared to subcutaneous immunization? This preliminary data suggests that both subcutaneous and mucosal immunization result in systemic and local adaptive responses with mucosal immunization having a less robust systemic response, but comparable local response.

DETECTION OF *MYCOPLASMA BOVIS* FROM MILK SAMPLES BY REAL-TIME POLYMERASE CHAIN REACTION

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Abstract

Mycoplasma bovis is considered a contagious pathogen with the ability to cause severe bovine mastitis. The objective of this study was to demonstrate the ability to detect *M. bovis* directly from milk samples using a newly developed real-time PCR (rt-PCR) assay. A total of 86 milk samples were examined. All samples were previously positive with *Mycoplasma* species as determined by standard culture method. Genomic DNA was extracted from all milk samples and used for both rt-PCR and nested PCR assays. All 86 samples were positive with *M. bovis*. The rt-PCR average Ct values were 32.35 $\pm$ 5.09 (range from 16.11 to 40.46). The nested PCR only identified 84 samples (97.67%) as *M. bovis*. PCR products of 24 positive samples were sequenced and all found to be identical to a region in *oppD* gene of *M. bovis*. The newly developed rt- PCR assay can accurately detect *M. bovis* directly from milk samples within 4 hours. This rapid detection has an advantage in that it reduces the time to properly identify this contagious pathogen as compared to conventional PCR. However, because other species of *Mycoplasma* are also mastitis pathogens the development of a multiplex real-time PCR is the ultimate goal to accurately identify the causative *Mycoplasma* species in mastitic milk.

Characterization of creatine kinase isoenzymes in canine cerebrospinal fluid

Amanda Brenna, Annie Chen-Allen, Tamara Wills

Creatine kinase (CK) is an enzyme that is often measured to evaluate tissue damage. Three isoenzymes of creatine kinase exist: CK-BB is found predominantly in the brain, CK-MB is found in cardiac muscle, and CK-MM is found in skeletal muscle. Studies indicate that CK-BB may be a useful tool in determining the extent of various forms of neurological damage. While extensive research has been done to examine normal and elevated CK-BB levels in the cerebrospinal fluid (CSF) of humans, limited work has been done to establish CK-BB levels in the CSF of dogs. The purpose of our study is to: 1) isolate CK isoenzymes from normal canine CSF using gel electrophoresis, and 2) determine the contribution of CK-BB, CK-MB, and CK-MM to total CK activity using densitometric scanning.

Dogs with no known medical problems and normal neurologic exam, between ages of one to six and over twenty pounds were used for this study. Atlantoaxial CSF was collected and analyzed to make sure the CSF was normal. A total CK value was measured spectrophotometrically. CK isoenzymes were isolated using the QuickGel® CK Vis Isoenzyme technique (Helena Laboratories, Beaumont, TX). Contribution of CK-BB, CK-MB and CK-MM was determined using a densitometric scanner. Estimation of each isoenzyme activity in U/L was determined based on its fraction of the total CK activity.

This study is currently in progress. Quantitative data for the second specific aim have not been fully established. CSF from four dogs has been collected thus far. Preliminary results indicate that CK-MM is the only isoenzyme in normal canine CSF. An atypical band that migrated cathodically to CK-MM has been identified as mitochondrial CK . An additional band that migrated anodically to CK-BB has yet to be identified. Albumin was ruled out as a possibility and further tests are being performed to identify the unknown band. While this technique is commercially available for use in humans, species differences must be accounted for before this gel electrophoretic technique can be used to identify CK isoenzymes in canine CSF.

Development of a Molecular Assay for Detection and Quantitation of Alcelaphine Herpesvirus 2

Angela K. Brooking, Cristina W. Cunha, Donald P. Knowles, and Hong Li

Malignant catarrhal fever (MCF) is a generally fatal disease primarily of ruminants caused by gammaherpesviruses. The viruses are carried subclinically in reservoir hosts and cause disease in poorly adapted species such as bison and cattle. Sheep-associated MCF, carried subclinically in sheep, is the leading cause of death of farmed bison and is a major economic concern. The lack of cell culture systems to propagate OvHV-2 has constrained MCF research, including the development of vaccines to protect bison from this disease. Recent studies suggest that OvHV-2 switches its cell tropism at multiple stages during the cycle of replication, which hinders the development of a culture system. Researchers at Washington State University are developing an alternative strategy for a vaccine using a particular related OvHV-2 virus strain (called Alcelaphine herpesvirus 2, AlHV-2) that does grow in culture. Characterization of AlHV-2 infection in animals is a prerequisite to vaccine development. My specific objective was to develop a molecular assay to detect and quantify AlHV-2 DNA. A real-time PCR (DNA-binding dye based) and a nested PCR were optimized to amplify AlHV-2 DNA using primers specific to a unique AlHV-2 region and reference samples. The efficiency of the real-time PCR was consistently between 90 and 110%; the assay showed low replicate variability ($R^2 \geq 99\%$) and was specific for the target sequence as determined by melting curve analysis. Amplicons from both PCR assays were confirmed to be specific by sequencing. The assays showed 100% of specificity against 13 other viral genomes present in clinical samples, some from very closely related viruses. The limit of detection of the two assays was determined using serial dilutions of plasmids containing the target sequence and it was found that the real-time PCR and the nested PCR can detect two and 95 AlHV-2 target molecules per micro liter, respectively. Based on the lower detection limit observed and considering that a real-time PCR is more reliable than a nested PCR because it is less prone to contamination issues, the real-time PCR optimized for ALHV-2 was considered the test of choice to be validated for detection and quantification of AlHV-2 DNA in clinical samples.

Obtaining a prevalence estimate for and identifying management practices associated with failure of passive transfer in dairy calves on Washington dairies

Julie Caldwell and JR Wenz

Failure of passive transfer (FPT) in dairy calves has been associated with increased morbidity and mortality and long-term decreases in productivity. A recent NAHMS study estimated theUSprevalence of FPT to be 19.2 %. Objectives of the current study were to estimate the herdand calf-level prevalence of FPT and identify associations between colostrum management and FPT on WA dairies. Lastly, the utility of Brix refractometry (BRX) was examined as a means of assessing passive transfer status. Serum total protein (TP) and BRX was determined by handheld refractometers on 18 calves from each farm. Calves with TP< 5.2 g/dL were considered to have FPT. A colostrum management survey was administered by interview. Serum IgG was measured by radial immunodiffusion and correlated with BRX and TP. Associations between management practices and TP were evaluated using multiple linear regression with repeated measures. The mean TP of 952 calves sampled from 56 farms was 5.5 g/dL and 34% were classified as FPT. Sixty-seven % of herds had FPT proportions greater than the national average of 19.2%. The IgG and BRX were well correlated (r^2= .81) and BRX of 8.3% was equal to 10 mg/mL IgG. Colostrum management surveys were completed for 30 (54%) herds in the study. Mean TP was associated with region of the state and higher if colostrum was collected by dairy personnel other than a milker. Mean TP was lower if the dairy saved more than the first milking as colostrum to feed to neonates and if supplements or replacers were added to maternal colostrum. Mean TP was higher if colostrum was administered by esophageal tube or if TP was monitored. This study will provide producers with statewide FPT statistics, as well as provide areas of focus to improve management of passive transfer. In addition, the correlations between Brix percentages and IgG, and TP measurements and IgG further support the plausibility of using these tools to measure passive transfer status in newborn dairy calves.

Generation of Peripheral Vagal Afferents and Disparities in Cell Morphology of the Nodose Ganglia Following Axotomy and Capsaicin Treatment in Adult Rats.

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Physical and chemical injury to peripheral neurons results in damage to nerve fibers and/or death to neurons in the nodose ganglia. With physical injury, such as axotomy, the majority of neurons tend to survive but sacrifice their processes; those that behave in this manner regenerate their axons. Conversely, chemical injury from capsaicin induces cytotoxicity and tends to kill the majority of affected neurons. Regeneration cannot account for recovery of axons in such populations. Therefore, new neurons must emerge after injury induced cell death. Since burgeoning cells typically appear bipolar and tripolar in neonatal rats, the purpose of this experiment was to evaluate the morphology of nodose ganglion cells, from dispersed cell cultures, following subdiaphragmatic vagotomy and intraperitoneal capsaicin injection. Immunocytochemistry and fluorescent microscopy were used to observe fixed tissue from cultures; treatment group neuronal and supporting cell populations differed in morphology from each other and controls, and new bipolar/tripolar cells were observed in both vagotomy and capsaicin but not in control conditions.

Defining the Mechanism of Proximity-Dependent Inhibition in *Escherichia coli*

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Previous work by our group showed that antibiotic selection pressure was not required to maintain high frequencies of antibiotic resistant *E. coli* in calves at the WSU dairy. Resistance to streptomycin, sulfadiazine, and tetracycline (SSuT) was the most common resistance pattern observed, and genetic traits linked to the SSuT phenotype were thought to confer a fitness advantage that allowed these bacteria to persist in this calf population. Recent analysis of the SSuT phenotype revealed an inhibitory property in a subset of isolates and this inhibition phenotype is independent of the SSuT phenotype. During competition "inhibitor strains" severely restrict or kill competing strains via what we have called "proximity-dependent inhibition" (PDI). PDI allows these bacteria to inhibit both commensal and pathogenic *E. coli,* including *E. coli* O157:H7, but inhibitor strains cannot limit other inhibitor strains, indicating that there is an immunity mechanism involved. We have excluded colicins and bacteriophage as possible mechanisms that could explain the PDI phenotype. Additionally, we have found that the trait is more pronounced under nutrient deficient conditions. We have also constructed and screened a fosmid library to identify the genetic basis for PDI, but we were unsuccessful identifying a clone that acquired the PDI phenotype. Our current study will employ transposon mutagenesis coupled with a moderate throughput screening assay to identify the trait of interest through a gene-knockout procedure. Clearly, the ability to inhibit a wide diversity of *E. coli* strains indicates that PDI may influence community composition and thus play a significant role in the dynamics of bacterial populations. Once identified it may be possible to exploit the PDI mechanism as a means to control pathogenic EHEC *E. coli* including O157:H7 and O26.

Identification of the Host-cell Contact-Dependent Induction Mechanism of *Vibrio parahaemolyticus* Type III Secretion System 1

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Vibrio parahaemolyticus is a Gram-negative, food-borne bacterial pathogen that causes more than 4500 infections annually in the U.S. Two distinct type III secretion systems (T3SS1 and T3SS2) have been identified in *V. parahaemolyticus* with T3SS1-dependent host-cell death implicated as a key virulence factor. Expression of T3SS1-associated genes is tightly controlled with induction occurring upon host-cell contact but the precise mechanism that signals host-cell contact has not been identified. Previous work in our lab has identified the proximal positive (ExsA and ExsC) and negative (ExsD and ExsE) regulators for the T3SS1 operon and this system appears to be functionally orthologous to the ExsACDE regulatory cascade of *Pseudomonas aeruginosa*. Unlike the *P. aeruginosa* model, however, deletion of *exsC* or over-expression of *exsE* in *V. parahaemolyticus* does not affect the production of ExsA in the presence of host-cell contact and these mutants retain the ability to induce Hela cell cytotoxicity. This indicates that there is an ExsCDE-independent pathway involved in signaling host-cell contact and upregulation of the T3SS1. We propose two independent approaches to identify the signaling mechanism. In the first approach, random transposon mutagenesis will be used to produce a library of mutants that will be screened for loss of T3SS1-dependent cytolysis in the presence of Hela cells. For the second approach, whole transcriptome shotgun sequencing (RNA-seq) by pyrosequencing will be used to identify genes that are actively transcribed during *in vitro* and *in vivo* infection. Putative signaling and regulatory genes will be confirmed by gene knockout and complementation. We surmise that analogous signaling systems operate in other Gram-negative pathogens with T3SSs and that these systems present opportunities for development of therapeutic interventions.

Neural proliferation and restoration of neurochemical phenotypes and compromised functions following capsaicin-induced neuronal damage in the nodose ganglion of the adult rat

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We previously reported that neuronal numbers within adult nodose ganglia (NG) were restored to normal levels 60 days following the capsaicin-induced destruction of nearly half of the neuronal population. However, the nature of this neuronal replacement is not known. Therefore, we aimed to characterize neural proliferation, neurochemical phenotypes, and functional recovery within adult rat NG neurons following capsaicin-induced damage. Sprague-Dawley rats received intraperitoneal injections of capsaicin or vehicle solution, followed by BrdU injections to reveal cellular proliferation. NG were extracted at multiple times post-treatment (up to 300 days) and processed for immunofluorescence, real-time RT-PCR, and dispersed cell cultures. Capsaicin-induced cellular proliferation, indicated by BrdU/Ki-67-labeled cells, suggests that lost neurons were replaced through cell division. NG cells expressed the stem cell marker, nestin, indicating that these ganglia have the capacity to generate new neurons. BrdU incorporation within beta-III tubulin-positive neuronal profiles following capsaicin suggests that proliferating cells matured to become neurons. NG neurons displayed decreased NMDAR expression up to 180 days post-capsaicin. However, both NMDAR expression within the NG and synaptophysin expression within the central target of NG neurons, the NTS, were restored to pre-injury levels by 300 days. NG cultures from capsaicin-treated rats contained bipolar neurons, normally found only during development. To test the functional recovery of NG neurons, we injected the satiety molecule, CCK. The effect of CCK on food intake was restored by 300 days post-capsaicin. This restoration may be due to the regeneration of damaged NG neurons or generation of functional neurons that replaced lost connections.

Canine Oral Fibrosarcomas: 65 cases

Heather Gardner

Fibrosarcomas are the third most prevalent oral neoplasm in dogs. They have been historically treated with a variety of different protocols/modalities with varying results. The objective of this retrospective study was to assess the utility of a variety of therapeutic modalities in the treatment of canine oral fibrosarcomas in relation to the median survival. A secondary objective was to assess whether grade was prognostic for survival in dogs with oral fibrosarcomas. Sixty-five dogs with oral soft tissue sarcomas presented to the Washington State University Veterinary Teaching Hospital between June 1998 and March 2010 and met the criteria for inclusion in this study. The medical records were examined and histological slides were reviewed when possible. Follow-up information was gathered from the medical record and conversations with the referring veterinarian and/or owner.

Results: Dogs with oral tumor stage three and palatine tumors had a shorter median survival. Dogs receiving a combination of surgery and radiation therapy had a longer median survival. Grade 2 tumors had a statistically significant shorter median survival than grade 1 and grade 3 tumors. Progression free survival was lower in patients with mandibular and palatine/oral tumors, patients not receiving a combination of radiation and surgery and in patients receiving palliative radiation therapy.

Conclusions: The treatment of oral fibrosarcomas with a combination of surgery and radiation therapy provided the most durable median survivals in this study. Curative intent radiation therapy appeared to prolong the progression free survival more so that palliative radiation and surgery. We also found that histological grade did not accurately predict survival, and further methods of elucidating the biological behavior of oral fibrosarcomas could be considered in order for more accurate prognostication.

The role of *rpoB* gene in the rifampicin attenuation of *Flavobacterium psychrophilum*

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Flavobacterium psychrophilum is the etiologic agent of Coldwater disease (CWD) with outbreaks when water temperature is between 3-15°C and mortalities can be ≥ 50%. The bacterium is capable of vertical and horizontal transmission and emerging antibiotic resistance is a growing concern in salmonid aquaculture. At present there are no licensed vaccines targeting the disease. Recently, a strain of *F. psychrophilum* (B17) was attenuated by passage on rifampicin plates and immunization trials demonstrated protection of young rainbow trout against the disease. Detailed analysis of the B17 strain revealed a mutation in the *rpoB* gene as compared to the parental CSF259.93 strain. *RpoB* encodes a β subunit of DNA-dependent RNA polymerase, a natural target of rifampicin and the mutated *rpoB* enables bacteria to grow in the presence of the antibiotic. Additionally, 2D gel electrophoresis and mass spectrometry demonstrated that the B17 strain exhibits a different protein profile as compared to the parental CSF259-93 strain. In prokaryotes protein expression is regulated mainly at the transcriptional level and with RNA polymerase being a nexus point in this process, we hypothesize that the mutated enzyme may exhibit abnormal function leading to a different protein expression "program" and attenuation. To determine the mechanism of rifampicin attenuation and the role of mutated *rpoB* in this process we will examine the native and mutated RNA polymerases and their interactions with specific promoters and different transcriptional regulators.

Detection of integron and gene cassettes of Arcanobacterium pyogenes in uterus of dairy cows

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The objective of the study to detect multidrug resistance of *A. pyogenes* isolated from the uterus of postpartum dairy cows using integron and gene cassettes.

Methods

All parity lactating Holstein cows (N=40) from a dairy farm at Sunnyside, WA were enrolled in this study. Uterine secretions were collected using cytobrush technique following a sterile procedure. The samples were collected twice at 2 weeks interval. The brush was stored in a Anaerobic Specimen Collector (Diagnostic Systems, Sparks, MD 21152) placed in a cooler with ice and transported to the laboratory within 2 h. In the lab, the uterine secretions were removed from brush to a vial with sterile glycerol saline. Initially, 50µL uterine secretion from each cow was inoculated onto blood agar plates supplemented with defibrinated sheep blood and 100 U/mL of polymixin and incubated with 5% CO_2 at 37°C for 48 h to isolate *A. pyogenes.* Initial identification is based on colony morphology. These colonies were further identified using API Coryne Vit system (bioMerieux Inc., Durham, NC 27712). To further identify and classify the isolated strains and analyze their evolution status, the 16S rRNAThe 16 S r RNA gene of *A. pyogenes* was amplified by PCR using universal primers as described.

Detection of integron gene and gene cassettes

The procedure for PCR detection of various types of integrons and gene cassettes were according to the procedure described previously. Specific primers used for PCR detection of the integron gene (*IntI I and IntI II)* and gene cassette were shown in Table 1.

Results

8 out of 40 (20%) cows were positive during first sampling time and 6 out of 40 (15%) were observed positive during second sampling time. Four new cases were recorded during the second sampling time and two cows were persistently infected during second sampling time. Five cows were *IntI I* positive and no cows were positive for *IntI II*. Four cows that were positive for *IntI I* were also positive for gene cassette. The 1048 and1608- bP amplicons revealed to contain *addA 5 and addA 24*-ORF1 gene respectively. The *addA 5 were resistant to sulfadiazine, bacitracin, florfenicol and ceftiofur. The addA 24*-ORF1 was resistant to sulfadiazine, bacitracin, penicillin, clindamycin and erythromycin. There was one cows which showed high resistance compared to others.

Conclusion

Detection of *intI I* and gene cassettes can be utilized to detect multidrug resistance. A. pyogenes isolates were positive for the presence of *intI I* gene and gene cassettes. Sequence analysis of gene cassettes revealed resistant determinants of sulfadiazine, bacitracin, penicillin, clindamycin, erythromycin, florfenicol and ceftiofur.

Molecular Epidemiological Strain Typing of *Mycoplasma ovipneumoniae* to Investigate the Etiology of Bighorn Sheep Pneumonia

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Mycoplasma ovipneumoniae (*M. ovi*) is a bacteriological agent associated with lethal bronchopneumonia in bighorn sheep. Strain typing of *M. ovi* has not been attempted or published in the US thus far in either domestic or bighorn sheep herds. One hurdle to performing strain typing of this finicky organism is culturing enough of the bacterium to be able to extract a sufficient amount of high-quality DNA. Therefore, the first goal of this study is to prepare fresh media for comparison with purchased media in the culturing of this organism, in order to maximize the growth of *M. ovi* in samples from recent bighorn sheep bronchopneumonia outbreaks. After culture and sufficient DNA extraction is achieved, molecular epidemiological strain typing is employed to provide insight into the role of this agent in the epidemiology of pneumonic disease in bighorn sheep herds. Culture results showed that the growth of *M. ovi* on freshly-prepared media was significantly faster than on purchased Mycoplasma agar media plates, especially those near their expiration dates. Strain typing using Multiple Locus VNTR (Variable Number Tandem Repeat) Analysis (MLVA) is still in development. Several VNTR loci have been identified that differ in repeat number; however further analysis for tandem repeat variability will require fluorescence-labeled primers and capillary electrophoresis to provide the necessary size discrimination. In addition, additional refinement of procedures to improve DNA template quality will be necessary for MLVA to provide reliable strain typing.

Evolution of *Anaplsma marginale* msp2 pseudogene repertoire

Pei-Shin Ku

Anaplasma marginale is a tick-borne pathogen of cattle. The organism contains an antigenically variable surface protein- Major Surface Protein 2 (MSP2), which allows the organism to evade the immune response and establish persistent infection. The *msp2* family is composed of a single expression site (ES) and five to eight pseudogenes depending on the strain. Each pseudogene is truncated compared to the full length ES and contains a central hypervariable region (HVR) located between 5' and 3'conserved sequence. Segments of the HVR from different pseudogenes can be recombined into the ES to create antigenically distinct mosaic variants. These antigentically distinct surface variants are the key for the organism to evade adaptive immunity. Thus, the pesudogenes play a very important role in immune evasion.

Previous research indicates that a distinct ES variant (due to a distinct pseudogene) allows for superinfection, is the ability of a second strain to infect a host with existing broad immunity to MSP2. Also, it has been shown that ES variants that correspond to whole pseudogene HVRs have a growth fitness advantage over the mosaic ES variants that are selected for immune evasion. Thus, there are dual selective pressures on the pseudogene repertoire: that of immune escape and growth fitness. We hypothesize that the evolution of the pseudogene repertoire is through a (pseudo)gene duplication process, with subsequent accumulation of mutations in the duplicated pseudogene. In this way the pathogen can maintain fitness while probing for a new "best fit" pseudogene to maintain in the repertoire. To test the hypothesis we have cloned the pseudogene repertoire from *A. marginale* strains and are in the process of testing the immune response of variant loci .

Identifying Signal Motifs for Transport through the Type III Secretion Systems of *Vibrio parahaemolyticus*

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The type III secretion system (T3SS) is an important virulence factor in many types of Gram-negative bacteria, including *Yersinia*, *Salmonella, E. coli* O157:H7 and *Vibrio*. Although well studied in organisms such as *Yersinia*, significant gaps remain in our understanding of its function, particularly with respect to the signals that enable transport of effector proteins that are secreted and translocated into host cells. One signal, the N-terminal export sequence, appears necessary for transport through the T3SS and sufficient for effector secretion, although its role in T3SS specificity and translocation is less clear. A second signaling motif involving chaperone protein interactions may be necessary for T3SS specificity and effector translocation. In this model the export sequence enables generalized T3SS transport while additional factors and proteins are responsible for effector specificity and translocation into host cells. The pathogen *V. parahaemolyticus* harbors two phylogenetically distinct T3SSs (T3SS1 and T3SS2) that traffic separate effector proteins and that are responsible for different pathogenic effects, and this presents a unique system to examine how different signaling components contribute to T3SS transport and specificity. Our studies are focused on known effector proteins in *V. parahaemolyticus* with the aim to determine how the export sequence and effector chaperones contribute to secretion efficiency, T3SS specificity and translocation. This work will contribute to our understanding of effector transport through the T3SS, which likely extends to other pathogens and provides opportunities to identify novel targets for therapeutic intervention.

Adiponectin levels in postpartum dairy cows: association with body condition score and uterine disease

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The objective of the study was to determine the association of mean serum adiponectin level, body condition score and uterine disease in postpartum dairy cows.

All parity lactating Holstein cows (N=40) from a dairy farm at Mabton, WA were enrolled in this study. All cows received a body condition score at enrollment (emaciated 1; Obese 5, with 0.5 score increment); Eight cows in each body condition score categories from 2 to 4, were included. Blood samples were collected by coccygeal venipuncture from 1 week prior to calving to 4 weeks after calving for serum adiponectin assay. Cows were monitored and were diagnosed for the presence of metritis, endometritis, subclinical endometritis based on criteria described by (Sheldon et al 2006 and Kasimanickam et al. 2004). Serum adiponectin levels were estimated by direct ELISA using absorbance module of Glomax multi detection system (Promega Corporation, Madison, WI 53711). Serum adiponectin level during week 1was used to determine the association of adiponectin and metritis, and adiponectin level during week 4 was used to determine the association of clinical endometritis, subclinical endometritis or normal. Results indicated that cows with metritis or endometritis had significantly higher serum adiponectin level compared to cows with subclinical endometritis or normal cows ($P<0.01$). Cows with body condition score 2 or 2.5 had significantly higher adiponectin level than cows with body condition score 3, 3.5 or 4 during pre- and post-calving.

In conclusion, we identified circulating adiponectin levels were in association with body condition score and postpartum uterine diseases. Further investigations are in progress to elucidate the mechanisms involved in different effects of adiponectin on dairy cow fertility and immune function.

Lesion of sap injection in arcuate nucleus alters circadian feeding pattern

Marjolein Oostrom

Ai-Jun Li, Qing Wang, Michael Wiater, Thu Dinh, Brandon Roberts, Sue Ritter

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ABSTRACT

Leptin is a hormone secreted by fat cells and which circulates in the blood to act on various tissues in brain and periphery. Leptin has important effects on food intake, body weight and metabolism that are known to be mediated primarily by its effects on the arcuate nucleus (Arc) at the base of the hypothalamus. Lesions in this area can produce a physiological response similar to that of genetic deletion of leptin or its receptors. In this experiment, we used a novel targeted toxin, leptin conjugated with saporin (Lep-sap), to produce a selective lesion of leptin receptor-expressing neurons in the Arc. Lep-sap or a control saporin conjugate, blank-saporin (B-sap) was injected bilaterally into the Arc and the feeding behavior and body weight were examined in various experiments. The Lep-sap injection caused the rats to eat considerably more and gain more weight than the control rats. One of the major findngs was that the circadian feeding pattern was severely disrupted. In my work, I analyzed the circadian distribution of food intake of these rats during a normal light-dark cycle and under two test conditions: constant light and constant dark. Results indicate an absence of circadian rhythm under these conditions in the Lep-sap group, suggesting that leptin receptor-expressing neurons in the Arc and perhaps leptin itself, may contribute to the circadian patterning of food intake. Results also replicate previous findings that Arc neurons are critical for maintenance of energy homeostasis.

Adiponectin and related gene expression in placenta and uterus in tocopherol supplemented late pregnant ewes

Lisa Pearson, Vanmathy Kasimanickam, Ahmed Tibary, Ram Kasimanickam

The objective of the study was to determine the effect of tocopherol on adiponectin and related gene expression in uterus and placentome of late pregnant ewes. Eighteen pregnant ewes during late gestation received daily oral supplementation of 500 mg of alpha-tocopherol (aT; N=6) or 1000 mg of gamma-tocopherol (gT; N=7) or placebo (CON; N=5) from 107 to 137 days post breeding. Serum was obtained at weekly intervals and tissue samples were obtained at the end of supplementation to evaluate relative mRNA expressions of adiponectin, adiponectin receptor (ADIPOR)1, ADIPOR2, peroxisome proliferator-activated receptor gamma (PPARγ), IGF-1, IGF-2 and leptin in uterus and placentomes. In cotyledon and caruncle, the mRNA expression of adiponectin, AdipoR1, IGF-2 and leptin expressions in ewes supplemented with gT were significantly higher compared to the CON group. The AdipoR2, PPARγ and IGF-1 expressions were not different between gT and the CON groups. In contrast, adiponectin, AdipoR1, PPARγ, IGF-1, IGF-2 and leptin expressions were lower in ewes supplemented with alpha tocopherol (aT) compared to control; however AdipoR2 was significantly higher in aT than control group. In uterus, Adiponectin, PPARγ, IGF-2 and leptin expression in ewes supplemented with gT were significantly lower compared to the CON group. The AdipoR1, AdipoR2, and IGF-1 expression were not different between gT and the CON groups. In ewes supplemented with aT, adiponectin, IGF1, IGF2 and leptin expression were lower compared to control; however AdipoR2 was significantly higher in aT than control group. Adiponectin and its related genes play a crucial role in placental angiogenesis especially in late pregnant ewes supplemented with gamma tocopherol.

Safety of stallion testicular biopsy performed by novice operators

Lisa Pearson, Jacobo Rodriguez, Shirley Sandoval, Charles Leathers, Ahmed Tibary

Testicular biopsy remains seldom used in stallions due to fear of complications. The aim of this study was to evaluate the efficacy and safety of testicular biopsy in stallions performed by novice operators. Six adult stallions scheduled for castration were used. Testicular biopsies were obtained aseptically using a 14 ga core self-firing instrument (Bard® Biopsy systems, AZ) by senior veterinary students with no prior experience. Students were given instructions in a 30-minute seminar regarding how to perform the technique. Stallions were placed in stocks and sedated with detomidine HCl (10 μg/kg; IV) or xylazine (0.5 mg/kg; IV) and butorphanol tartrate (0.01 mg/kg; IV). Testicular ultrasonography was performed on all stallions before, and daily for five days after the procedure. The marginal part of the testicular artery was evaluated using pulsed-wave color Doppler, and the peak systolic velocity (PSV) and end diastolic velocity (EDV) were recorded. Measurements before and after biopsy sampling were compared within and between testicles using ANOVA/AOCV after log transformation. Biopsy samples were evaluated for diagnostic validity. Stallions were castrated 10 days after biopsy and testes were grossly examined. Representative samples were submitted for histopathological evaluation. No systemic effects were observed during the study period. Ultrasound evaluations revealed subcutaneous/albuginea hematomas in 3 stallions, which resolved within 1 week. After 10 days, the biopsy site was identified on each excised testicle as a pin point. There was no significant effect of biopsy sampling on blood flow ($P< 0.05$) when paired testicles were compared. There was no difference between biopsied and non-biopsied testicles for PSV ($P=0.39$) and EDV ($P=0.47$). Testicular biopsy sampling, using this technique, is a safe and reliable procedure in stallions even in the hand of novice operators if they follow simple instructions.

Comparison of 1 vs. 2 doses of Prostaglandin F2 administration at CIDR removal in a 5-day CIDR CO-Synch protocol on AI Pregnancy Rate in Angus Cross Beef Heifers

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The objective was to determine the effect of two vs. one PGF administration at CIDR removal on day 5 on AI pregnancy rate in beef heifers synchronized with a 5 day CIDR-CO-Synch protocol. Angus cross beef heifers (N = 562) at six locations from WA and ID were included in this study. All heifers received 100 mg of gonadorelin hydrochloride (GnRH) and a controlled internal drug release insert (CIDR) on Day 0. Within farm, cows were randomly allocated to receive 25 mg of dinoprost (PGF) at the time of CIDR insert removal on Day 5 (1 PGF; N = 264) or two 25 mg doses of PGF, the first given on Day 5 at the time of CIDR removal and the second administration 6 h later (2 PGF; N = 298). Heifers (N=415) received heat detection patches at the time of CIDR removal. After CIDR removal, heifers were observed twice daily through Day 7 for estrus and heat detector patch status (activated, partially activated and lost vs. intact) was recorded. On Day 8, heifers were given 100 mg of GnRH, heat detector aid status was recorded, and heifers were inseminated at 72 h from CIDR removal. Mixed procedure of SAS was used to examine the effect of treatments (1 PGF vs. 2 PGF) on timed-AI pregnancy rates. Variables included in the timed-AI pregnancy model were body condition scores (<5, ≥5; on a scale from 1–9; 1– emaciated; 9 – obese), heifers in estrus at or prior to AI (estrus = activated, partially activated and lost heat detector aids or no estrus = intact heat detector aids), location (1–6) and appropriate interaction of main effects. AI sires offered as random effect in the model. Accounting for significant variables such as location, heifers in estrus at or prior to AI and treatment by location interaction, 2 doses of PGF administration had higher fixed time AI pregnancy rate compared to 1 dose of PGF administration on day 5 at CIDR removal (P = 0.058; 62.1 vs. 54.2%). In conclusion, 2 doses of PGF administration at CIDR removal on day 5 in a 5-day CIDR-CO-Synch protocol yielded 8% higher timed-AI pregnancy compared to 1 dose of PGF administration.

Effect of the testicular size of the sire group on the pregnancy rate in alpacas (*Vicugna pacos*)

J. Sumar[a] , Y. Picha[a,b], A. Tibary[b]

The positive correlation between size and weight of the paired testicles and fertility is well established in all traditional livestock species. We have observed a large variation in testicular size of adult breeding alpacas used in the Peruvian Altiplano herds. We hypothesized that in the group mating system practiced in this area, pregnancy and birth rate will be affected by the mean testicular size of sires in mating groups. Our objective was to determine if females joined to males with smaller testicular size will achieve lower overall birthing rates than females joined with larger testicular size males.

Eighty (80) parous healthy females were randomly assigned to one of two mating groups. Group 1 (n=40) was joined to 4 adult males with large testicles with a mean testicular length and width of 4.8 ± 0.25 cm and 3.6 ± 0.16 cm respectively. Group 2 (n=40) was joined to 4 adult males with small testicles with a mean testicular length and width of 3.0 ± 0.08 cm and 1.8 ± 0.5 cm, respectively. The mean testicular size was significantly different ($P<0.05$) between the male groups. The breeding trial was conducted at the end of the traditional breeding season over a 42 day period at the Sumac Tarpuy Station, Ayaviri, Puno, Peru, at 3,900 meters above sea level. Both groups of females were managed similarly following the breeding season. Parturition rates were recorded during the birthing season for both groups and compared using chi-square analysis.

The parturition rate in Group 1 and Group 2 were 47.5% and 30%, respectively. Although females mated to large testicular sized males had a higher odd ratio to become pregnant, this difference was not statistically different ($P=0.108$). The overall low fertility observed in this trial (38.8%) and the relatively small number of females used may be a reason for this lack of statistical significance. Fertility tended to be higher when female alpacas were bred to males with normal sized testicles which justifies considering this trait in selecting future sires. Further experiments are planned to determine the effect of other factors such as the female to male ratio and length of the breeding season on the performance of males with different testicular sizes.

Effect of Recipient Lactation Status on Pregnancy Rate Following Embryo Transfer in Alpacas

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The objective of the present study is to determine the effect of the lactation status (LS) on the pregnancy rate following the transfer of embryos in alpacas.

Recipients alpacas from traditional herds in different communities were selected and placed on natural pastures with periodic supplementation of oats and alfalfa hay. The body condition score (BCS) ranged from 1.5 to 3.5 (1 to 5 scale). Only females with a BCS $\geq$ 2.5 were used as recipients. During two breeding seasons (2007 and 2008), 705 embryos were collected non-surgically from an elite herd of 66 females and 19 breeding males. Of these, 54 (7.7%) were eliminated from the program after evaluation and 651 were transferred either to alpacas with cria at foot (lactating, n=291) or to non-lactating alpacas (n=360). Recipient follicular activity was monitored by ultrasonography and suitable recipients were induced to ovulate and matched to bred donors when they had a mature follicle ($\geq$ 8 mm and $\leq$12 mm). Ovulation was induced with buserelin (8.4 µg, IM, Conceptal®, Intervet®, Lima, Peru). Ovulation was confirmed at the time of transfer by transrectal ultrasonographic visualization of the corpus luteum. All embryos were collected on day 7.4 post-breeding and transferred non-surgically within 20 minutes of collection. Pregnancy diagnosis was performed by ultrasonography 8 days post transfer. Pregnancy rates in lactating and non-lactating alpacas were compared by chi-square analysis.

The overall pregnancy rate following transfer was 33%. The pregnancy rates for non-lactating (44.4%) and lactating (18.2%) recipients alpacas were significantly different ($P<0.001$). These results clearly demonstrate the effect of lactation on pregnancy establishment and maintenance. This effect may be due to negative energy balance and weight loss or other mechanisms that may interfere with corpus luteum function. These factors are being as well as the effect of nutritional supplementation of lactating recipients under Peruvian pasture conditions on pregnancy rate following embryo transfer are being investigated at present in our laboratory.

Understanding how brain glucose and neuronal pathways in the brainstem control appetite.

B. L. Roberts, and S. M. Appleyard.

Even a slight imbalance between food intake and exercise can result in significant weight gain over time and the development of obesity. Obesity now afflicts approximately one third of adults in the United States and is a major threat to health. Before we can understand the treatment and prevention of obesity, we must understand the physiology and neural circuitry that regulates these processes. Appetite is controlled by a constant communication between the enteric nervous system and the brain. For example, when a meal is eaten the stomach expands and nutrients are detected causing the vagus nerve to be stimulated. The vagus nerve then activates specific groups of neurons in the brainstem that send this signal throughout the rest of the brain to produce a feeling of fullness, or satiety. We have previously demonstrated how hormones that reduce appetite increase the amplitude of activation in a group of brainstem neurons that contain and release catecholamines. In contrast, hormones that increase appetite decrease activation of these catecholamines neurons. In this study we investigate how changes in brain glucose levels alter the activation of brainstem catecholamine neurons and their response to vagal stimulation. The relationship between brain glucose levels and neuronal firing is one potential mechanism influencing our ability to feel satiated or hungry.
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Cryopreservation and fertility of Bighorn (*Ovis canadensis c.*) cauda epididymis semen

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Production of hybrid pregnancies between the two species using laparoscopic insemination (LAI) with fresh semen have been reported.[1] The objective of the project was to determine the fresh and post-thaw quality and fertility cauda epididymis semen collected postmortem from 4 Bighorn rams suffering from pneumonia.

Semen was harvested from cauda epidiydmis by float-up technique using a commercial ovine freezing extender (IMV, St Paul, MN) with 20% egg yolk. Semen was diluted at 100 million spermatozoa per mL and equilibrated 3 hours at 5 °C before freezing. To evaluate fertility frozen-thawed samples, 8 estrous synchronized ewe-lambs were LAI. Pregnancy diagnosis was performed by ultrasonography at 30 days.

There was no significant difference in semen quality amongst rams ($P<0.05$) despite the fact that some had fever of 105 °F for up to 5 days before euthanasia. The mean (±SEM) percent post-thaw progressive motility was 63.7 ± 1.8. Three ewes became pregnant following LAI. This technique could be used to preserve genetic diversity in Bighorn flocks if the testes are collected in a timely manner.

1- Rodriguez JS et al. Production of hybrid (Bighorn x domestic sheep) lambs by laparoscopic artificial insemination using Bighorn fresh semen collected by electroejaculation. *Clinical Theriogenology* 2009;1:243.

Highly pathogenic strains of *Salmonella* Enteritidis show enhanced tolerance to acid, oxidative stress and better survival in egg albumen.

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Purpose: *Salmonella* Enteritidis (SE) is a major cause of food-borne gastroenteritis in the USA with contaminated eggs being the major source of human infection. Nevertheless, not all poultry-associated SE strains are equally pathogenic. In a previous study, we identified SE strains with low- (LP) and high-pathogenic (HP) potential using a mouse infection model. The objective of the present study was to further examine the link between observed differences in pathogenicity and tolerance of these SE strains to adverse environments encountered in the chicken host.

Methods: The stationary phase cultures of HP (n=4) and LP (n=3) strains of SE were tested for their ability to survive in acidic environment similar to that of chicken stomach (pH 2.6), in oxidative stress (15 mM H_2O_2) that SE strains encounter within the chicken macrophages, and growth in egg albumen (pH 9).

Results: We found that the HP strains survived in significantly high numbers (P <0.05) than LP strains in acidic environment and in the presence of 15 mM H_2O_2. All HP strains grew well in egg albumen whereas two LP strains did not survive in this condition. In addition, all HP strains were more invasive after oral inoculation in 3-day old chickens with high numbers of bacteria being recovered from spleen and liver. In contrast, none of the LP strains were found to be invasive.

Conclusions: These observations indicate that HP strains with enhanced tolerance to acid and oxidative stress may survive better in the acidic environment of chicken stomach and within chicken macrophages. In addition, differential survival of SE strains in egg albumen indicates that not all SE strains may have the ability to contaminate internal content of eggs. We hypothesize that differential transcriptional or translational regulation account for the observed differences in virulence and other phenotypes observed in this study.

Transposon mutagenesis in a highly invasive isolate of *Salmonella* Enteritidis reveals a number of genes with potential roles in cell invasion.

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Background: *Salmonella* Enteritidis (SE) has recently surpassed *S.* Typhimuium (ST) to become the number one cause of food-borne gastroenteritis in the USA and other developed nations. Poultry is the major reservoir of SE and contaminated poultry products such as eggs are the primary source of human infection. Recent studies have shown that there are remarkable differences in the genetic and phenotypic properties of SE and ST. However, unlike *S.* Typhimurium little is known about the genetic basis of pathogenesis of SE infection in both human and chicken hosts. The aim of this study was to investigate the molecular basis of host cell invasion in SE by applying transposon mutagenesis to a highly invasive strain.

Methods: A random transposon mutant library consisting of a total of 4,992 SE mutants was constructed. A high throughput *in vitro* assay of invasion using well differentiated Caco-2 cells grown in 96-well tissue culture plates was adapted to identify invasion attenuated mutants. The mutated genes in invasion attenuated mutants were identified by cloning and sequencing the DNA regions flanking the transposon insertion.

Results: A total of 79 invasion attenuated mutants with ~10-fold to 100-fold lower invasiveness were identified. The location of the transposon insertion in 11 of these mutants has been determined. Four mutants had transposon insertions in genes encoding FljB (flagellin), FliH (flagellar assembly protein), SipC (T3SS effector protein), SpvR (plasmid virulence regulator protein). Five mutants had transposon insertions in previously uncharacterized genes encoding hypothetical proteins whereas two mutants carried insertions in the interspacer regions.

Conclusions: This is the first report of large scale genome wide mutagenesis study to identify genes involved in cell invasion of SE. While identification of location of transposon insertions in several of the mutants is ongoing, the results suggest that flagella, T3SS and virulence plasmid plays an important role in the cell invasion. In addition, we have identified several previously uncharacterized virulence-associated genes. Further studies on elucidating the precise roles of these genes are currently ongoing.

Not all antibiotics retain their biological activity in soil

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The emergence and persistence of antibiotic resistance in bacterial populations is an inevitable outcome of using antibiotics in human and agricultural animals. Besides selection *in vivo*, a major portion (25-75%) of antibiotics used in agricultural animals is excreted into the environment *via* manure and urine where they can, presumably, exert continued selective pressure on microbial communities. In light of the total mass of antibiotics that are used in agricultural animals and that are then excreted into the environment, it becomes important to determine if these excreted drugs exhibit a biological effect on the microbial flora and for how long this biological effect is retained. In this study we established a simple soil slurry assay to determine how biological activity of different antibiotics changes after brief (24 hr) exposure to different soils. Biological activity was assessed by determining how well an indicator strain of *E. coli* would grow in supernatant from the soil slurry containing antibiotics. These experiments demonstrate that from a biological perspective, many of the antibiotics used in U.S. agriculture (tetracycline, ciprofloxacin, neomycin and erythromycin) are effectively neutralized upon contact with soil. Loss of biological activity is due to adsorption onto particle surfaces, hydrolysis, or conversion to an insoluble form. Importantly, however, representative drugs from beta-lactams and phenicols (ampicillin, cephalothin, cefoxitin, ceftiofur and florfenicol) retained biological activity much longer than other drugs and consequently may represent an important and unappreciated factor in selection of antibiotic resistance in soil microflora.

The potential cardioprotective role of adiponectin in hibernating grizzly bears

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Grizzly bears undergo a unique set of physiologic changes during hibernation in order withstand their heart rate and cardiac output dropping to extremely low levels. This would normally lead to congestive heart failure in most species, yet the bear is able to withstand these conditions and make a full recovery in the spring as they emerge from hibernation. One potential mechanism the bears may be utilizing is an anti-inflammatory cytokine secreted by fat cells, adiponectin (ADPN), which is commonly known for its role in energy homeostasis, but it also plays a cardioprotective role in human clinical studies. Our first goal was to determine if the serum concentrations of ADPN and the mRNA expression of ADPN's two receptors in cardiac tissue, AdipoR1 and AdipoR2, vary throughout the year in grizzly bears. Our second goal was to assess seasonal changes in cardiac ADPN and AdipoR1 protein using western blots. ADPN protein in cardiac tissue was further characterized by the concentrations of high and low molecular weight forms (HMW and LMW respectively). We found serum ADPN in bears to be greatest in September (12.1 ± 0.6 ug/ml) just prior to hibernation when their rate of weight gain is greatest. Serum levels were lowest in during hibernation, December-February (2.5 ± 0.3 ug/ml). They gradually increased throughout the active period from March-August, (2.9 ± 0.5 ug/ml to 7.7 ± 0.5 ug/ml respectively). Both mRNA and protein expression of AdipoR1 in cardiac tissue were significantly down-regulated during hibernation ($p < 0.005$). Despite the marked difference in serum levels, there was no significant difference of cardiac ADPN protein between active and hibernating bears found. All bears had much higher concentrations of the active form of ADPN (the HMW form) relative to the LMW form. In conclusion, circulating ADPN changes dramatically throughout the year, but local ADPN is maintained. This finding suggests local cardiac regulation of ADPN may be necessary, which may play a protective role in conditions of cardiac stress or disease.

Selective antagonism of hindbrain NMDA-type glutamate receptors reverses reduction of food intake by cholecystokinin.

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Systemically cholecystokinin (CCK) reduces food intake. This effect is mediated by abdominal vagal afferents synapsing in the hindbrain nucleus of the solitary tract (NTS). Systemic antagonism of NMDA-type glutamate receptors, which are expressed by vagal afferent neurons, attenuates reduction of food intake by CCK (Covasa et al., 2004; Guard et al., 2009). We hypothesized that NMDA receptor function in the NTS, where vagal afferents synapse, is necessary for reduction of food intake by CCK. Therefore, we measured reduction of food intake by CCK subsequent to NMDA receptor antagonist injection either into the fourth ventricle of the hindbrain, or directly into the NTS. We found that both fourth ventricle (6 µg) or direct NTS injection (500 ng) of the non-competitive NMDA antagonist MK-801 prior to intraperitoneal CCK administration (2 µg/kg) reversed CCK-induced reduction of feeding, while the same antagonist doses injected subcutaneously did not. Similarly, fourth ventricle injection of D-cppene, a competitive NMDA receptor antagonist, also attenuated reduction of food intake following IP CCK. Finally, we found that fourth ventricle D-cppene significantly reduced expression CCK-induced expression of immediate-early gene cfos in the NTS and area postrema following injection of CCK. We conclude that NMDA receptors in the hindbrain, perhaps on presynaptic vagal afferent terminals, are necessary for responses to gastrointestinal satiation signals, such as CCK. Supported by NIH grants DK052849 and NS 020561 to R.C. Ritter.

DPYD as a candidate gene for 5-fluorouracil toxicity in dogs

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The chemotherapeutic agent 5-fluorouracil (5-FU) is used in veterinary oncology for the treatment of a variety of tumors including carcinomas, sarcomas, and mast cell tumors. 5-FU is an anti-metabolite which is catabolized by dihydropyrimidine dehydrogenase (DPD), encoded by the DPYD gene. The enzyme is responsible for the rate limiting step of 5-FU catabolism and elimination of roughly 80% of administered drug.

Deficiency of DPD has been noted in many human patients who have experienced severe and even fatal adverse reactions after 5-FU administration. Researchers have identified 39 different polymorphisms and mutations in human DPYD. Of those, the splice site mutation IVS14 +1G>A is the most common. This mutation results in the deletion of exon 14 and a DPD mRNA sequence that will fail to code for amino acids 581-635. The deletion renders the enzyme non-functional, resulting in accumulation of 5-FU and toxicity in affected patients.

Recently, neurological toxicity was identified in several dogs included in a clinical trial of 5-FU for cutaneous mast cell tumors.[1] These canine patients experienced severe neurotoxicity after administration of 5-FU. The patients' signs ranged from peripheral neuropathies to seizures. Because polymorphisms in human DPYD predispose patients to 5-FU neurological toxicity, canine DPYD is a logical candidate gene for 5-FU neurologic toxicity in dogs.

We sequenced all 22 exons of the canine DPYD gene using cDNA from healthy dogs (unknown phenotype). Primers were designed based on the human DPYD gene sequence. Canine DPYD was found to be 93% conserved compared to the human DPYD gene. We identified 204 bases that differed between the human and canine cDNAs. The nature of the nucleotide changes will be presented in the poster. Future research will involve sequencing phenotyped dogs: Affected = dogs that developed 5FU-induced neurological toxicity; Unaffected = dogs that received 5FU but did not develop neurotoxicity.

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[1] Veterinary Cancer Care P.C., 2001 Vivigen Way, Santa Fe, NM 87501