M49 Comparison of antibody response, bacteriological culture and PCR based diagnostic methods in *Brucella ovis* inoculated rams. Ariel O. Miranda*[1], Hernán Romero Harry[1], Valeria N. Baldone[1], Marcy Owens[2], and Scott Pratt[2], [1]*INTA, Anguil, La Pampa, Argentina,* [2]*Clemson University, Clemson, SC.*

Twenty-seven *Brucella ovis* (*B. ovis*)-negative adult Pampinta rams were used to evaluate 3 diagnostic methods for *B. ovis* detection post-inoculation. Twenty-four rams were inoculated by the conjunctival route and intraprepucially with a total of 3×10^9 cfu/ram of *B. ovis* (strain, INTA Bariloche, Argentina). Three rams were inoculated with a saline placebo. Blood and semen samples were taken for serological antibody detection, bacteriology culture and PCR at 0, 12, 24, 62 and 97 d post inoculation. Modified Thayer Martin medium was used to detect presence of *B. ovis* in semen. Serum samples were tested for antibodies to *B. ovis* using a commercial ELISA kit. *B. ovis* DNA from semen samples was extracted using a commercial kit (Qiagen) and PCR performed using primers pairs specific to *B. ovis*. The proportion of agreement (Kappa value, k) was calculated. *B. ovis* was not detected 12 d post-inoculation by any detection method. Nine rams were positive for *B. ovis* 24 d post-inoculation detected by ELISA; however, the rams were negative for *B. ovis* using the other 2 detection methods. Further, we observed a continued increase toward the end of the study. The intermittent elimination of *B. ovis* by semen agrees with the lower result response in culture and PCR. The low percentage of positive animals in culture on the 97 d could have been given to false negatives or methodology. Control group was negative to the 3 methods over the study. Seroconversion confirmed by ELISA is the most sensitive diagnostic measure of *B. ovis* exposure.

Table 1 (Abstr. M49). Number and percentage of positive rams by different methods and k value between them throughout the study

	Sampling day				
Test diagnostic	0	12	24	62	97
ELISA positive	0	0	9 (38%)	21 (88%)	23 (96%)
Culture positive	0	0	0	14 (58%)	2 (8%)
PCR positive	0	0	0	18 (75%)	17 (71%)
	Agreement (%), k-value (SE)				
ELISA vs. culture	100, 1.0 (–)	100, 1.0 (–)	62, 0.11 (0.10)	70, 0.33 (0.16)	12, 0.00 (0.01)
ELISA vs. PCR	100, 1.0 (–)	100, 1.0 (–)	62, 0.11 (0.10)	70, 0.07 (0.20)	75, 0.19 (0.17)
Culture vs. PCR	100, 1.0 (–)	100, 1.0 (–)	100, 1.0 (–)	83, 0.63 (0.15)	37, 0.07 (0.05)

Key Words: *Brucella ovis*, diagnostic tools, PCR diagnostic

M50 Prevalence of brucellosis in Iraq and control through a vaccination campaign. Alaa Khalil Ismaiel*, *Ministry of Agriculture, Veterinary Directorate, Central Veterinary Laboratory, Baghdad, Iraq.*

Brucella melitensis is primarily a cause of abortions in sheep and goats. It can be isolated from cattle, water buffalo, and camels, and transmits to humans through unpasteurized milk and cheeses. Little was known about the distribution of this organism around Iraq, which was needed for developing an effective control campaign. The goals of this study, initiated in 2005 in collaboration with the Food and Agriculture Organization (FAO), were to determine the prevalence of *Brucella* in sheep, goats, cattle, water buffalo, and camels across Iraq, and to test the effectiveness of a whole cell vaccine. Villages for sampling were selected from 15 governorates that had more than 2000 animals. In each governorate, goats and sheep were sampled in 17 villages, cattle and water buffalo in 11 villages, and camels in 9 villages; 60 animals were sampled in each category per village. Serum samples initially screened by the Rose Bengal test were further confirmed by ELISA. These results indicated that 6.5% (6.1–6.9, 95% CI), 1.0% (0.78–1.2, 95% CI) and 1.5% (1.2–1.8, 95% CI) of sheep and goats, cattle, and water buffalo were positive for *B. melitensis*, respectively. Prevalence for sheep and goats ranged from 0.2% in Murhana to 16.4% in Kirkuk, for cattle from 0.02% in Thyqar to 11.1% in Muthana, and for water buffalo from 0% in Babylon to 5.4% in Basrah. Approximately 1 to 2×10^9 cfu of the live attenuated *B. melitensis* vaccine, Rev1, was used to vaccinate 1.8 to 2.5 million lambs and kids each year, across all 15 governorates, from 2009 to 2014. During this time, cases of abortion in sheep and goats fell from 5090 in 2011, to 322 in 2013. This study concluded that the incidence of *Brucella* varies across different regions of Iraq, which can be related to animal handling practices. An aggressive strategy of vaccination was effective in reducing abortions in sheep and goats by over 90%. During the course of the vaccine intervention in this study, the Iraqi Ministry of Health reported that the incidence of brucellosis in humans decreased from 24 to 7 incidences per 100,000 people, further indicating an effective vaccination program.

Key Words: *Brucella*, vaccination, pathogen control

M51 Maternal undernutrition increases acylated ghrelin concentrations in the umbilical artery and vein of the twin ovine fetus. Sahng-Wook Hahm*[1], Meghan Field[1], Russell V. Anthony[2], and Hyungchul Han[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.*

Maternal undernutrition can induce intrauterine growth restriction and contribute to the development of adult metabolic diseases. Ghrelin, a peptide hormone purified from the gastric mucosa, activates food intake and energy homeostasis. Ghrelin exists in 2 forms, the non-acylated ghrelin and acylated ghrelin (active form). The acylation of ghrelin is mediated by membrane bound O-acyltransferase 4 (Mboat4). We hypothesized that maternal undernutrition would increase the acylated ghrelin in the blood of the fetus during gestation. Twin bearing western whiteface ewes were either fed 100% (C, n = 12), or 50% of their global nutrient requirements from 28 to 78 d of gestational age (dGA) and readjusted to 100% beginning at 79 dGA (LC, n = 12), or continuously restricted until 135 dGA (LL, n = 12). At 135 dGA, umbilical artery and vein plasma, and fetal abomasum samples were collected. Umbilical arterial and venous ghrelin concentrations were measured by radioimmunoassay. Mboat4 gene expression in fetal abomasum was measured using quantitative real-time PCR. Mboat4 protein concentration in the fetal abomasum was analyzed by Western blot analysis. Each ewe was treated as one experimental unit and twin fetuses were nested within the ewe. All data are presented as least squares means ± SEM using the PROC MIXED model of SAS. The umbilical arterial active ghrelin concentration tended to be greater in LL fetuses (41.63 ± 5.49 pg/mL) compared with control (27.17 ± 5.76 pg/mL; $P = 0.0523$) and LC fetuses (22.81 ± 5.28 pg/mL; $P = 0.0197$). The active to total ghrelin ratio was

higher in LL than LC fetuses ($P < 0.05$). Umbilical vein active ghrelin concentration tended to be greater in LL, when compared with LC fetuses ($P = 0.0812$). No significant difference was observed in Mboat4 mRNA expression in fetal abomasum. Mboat4 protein concentration tended to be greater in fetal abomasum of LC and LL fetuses compared with control fetuses ($P = 0.0584$). These results are interpreted to mean that elevated active ghrelin but not total ghrelin in fetal circulation may be an adaptation of the fetus to prolonged undernutrition during gestation.
USDA-AFRI Grant no. 2009–65203–05670.

Key Words: fetus, ghrelin, membrane bound O-acyltransferase 4 (Mboat4)

M52 Evaluating udder health in dairy goats: An old but still unsolved issue. Andrea Bezerra[1], Candice De Leon[1], Magda Fernandes[1], Bryan White[2], Juan Loor[2], and Celso Oliveira*[1,2], *[1]Federal University of Paraiba (UFPB), Brazil, Areia, PB, Brazil, [2]The University of Illinois at Urbana-Champaign, Urbana, IL.*

Despite the high economical importance of goat milk production in certain regions, the real burden of subclinical mastitis caused by intramammary infections (IMI) is still unknown. This is mainly caused by the lack of reliable diagnostic tests, since the accuracy of somatic cell count (SCC) as an indirect indicator of udder health is questionable in this species. This study aimed to investigate the correlation among and somatic cell count (SCC), total bacterial count (TBC), California Mastitis test (CMT) and microbiological culture (MC) as indicators of udder health in dairy goats in Northeastern Brazil, the leading goat milk producing region in South America. From 6 farms, a total of 396 milk samples were individually collected from each teat of 66 goats at different lactation periods (begining, mid, end). Out of 146 (37%) positive samples, coagulase negative (CoNS, 73%) and positive *Staphylococcus* (21%), gram-positive bacilli (6%), streptococci (3.4%) and Enterobacteriaceae (1.4%) were identified. SCC and TBC were correlated (r = 0.47; $P < 0.01$) but a weak correlation (0.20; $P < 0.01$) was observed between SCC and infection by CoNS infection. Although a positive association ($P < 0.05$) was seen between SCC and MC using percentile 75 (6.05 log SCC/mL) as threshold, SCC was weakly (0.29) correlated with MC. Interestingly, the correlation was strong at the beginning of lactation (0.49, $P < 0.001$) but negligible at mid (0.3; $P = 0.79$) and end lactation (0.12; $P = 0.14$). Using MC as the gold standard, sensitivity values for CMT and SCC were 39.2 and 40.2%, whereas specificity reached 80 and 89.5%. SCC in non-infected goats increased ($P < 0.01$) at the end lactation. The low agreement among the diagnostic methods and the large physiological variations in SCC during lactation reinforce the limitations of the current methods to accurately predict udder health in goats, especially at the animal level. A better knowledge about the glandular tissue responses against IM agents is strongly needed. We have currently been using metagenomic approaches to investigate in-depth changes in the microbiome of naturally infected animals to bring new insights about mastitis in goats.

Key Words: goat mastitis, somatic cell, goat milk

M53 Effect of FMD vaccine on seminal traits of HF bulls. Mohua Das Gupta*[1], Shivaji Hanmantrao Sontakke[1], Gunjan Rathi[1], Vinod Haribhau Shende[1], Mohammed Mushtaque[1], Samir Kumar Dash[1], Suresh B. Gokhale[1], Arun P. Phatak[2], Hemant Dasharath Kadam[1], Narayan Laxman Phadke[1], and Jayant Ramachandra Khadse[1], *[1]BAIF Development Research Foundation, Central Research Station, Uruli Kanchan, Pune, Maharastra, India, [2]601 Curran Drive, Waterford, CA.*

An attempt was made to study the effect of Foot and Mouth Disease vaccination on seminal traits of 30 Holstein Friesian bulls ranging between 3 and 5 years age maintained at BAIF Central Research Station Uruli Kanchan, Pune, India. Study period was from August to September 2013 and observations on semen were of bulls maintained under identical feeding and management regimens. Semen collections 15 d before vaccination, 15, 30 and 45 d after vaccination were evaluated for seminal traits such as fresh and post-thaw sperm motility, sperm concentration, semen volume, live and dead count, plasma integrity. Data generated were analyzed using ANOVA. It revealed that vaccination had significant ($P < 0.05$) effect on post-thaw motility and highly significant ($P < 0.01$) deleterious effect on host test, head and mid piece abnormalities as it causes derangement in spermatogenesis and epididymal function due to rise in testicular and body temperature; however, no effect was noted on seminal traits such as volume, concentration, initial motility viability and tail abnormalities. There was general decline observed in performance of seminal traits of bulls, confirming the reports of previous studies, which state that the secondary activities following vaccination remain unaltered. Therefore it can be concluded that FMD vaccination causes alteration in spermiogram but functions of accessory sex glands remain unaltered. Hence for better semen picture and to counter the effect, preventive immune enhancement before vaccination and sexual rest for highly affected bulls can be adopted.

Table 1 (Abstr. M53). Sperm characteristics before and after vaccination with FMD vaccine

Parameter	Pre-vaccination	Post-vaccination	*P*-value
Volume (mL)	6.63 ± 0.31	6.42 ± 0.17	0.59
Concentration (millions/mL)	1705.69 ± 86.09	1688.40 ± 43.29	0.88
Initial motility (%)	76.53 ± 0.38	76.3 ± 0.21	0.41
Live and dead (%)	82.89 ± 1.63	80.64 ± 1.03	0.93
Post-thaw motility (%)	58.19 ± 0.41	57.5 ± 0.24	0.03*
Host test (%)	61.53 ± 0.43	60.59 ± 0.21	0.0003**
Morphology			
Head (%)	2.78 ± 0.17	3.3 ± 0.12	0.01**
Mid (%)	1.78 ± 0.16	2.41 ± 0.11	0.0001**
Tail (%)	2.08 ± 0.18	2.19 ± 0.09	0.61

Key Words: FMD vaccine, seminal trait

M54 Development of an effective oral animal vaccine using M cell targeting strategy. Sangkee Kang*[1,2], Yoonseok Lee[2], Jinduck Bok[2], Chongsu Cho[3], and Yunjaie Choi[2,3], *[1]Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang, Republic of Korea, [2]Institute of Green-Bio Science & Technology, Seoul National University, Pyeongchan, Republic of Korea, [3]Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea.*

Development of oral vaccine is necessary in animal husbandry field because of not only its convenience in treatment but also its capability inducing mucosal immune response. M cells are well-known as antigen collecting portals for GALT (gut associated lymphoid tissue) in intestinal tract, thus targeted delivery of vaccine molecules to the M cells could be a promising strategy to improve efficiency of oral vaccine. Previously, our research group have identified an M cell targeting peptide moiety, CKS9, by phage display technique using in vitro M cell model consisting of coculture system with Caco-2 (human colon carcinoma cells) and human Raji B cells. In this study, we constructed a recombinant lactic acid bacteria, *Lactobacillus plantarum* producing a model

antigen, M-BmpB, the BmpB (surface membrane protein originated from *Brachyspira hyodysenteriae*) conjugated with CKS9, to validate its potency as an efficient animal oral vaccine. We ascertained that the fusion protein, M-BmpB, was expressed as soluble form in the cytoplasm of *L. plantarum* by SDS PAGE and Western blot and confirmed its M cell targeting property in contrast to original BmpB (without CKS9) and P-BmpB (with unrelated peptide ligand) by in vivo closed ileal loop assay. In in vivo immunization assay (Balb/C, n = 5 in each group), Oral administration of *L. plantarum* producing M-BmpB (LP25-M-BmpB) to mice revealed significant improvement in induction of both serum IgG ($P < 0.05$) and fecal IgA ($P < 0.01$) against BmpB compared with control groups. Our results suggest that the recombinant lactic acid bacteria, such as *L. plantarum*, producing certain pathogenic antigen with M cell targeting strategy could have a great potential to develop an effective and convenient oral animal vaccine system.

Key Words: oral vaccine, M cell targeting, mucosal immunity

M55 Amino acid supplementation and lipopolysaccharide challenge alters bovine blood polymorphonuclear leukocytes response in vitro. M. Garcia*[1], T. H. Elsasser[2], Y. Qu[1], L. Juengst[1], B. J. Bequette[1], and K. M. Moyes[1], *[1]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, [2]Agricultural Research Service, Animal Biosciences and Biotechnology Laboratory, U.S. Department of Agriculture, Beltsville, MD.*

Glutamine is the preferred amino acid (AA) utilized by polymorphonuclear leukocytes (PMNL) during the inflammatory response. However, the effect of other AA on bovine PMNL response during inflammation and how this is altered by stage of lactation are currently unknown. The objective of this study was to determine the effect of additional AA supplementation (pool of AA excluding glutamine) on AA profile, transcriptomic, and inflammatory function of PMNL from dairy cows in early and mid-lactation in vitro. Twenty Holstein dairy cows in early (n = 10; DIM = 17 ± 3.1) and mid-lactation (n = 10; DIM = 168 ± 14.8) were used for this study. PMNL were isolated and diluted using RPMI, containing basal concentrations of glucose (7.2 m*M*) and amino acids (3.1 m*M*). Working solutions of AA (0 m*M* or 4 m*M* of AA) and LPS (0 or 50 μg/mL) were added and tubes were incubated for 2 h at 37°C and 5% CO_2. Data were analyzed as a randomized block design. Stage of lactation did not alter PMNL responses in vitro. AA in combination with LPS increased ($P \leq 0.02$) the concentration of alanine and methionine and tended ($P < 0.10$) to increase that of leucine, isoleucine, threonine, and phenylalanine. Regardless of LPS challenge, AA supplementation downregulated ($P < 0.05$) the expression of genes associated with inflammation such as *NFKB1*, *IL10*, *IL1B*, *IL6*, *TNFA*, *LYZ*, *SOD2*, and *SLC2A3* but tended ($P < 0.10$) to increase the expression of *TLR6*, *G6PD*, *LDHA*, and *PDHA1*. Supplementation of AA reduced the concentration of TNF-α (104.0 vs. 34.9 ng/mL, $P = 0.01$) in medium but did not affect chemotaxis and phagocytic functions of PMNL. Metabolic profiles for cows in early lactation did not parallel those for cows during the early postpartum period and may partly explain the lack of stage of lactation effects. This study identified AA other than glutamine that may alter PMNL response during inflammation in vitro that may lead to new avenues to improve immune response during inflammation in vivo.

Key Words: amino acid, cow, polymorphonuclear leukocyte

M56 In vitro activity of *Pithecellobium dulce* and *Lysiloma acapulcensis* on exogenous development stages of sheep gastrointestinal strongyles. A. Olmedo-Juárez[1], R. Rojo-Rubio*[1], J. Arece-García[2], C. Marie-Magdeleine[3], and J. F. Vázquez-Armijo[1], *[1]Centro Universitario UAEM Temascaltepec, Universidad Autónoma del Estado de México, Temascaltepec, Estado de México, México, [2]Estación Experimental de Pastos y Forrajes, España Republicana, Matanzas, Cuba, [3]Instituto de Investigaciones Agronómicas (INRA), Guadaloupe, France.*

Parasitic nematodes of the digestive tract remain one of the main constraints in small ruminants in subtropical countries. In these regions there are a lot of browsed plant species with antihelmintic activity; in this way an experiment was conducted to evaluate the effects of 2 lyophilised aqueous extracts of *Lysiloma acapulcensis* (LAE) and *Phitecellobium dulce* (PDE) tree leaves on in vitro assessment of hatching of eggs, larval development and migration of gastrointestinal nematodes of sheep using a general linear model. Treatments contained extracts from both species at concentrations of 0, 125, 250 and 500 μg/mL. Both albendazole and levamisole were used at a level of 1% as positive control. The extract of LAE, compared with PDE, showed better inhibition ($P < 0.05$) of egg hatching. Different doses of both the LAE and PDE extracts showed a larvicidal effect ($P < 0.05$) on all larvae exposed to different doses of the extracts. In the larval migration assay, a similar effect with levamisole occurred with the LAE extract at doses of 250 and 500 μg/mL. The extract of *P. dulce* had a lower larvicidal effect ($P < 0.05$) than levamisole and *L. acapulcensis* extracts. The use of aqueous extract of *L. acapulcensis* could be a promising alternative to synthetic anthelmintics as treatments of gastrointestinal nematodes of sheep in organic and conventional production systems under subtropical conditions.

Key Words: anthelmintic, extract, sheep

M57 Changes in transcriptome of bovine monocytes-derived macrophage challenged with *Mycobacterium bovis*. Dianelys Gonzalez-Pena*, Robmay Garcia, and Andrew J. Steelman, and Sandra L. Rodriguez-Zas, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Macrophages pertain to the mononuclear phagocytes system as part of the innate and acquired immunity responses. The interaction of macrophages with *Mycobacterium bovis* concludes with the formation of granulomas. However, the host-immune response to *M. bovis* could be compromised by the suppression of immuno-regulatory pathways leading to active tuberculosis. Therefore, transcriptome analysis of the macrophage response to *M. bovis* can offer insight into the host-pathogen interaction. Bovine monocyte derived macrophage (MDM) of peripheral blood was obtained from 7 non-infected Holstein cows, cultivated and infected with *M. bovis*. The RNA was isolated 24 h after infection and single-end reads were mapped to the *Bos taurus* (UCSC_bosTau7) reference genome using Tophat v2.012. Using Cufflink v2.2.1, 7,664 transcripts from 7,505 genes were tested and 1,192 transcripts from 1,187 genes were differentially expressed between the infected and non-infected MDM (false discovery rate adjusted *P*-value <0.05). Among the transcripts, 61% were upregulated in the infected relative to non-infected MDM. Interferon gamma (IFNG), indoleamine 2,3-dioxygenase 1 (IDO1), chemokine (C-X-C motif) ligand 10 (CXCL10), and chemokine (C-X-C motif) ligand 9 (CXCL9) were overexpressed in infected relative to non-infected MDM. The IFNG is a potent activator of macrophages, while IDO1 encoded an important rate-limiting enzyme in the kynurenine pathway, activated during the inflammation process that favors immune suppression and tolerance. The CXCL9 and CXCL10 are critical during the early stages of the immune response to *M. bovis*. The top 5 ranking category clusters detected by the functional analysis of the differentially expressed genes using DAVID (enrichment score >5) included immune defense, and the activation and proliferation of leukocyte, lymphocyte, mononuclear cells, and T and B cells as well

as cytokines production and apoptosis regulation. This study unveils details of the host response of MDM to *M. bovis*.

Key Words: transcriptome, macrophage, *Mycobacterium*

M58 Phytochemicals in corn distillers grains. Adebola Daramola and Byungrok Min*, *University of Maryland Eastern Shore, Princess Anne, MD.*

Corn is a major feedstock for the production of fuel ethanol as well as corn distillers grains that are widely used for animal feed. Corn is one of the most abundant sources of health-beneficial phytochemicals among crops. Little information is, however, available for phytochemicals in corn distillers grains. The objective was to determine amounts, availability, and antioxidant capacities of phytochemicals in corn distillers grains: dried distillers grains with solubles (DDGS), wet or modified wet distillers grains (WDG/MWDG), and condensed distillers solubles (CDS), compared with corn. Freeze-dried samples were extracted with 80% ethanol to obtain soluble phenolics. Residues were alkali-treated and extracted with ethyl acetate for cell-wall-bound phenolics. Amounts (total phenolic and flavonoid contents) and antioxidant capacities (DPPH radical scavenging, oxygen radical absorbance, hydroxyl radical averting, and iron chelating capacities) in both phenolics were evaluated. Phenolic acids and carotenoids were also identified and quantified using HPLC. Data were analyzed by ANOVA and sample means were compared using SNK multiple range test. Amounts and antioxidant capacities of soluble phenolics were the highest in DDGS, followed by CDS, WDG/MWDG, and corn ($P < 0.05$). Those of cell-wall-bound ones were the highest in DDGS, followed by WDG/MWDG, corn, and CDS ($P < 0.05$). Ferulic acid was the predominant phenolic acid (~90%) and its amount was the highest in DDGS ($P < 0.05$). Carotenoid content was the highest in DDGS, followed by WDG/MWDG, CDS, and corn ($P < 0.05$). Total phenolic, phenolic acid, and carotenoid contents in DDGS were over 3 times higher than in corn. It is known that, due to conversion of starch to ethanol and CO_2, macronutrients in corn are generally concentrated into DDGS over 3 times. The results indicate that phytochemicals in corn are well concentrated during the processing, thus DDGS have great potential as a source of phytochemicals to improve farm animal health and performance. However, cell-wall-bound phenolic contents in DDGS were 2–5 times higher than those of soluble ones, indicating that the majority of phenolics in DDGS are not readily absorbable. Hence, technologies to liberate them are needed to maximize health-beneficial potentials of DDGS.

Key Words: phytochemical, DDGS, corn

M59 Characterization of the binding potential of pathogenic bacteria to yeast probiotics and paraprobiotics. Janet R. Donaldson*[1], Gabe Posadas[1], Jeffery A. Carroll[2], Paul R. Broadway[2], Amanda Lawrence[1], and Jimmie Corley[3], *[1]Mississippi State University, Mississippi State, MS, [2]USDA-ARS, Lubbock, TX, [3]Phileo, Lesaffre Animal Care, Milwaukee, WI.*

Probiotics and their associated derivatives (paraprobiotics) are frequently utilized to improve animal health and productivity. However, their mechanisms of action are not fully characterized, especially in regards to the interactions with pathogenic bacteria in the gastrointestinal tract. This study tested the hypothesis that yeast probiotics and paraprobiotics directly interact with pathogenic bacteria differently. To test this hypothesis, the binding capability of 5 different yeast probiotics or paraprobiotics to gram-negative bacteria (*Salmonella* sp. and *Escherichia coli* O157:H7) and gram-positive bacteria (*Listeria monocytogenes* and *Clostridium* sp.) were analyzed. Yeast and bacteria were co-incubated on coverslips, washed extensively, and examined by scanning electron microscope to determine the extent of binding between products and pathogens. Membrane filtration was also used to quantitate the amount of bacteria capable of binding to the yeast product; yeast products and bacteria were co-incubated, filtered using 3μM membrane filters, and the resulting filtrates were assessed for viable bacteria by plate counts. All bacteria tested bound with nearly equivalent efficiencies ($P > 0.05$) against the live yeast probiotics tested (~26%). However, much variation was observed in the binding efficiencies with the paraprobiotics. The gram-positive bacteria had, as a group, a preference for binding to one paraprobiotic in comparison to the other 2 products analyzed (25% adhered vs. 47% adhered; $P < 0.05$), whereas the gram-negative bacteria had greater efficiency to bind to 2 paraprobiotics (40% adhered; $P < 0.001$). These data suggest that the use of probiotics and paraprobiotics as therapies needs to be specific to the pathogen of interest; thus indicating a need for "designer" probiotic/paraprobiotic feeding strategies. Further research is needed to analyze specific binding efficiencies of probiotics and paraprobiotics against infectious agents in vivo.

Key Words: probiotic, bacteria, paraprobiotic

M60 Effects of polybrene and puromycin on equine infectious anemia virus replication. Dustin A. Therrien, Rebecca D. Parr, and Sarah C. Canterberry*, *Stephen F. Austin State University, Nacogdoches, TX.*

Equine infectious anemia virus (EIAV) is a lentivirus that infects members of the family Equidae. Despite extensive study, there is no method for prevention or treatment of this disease. Novel mechanisms, such as RNA interference (RNAi), have been used to decrease replication of many viruses. Results of previous in vitro studies have indicated that in the process of generating transgenic cells, EIAV replication was inhibited. Thus, these data were inconclusive as to the efficacy of RNAi against EIAV. To further investigate the effectiveness of RNAi, it is essential to discern which aspects of generating transgenic cell lines affects viral replication. Here, we have investigated 2 common reagents: polybrene (Pol), a polycationic polymer used to increase initial cell transduction, and puromycin (Pur), an antibiotic selecting agent. Non-transgenic feline adenocarcinoma cells, persistently infected with $EIAV_{19}$ (a laboratory adapted strain of the virus), were exposed to high and low concentrations of Pol (8 and 16 μg/mL), and Pur, (1 and 2 μg/mL). Control cell lines were exposed to neither reagent. Supernatants were collected for 17 d and viral replication was quantified using a reverse transcriptase (RT) Assay. The RT values were analyzed using R (R, v. 2.15.3, R Development Core Team, 2012) to generate 9999 permutations of the data. Mean RT values (Table 1) for experimental groups were not significantly different from the mean RT values for the control groups. These data indicate that Pol and Pur alone have no effect on viral replication in these cells. Additional studies are needed to determine which reagents used in establishing transgenic cells is responsible for the observed decreases in viral replication.

Contd.

Table 1 (Abstr. M60). Mean RT values per 100,000 cells per day of viral accumulation

Day	Control	Pol High	Pol Low	Pur High	Pur Low
2	3.2E6 ± 0.5E6	3.8E6 ± 0.4E6	4.9E6 ± 0.9E6	13.6E6 ± 0.4E6	13.4E6 ± 4.9E6
4	12.7E6 ± 2.7E6	13.7E6 ± 1.5E6	15.2E6 ± 1.3E6	720.6E6 ± 341.6E6	59.1E6 ± 21.8E6
7	8.6E6 ± 1.7E6	13.8E6 ± 2.5E6	17.4E6 ± 3.3E6	527.1E6 ± 210.1E6	21.6E6 ± 5.9E6
9	27.9E6 ± 1.8E6	27.7E6 ± 1.5E6	28.4E6 ± 6.5E6	529.4E6 ± 104.4E6	27.4E6 ± 1.5E6
13	9.7E6 ± 0.8E6	12.0E6 ± 3.1E6	9.9E6 ± 0.9E6	215.0E6 ± 13.5E6	6.5E6 ± 0.8E6
17	5.2E6 ± 1.0E6	7.0E6 ± 1.7E6	6.4E6 ± 0.8E6	198.6E6 ± 11.7E6	4.2E6 ± 0.3E6

Key Words: equine infectious anemia virus (EIAV), RNA interference (RNAi)

M61 Co-aggregation ability of cell wall components of *Saccharomyces cerevisiae* to pathogenic bacteria. Marlén Rodríguez[1], Ana Julia Rondón[1], Yadileiny Portilla[1], Ramón Bocourt[2], María José Ranilla*[3,5], María Dolores Carro[4], Alexey Díaz[3,5], and Grethel Milián[1], *[1]Center for Biotechnological Studies, University of Matanzas, Matanzas, Cuba, [2]Institute of Animal Science, Mayabeque, San José de las Lajas, Cuba, [3]Animal Production Department, University of León, León, Spain, [4]Agriculture Production Department, Technical University of Madrid, Madrid, Spain, [5]5IGM (CSIC-ULE), Finca Marzanas s/n, Grulleros, León, Spain.*

Autoaggregation in bacteria is the phenomenon of aggregation between cells of the same strain, whereas coaggregation is due to aggregation occurring among different species. Aggregation ability of prebiotic bacteria is related to adhesion ability, which is a prerequisite for the colonization and protection of the gastrointestinal tract in all animal species; however, coaggregation ability of prebiotic bacteria offers a possibility of close interaction with pathogenic bacteria. Coaggregation ability of cell wall components of *Saccharomyces cerevisiae* is known, because of their mannan content, but literature offers little information on this topic. The aim of this experiment was to assess the ability of coaggregation of 2 preparations of *S. cerevisiae* cell walls to 3 pathogenic bacteria (*Staphylococcus aureus* hemolytic enterotoxin A, *Salmonella enteritidis* and *Escherichia coli* serotype O157:H/7). Cell wall preparations consisted on either the distillery cream (DT), a byproduct of sugar cane, or a hydrolyzate (HT) obtained by enzymatic methods. Pathogens were grown in nutritive broth medium for 18 h at 37°C. After that, cultures were diluted (1:1) with DT and HT, and absorbance (560λ) was measured at 0 and 5 h. Both DT and HT showed the ability of coaggregate to the 3 pathogenic strains, and no bacterial strain × cell wall preparation interaction ($P = 0.379$) was detected. Coaggregation was higher ($P < 0.001$; SEM = 0.36) with HT (mean values of 85.3, 78.6 and 77.8% for *S. aureus*, *S. enteritidis*, and *E. coli*, respectively) compared with DT (mean values of 16.5, 5.8 and 6.0% for *S. aureus*, *S. enteritidis*, and *E. coli*, respectively). If confirmed with other pathogen species, these results support further research on the use of the HT from *S. cerevisiae* as a possible prebiotic additive for animal feed.

Key Words: coaggregation, pathogenic bacteria, *Saccharomyces cerevisiae*

M62 In vitro efficacy of chitosan against *Cryptosporidium parvum* and validation on infected goat kids. Karim Adjou[1], Jean-Philippe Marden*[2], Eric Auclair[2], Christian Mage[3], and Isabelle Vallée[1], *[1]UMR BIPAR Anses-ENVA, Maisons-Alfort, France, [2]Phileo Lesaffre Animal Care, Marcq en Baroeul, France, [3]Mage Consultant, Estivaux, France.*

The aims of this study were to investigate (1) the efficacy of chitosan in 2 forms, the monomer *N*-acetyl glucosamine (NAG) and a chloride salt of chitosan (MIX) in culture systems HCT-8 and Caco-2 cell lines in vitro for *Cryptosporidium parvum* compared with a positive control, paromomycin (PARO) a classical drug used in veterinary medicine; (2) the action of a chitosan-yeast-bacteria based product on neonatal diarrhea and mortality in goat kids. Cryptosporidiosis is considered as an economically important disease with clinical signs and death in young ruminants. The usual clinical symptom is acute diarrhea affecting animals from 1 to 3 weeks old. As no drugs are fully effective in the treatment of cryptosporidiosis in man and animals, the research for new therapeutic agents is crucial. Chitosan is a sugar that is obtained from the hard outer skeleton of shellfish, including crab and shrimp and it is used in medicine. It has been found to be active against a variety of diseases including antimicrobial and anti-tumoral effects. Immunofluorescence technique was used for the identification and enumeration of the parasites. The results showed a significant reduction of viability of Cryptosporidium oocysts (>95%) after pre-incubation of 24h at 37°C with PARO ($P < 0.001$), MIX and NAG ($P < 0.001$). Additionally, PARO, MIX and NAG inhibited significantly the development of *C. parvum* in HCT-8 and Caco-2 cell lines ($P < 0.005$). These effects were dose-dependent. Synergistic effects were obtained when NAG treatment was associated with Paromomycin. The efficacy of MIX in combination with yeast and bacteria (Optisaf FIRST, Phileo, France) was evaluated experimentally in goat neonates inoculated with *C. parvum* oocystes (10^6 oocytes/mL) per oral route. Preliminary results showed a significant reduction in oocyst shedding and diarrhea score in goat kids and mortality was significantly reduced (36%) in treated animals ($P < 0.05$) compared with the control group (90%). In conclusion, these findings provide evidence of in vitro inhibitory activities of chitosan against *C. parvum* and its combination with yeast-based products revealed promising in lessening the incidence of neonatal diarrhea in young ruminants.

Key Words: chitosan, yeast, goat