Clinical and Applied Science Research Presentations
Food Animal, Group B
Phi Zeta Research Day
March 10, 2015, 1:15-4:00pm
301 Trotter Hall

1:15 – 1:30   **Hailey Clemons** – Assessment of Ornithodoros Tick Parasitism: Lipocalin Proteins as Targets for Detecting Anti-tick Antibodies in Domestic and Feral Swine

1:30 – 1:45   **Mohammad M. Hossain** – Fluorescence Microsphere Immunoassay (FMIA) for the Detection of Envelope Glycoproteins E2 and E^{rns} Specific Antibodies in Bovine Viral Diarrhea Virus (BVDV) Infected Cattle

1:45 – 2:00   **Felicia Giok** – Antimicrobial resistance in probiotic preparations used in cattle

2:00 – 2:15   **L. Paulina Maldonado** – Characterization of *Salmonella enterica* Isolates from Feces of Feedlot Cattle Using Pulsed-Field Gel Electrophoresis

2:15 – 2:30   **Charley A. Cull** – Feedlot- and pen-level prevalence of Shiga toxin-producing *Escherichia coli* in feces of commercial feedlot cattle in two major cattle feeding states

2:30 – 2:45   **Break**

2:45 – 3:00   **Pragathi B. Shridhar** – Spiral plate- and real-time PCR-based quantification of non-O157 Shiga toxin-producing *E. coli* in cattle feces

3:00 – 3:15   **Jacob Hagenmaier** – The Effects of High-stress verses Low-stress Cattle Handling at the Time of Shipping to Slaughter on Physiological Responses in Cattle fed Ractopamine Hydrochloride

3:15 – 3:30   **Margaret Stephens** – A comparison of performance, carcass characteristics and meat quality from intact male beef cattle relative to castrated male beef cattle administered growth promotion technology

3:30 – 3:45   **Elsie Suhr** – Horn Growth during Feedlot Finishing Period

3:45 – 4:00   **Tiffany L. Lee** – Current Feedlot Cattle Health and Well-Being Program Recommendations in the United States and Canada: The 2014 Feedlot Veterinary Consultant Survey

5:00 – 6:00 pm **Reception and Awards Ceremony** Frick Auditorium and Foyer, 2nd Floor, Mosier Hall

- Initiation of New Members to Phi Zeta
- Announcement & Presentation of Awards Recognizing Research & Scholarship Accomplishments
- Closing Comments

Assessment of Ornithodoros Tick Parasitism: Lipocalin Proteins as Targets for Detecting Anti-tick Antibodies in Domestic and Feral Swine

Hailey Clemons

Author(s): Hailey Clemons & Dr. R. R. Rowland

Ornithodoros argasid (soft body) ticks are capable of transmitting a variety of infectious agents, including African swine fever virus (ASFV), East African human relapsing fever virus, blue tongue virus, epizootic bovine abortion virus, infectious bovine rhinotracheitis virus, and under some circumstances, West Nile virus. Experimental infections using several native North American *Ornithodoros* species have demonstrated the ability to become infected with and transmit ASFV. The purpose of this project is to use salivary lipocalin proteins as targets for detecting anti-tick antibodies for US ticks in both domestic and feral swine. It is predicted that tick feeding will largely reflect the geographical distribution of the tick species that serve as vectors. Lipocalin sequences from *Ornithodoros Coriaceus* and *Ornithodoros Parkeri* were selected based on the presence of the Biogenic Amine Binding motif (CDVX$_{7-17}$ EL [W/Y] X$_{3-30}$ C).The same motif is present in a South African species, *O. Moubata*. Nucleotide sequences for lipocalins were codon optimized, commercially synthesized, then cloned into pCR2.1 and pHUE plasmids. This resulted in a histidine tag addition to be used for affinity purification. Expression was then done in E. coli (BL21(DE3) competent cells). Purification was completed using a modified denaturing CAPS- Sarkosyl protocol with nickel columns. SDS-PAGE results show the proteins at the expected size of 27kDa. Incorporation into ELISA and Luminex multiplex serological assays is now in progress. In the future, work to acquire lipocalin sequences from other species that are present in North America will be done for use in both ELISA and Luminex platforms.

Fluorescence Microsphere Immunoassay (FMIA) for the Detection of Envelope Glycoproteins E2 and E^{rns} Specific Antibodies in Bovine Viral Diarrhea Virus (BVDV) Infected Cattle

Mohammad M. Hossain

Author(s): Mohammad M. Hossain[1,2], William C. Wilson[2], Izabela Ragan[1], Rachel Palinski[1], Nicholas A. Monday[1], Raymond R. R. Rowland[1]

Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506 USA[1]; USDA-ARS Arthropod-Borne Animal Disease Research Unit (ABADRU), Center for Grain and Animal Health Research, Manhattan, Kansas 66502 USA[2]

Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus*, of the family *Flaviviridae*. It is an infectious disease of cattle that can cause significant economic losses within the livestock industry throughout the world. BVDV is a positive strand enveloped RNA virus with a genome size approximately 12,500 nucleotides. E2 and E^{rns} are glycoproteins found on the surface of the virion, E2 is immunodominant in eliciting antibody response to virus infection whereas E^{rns} plays an important role in immune response. The majority of the viral epitopes related to virus neutralization are located on glycoprotein E2 (gp53). Detection and elimination of animals persistently infected (PI) with BVDV is essential for the control of bovine viral diarrhea (BVD) and eradication of BVDV. The purpose of this study was to develop a microsphere-based multiplex Luminex assay for the detection of antibodies specific to envelope glycoproteins E2 and E^{rns} in BVDV infected cattle. E2 and E^{rns} genes have been fragmented into 7 and 5 small pieces respectively and recombinant proteins expressed in *E. coli*. After successfully conjugation of affinity purified proteins to MAGPIX beads, the target antigens were assembled into a single multiplex and tested for antibodies in cattle infected with BVDV. The results demonstrate that all the protein fragments of E2 (8 including full length) and E^{rns} (6 including full length) were highly responsive to antibodies in BVDV infected cattle. However, the full length E2 and E^{rns} showed full potency of antibody response relative to the fragmented proteins. The results show the utility in FMIA as a multiplex platform for diagnosis of BVDV infection in cattle.

Antimicrobial resistance in probiotic preparations used in cattle.

Felicia Giok

Author(s): Felicia Giok, Deepti Pillai, <u>Sanjeev Narayanan</u>

Use of antimicrobials as feed additives in food animals has come under increasing scrutiny from the public and regulatory agencies. Probiotics are considered as valuable alternatives to antimicrobials in food animal nutrition. Probiotics are live organisms, when given orally, have shown to improve the gut microbial balance. However, studies in Europe report antimicrobial resistance (AMR) in probiotic organisms. This is of serious concern because of their potential to transfer resistance to pathogenic bacteria in the gut. The aim of the present study was to assess the AMR phenotypes of bacterial strains isolated from commercially available probiotics to 18 different antimicrobials. Two methods, disc diffusion and broth micro-dilution based MIC assay were performed to determine AMR. By disc diffusion assay, an *Enterococcus faecium* isolate showed resistance to erythromycin, kanamycin, metronidazole, rifampin and clindamycin. The MIC of erythromycin was >18ug/mL. A *Propionibacterium freudenreichii* isolate displayed no zone of inhibition for clindamycin, erythromycin, ceftriaxone, metronidazole, kanamycin and streptomycin. A *Lactobacillus acidophilus* isolate (from Probios) showed no inhibition zone for kanamycin, streptomycin, metronidazole and sulfamethoxazole/trimethoprim while another *L. acidophilus* isolate (from Bovamine) showed no inhibition zone for lower-level gentamicin, kanamycin, streptomycin, vancomycin and metronidazole. These studies show AMR is prevalent among probiotic bacteria used in cattle industry in the U.S. and justifies further characterization of such resistance.

Characterization of *Salmonella enterica* Isolates from Feces of Feedlot Cattle Using Pulsed-Field Gel Electrophoresis

L. Paulina Maldonado

Author(s): L. Paulina Maldonado, Xiaorong Shi, T.G. Nagaraja, David G. Renter, Natalia Cernicchiaro

Salmonella is one of the leading causes of foodborne illness in humans in North America and a significant food safety issue in the beef industry. Yet there are limited data on the distribution and variability of fecal shedding of this pathogen in commercial feedlots operations. A recent study conducted by our group evaluated the efficacy of an intervention for reducing fecal shedding of *Salmonella* at harvest. In this study, we found that fecal shedding of *Salmonella* was high among cattle (73.2%; 95% CI: 59.6 – 83.5%), and that prevalence within pens varied considerably (range: 24 to 96%). **Objective:** to genetically type *Salmonella enterica* strains isolated from feces of cattle, to further assess genetic relatedness among cattle pens using pulsed-field gel electrophoresis (PFGE). **Methods:** Out of 424 *Salmonella* fecal isolates obtained in the original study, 10 isolates per pen, were selected, from each of 12 unvaccinated pens, to subject to PFGE typing. Strains were analyzed by PFGE separation of genomic DNA digested by *Xba*1 in accordance with the CDC protocol. **Results:** Molecular relatedness of *Salmonella* strains and their distribution among feedlot pens will be discussed. These findings will help our understanding of the epidemiology and ecology of *Salmonella* within feedlot cattle.

Feedlot- and pen-level prevalence of Shiga toxin-producing *Escherichia coli* in feces of commercial feedlot cattle in two major cattle feeding states

Charley A. Cull

Author(s):   C.A. Cull, D.G. Renter, S.E. Ives, D.M. Dewsbury, L.W. Noll, P. Belagola Shridhar, T.G. Nagaraja, and N. Cernicchiaro

The objective of the study was to determine feedlot- and pen-level fecal prevalence of seven Shiga toxin-producing *Escherichia coli* (STEC) serogroups (O26, O45, O103, O111, O121, O145, and O157) and their associated virulence genes (*stx*1, *stx*2, and *eae*) in feces of feedlot cattle. Cattle pens from four commercial feedlots in each of two major cattle feeding states, Nebraska and Texas, were sampled. Up to 16 pen-floor fecal samples were collected from each of 4 to 6 pens per feedlot, per visit with three total visits per feedlot from June to August, 2014. Detection procedures included fecal enrichment in *E. coli* broth, immunomagnetic separation, and plating on selective medium, followed by PCR for confirmation of STEC serogroups and virulence genes. Overall, 100% of feedlots (n=8) and 73% of pens (n=126) had at least one fecal sample that tested positive for a STEC of interest. Crude cumulative feedlot-level prevalence estimates of STEC by serogroup were 25.0% (2/8) for O26, 25.0% (2/8) for O45, 100.0% (8/8) for O103, 25.0% (2/8) for O111, 0.0% (0/8) for O121, 62.5% (5/8) for O145 and 100.0% (8/8) for O157. Pen-level prevalence estimates of STEC were 2.4% (3/126) for O26, 3.2% (4/126) for O45, 18.3% (23/126) for O103, 1.6% (2/126) for O111, 0.0% (0/126) for O121, 5.6% (7/126) for O145 and 62.4% (78/125) for O157. These descriptive results provide insight on the feedlot- and pen-level fecal prevalence of STEC in pre-harvest commercial feedlot cattle and will be beneficial for quantitative microbial risk assessments.

Spiral plate- and real-time PCR-based quantification of non-O157 Shiga toxin-producing *E. coli* in cattle feces

Pragathi B. Shridhar

Author(s): Pragathi B. Shridhar, Lance Noll, Ellen Kim, Charley Cull, Diana Dewsbury, Xiaorong Shi, Natalia Cernicchiaro, David G. Renter, Jianfa Bai and T. G. Nagaraja

Insert abstract (250 words or less): Cattle are a major reservoir of six major 'non-O157' Shiga toxin-producing *E. coli* serogroups (O26, O45, O103, O111, O121, and O145) responsible for foodborne illnesses in humans. However, there is limited data on fecal concentration of these organisms in cattle because of lack of well-developed quantification methods. The objective of our study was to estimate the concentration of six non-O157 *E. coli* serogroups in cattle feces by spiral plating method and multiplex quantitative real-time PCR (qPCR). Cattle fecal samples (n=1,152), collected from eight commercial feedlots was suspended in *E. coli* broth. Diluted fecal suspensions were spiral plated onto a selective chromogenic medium and colonies were counted using a counting grid. Ten randomly picked chromogenic colonies were tested by PCR to identify the serogroup and virulence genes. DNA extracted from fecal suspensions were subjected to two qPCR assays targeting serogroup specific genes of O26, O103, O111 (assay 1) and O45, O121, O145 (assay 2). Of the 1,152 samples, 139 (17.7%; >log 2.9) and 493 (42.8%; > log 4) samples were quantifiable for one or more serogroups by spiral plating method and qPCR assays, respectively. *E. coli* O103 was the predominant serogroup quantified by spiral plating method (8.7%) and qPCR (25.5%). Only one sample was positive for Shiga toxigenic serogroup by spiral plating method. Real-time PCR quantified a higher number of samples compared to spiral plating method. However, the limitation of qPCR is that it does not distinguish between Shiga toxigenic and non-Shiga toxigenic *E. coli*.

The Effects of High-stress verses Low-stress Cattle Handling at the Time of Shipping to Slaughter on Physiological Responses in Cattle fed Ractopamine Hydrochloride

Jacob Hagenmaier

Author(s): Hagenmaier, J.A., S.J. Bartle, C.D. Reinhardt, M.J. Ritter, C.A. Guthrie, G.J. Vogel, D.U. Thomson

Feedlot steers fed ractopamine hydrochloride (n = 80; BW = 668 ± 36 kg) were used to measure the effects of handling at the time of shipping on physiological response and blood parameters. Cattle were randomly assigned to 1 of 2 treatments: 1) Low-stress handling (LSH; walked around a 1,600 m course) or 2) High-stress handling (HSH; ran around a 1,600 m course). Rectal temperature (RT), heart rate (HR), respiratory rate (RR) and blood samples were collected prior to handling (baseline) and post-handling. Blood samples were also collected at exsanguination at the abattoir. High stress handled cattle had higher HR than LSH cattle post-handling ($P = 0.01$); however, RR and RT did not differ. Blood pH, bicarbonate, and base excess were all decreased post-handling in the HSH cattle ($P < 0.0001$). Blood lactate was greater in the HSH cattle post-handling (15.1 vs. 5.2 ± 1.93 mmol/L, $P = < 0.0001$). High-stress handled cattle had greater post-handling levels of plasma epinephrine, norepinephrine, and cortisol ($P < 0.05$) than LSH cattle. However, no differences were observed in these hormones at exsanguination ($P > 0.05$). High-stress handled cattle had greater serum glucose post-handling (260 vs. 102 ± 10.3 mg/dL; $P < 0.0001$) than LSH cattle, however there was no difference at exsanguination. High-stress handling increased HR and stress hormones, as well as causing depletion of inspired oxygen leading to increased anaerobic glycolysis and acute acidosis. However, HSH cattle recovered and no differences were seen in blood parameters at exsanguination.

A comparison of performance, carcass characteristics and meat quality from intact male beef cattle relative to castrated male beef cattle administered growth promotion technology

Margaret Stephens

Author(s): Stephens, M.E., S.J. Bartle, D.N. Rethorst, C.D. Reinhardt, M.G. Siemens, and D.U. Thomson

Painful surgeries are raising animal welfare concerns. A portion of yearling bulls raised for breeding purposes fail the breeding soundness exam are routinely castrated. This study compared the performance, carcass characteristics and meat quality of intact male verses castrated male yearlings. Twenty four Angus bulls (605 ± 37 kg, age = 16 mo) were stratified by weight and randomly assigned to one of two treatments: uncastrated control (BULL) and castrated with growth promotion technology (STR). Cattle on the STR treatment were implanted with 120 mg trenbolone acetate and 24 mg estradiol, and fed ractopamine hydrochloride 300mg/d the last 28 days. Cattle were fed for 62 d (final wt = 680 +/-37 kg) then slaughtered. Cattle on the BULL treatment tended to have a higher ADG (1.40 vs. 1.05 kg; $P < 0.10$) and increased G:F (0.09 vs 0.07 kg; $P < 0.10$). The LMA was greater in BULL than STR cattle (100.1 cm^2 vs. 89.3 cm^2; $P < 0.05$). There was no difference between treatments for other carcass measures. Warner Bratzler shear force tenderness measures were similar for BULL and STR cattle (4.82 and 4.32 kg of force; $P < 0.05$). Sensory panel evaluation (1 to 8 scale, 8 = extremely desirable) showed no difference between treatments among the 6 categories; myofibrillar tenderness (5.24 vs 5.43), juiciness (5.18 vs 4.98), connective tissue (5.97 vs 6.26), beef flavor intensity, overall tenderness, and off flavor intensity. This study suggests that castration is an unnecessary procedure for bulls of this age.

Horn Growth during Feedlot Finishing Period

Elsie Suhr

Author(s): Elsie Suhr, Maggie Stephens, Dr. Steven Bartle, Dr. Dave Sjeklocha, Dr. Daniel Thomson

Horned cattle are often associated with human and cohort injury, therefore the current industry standard is to tip horns at the feedlot. Tipping is removing only a portion of the horn, but is often accompanied by pain issues. According to the National Beef Quality Audit, 2011, the prevalence of horns was 23.8%. The objective of this study was to determine horn growth during finishing phase in feedlot cattle. Measurements taken on heifers upon arrival to the feedlot and again at the abattoir included tip-to-tip, length of one horn, and base circumference of one horn. The cattle (n = 30) were on feed 194 d. Some horns were damaged during the feeding period and at the abattoir before measurements were taken. The average initial and final measurements for tip-to-tip length were 33.0 and 44.4 cm, length of one horn were 9.5 and 17.0 cm, and for base circumference were 13.1 and 16.2 cm, respectively. The average tip-to-tip length growth was 11.4 cm, the average length of one horn growth was 7.5 cm, and the average base circumference growth was 3.2 cm. Animals were categorized into 25, 50, 75, and 100 percentile groups by initial measurements and growth measurement averages were compared. Measurements for tip-to-tip growth were 12.6, 14.7, 13.7, and 10.8 cm, for one horn length growth were 8.1, 8.1, 6.9, and 7.1 cm, respectively. Initial measures were not indicative of growth during the feeding period. Horn growth during the feeding period should be considered during tipping decisions on incoming cattle.

Current Feedlot Cattle Health and Well-Being Program Recommendations in the United States and Canada: The 2014 Feedlot Veterinary Consultant Survey

Tiffany L. Lee, DVM

Author(s): Tiffany L. Lee, DVM; Shane P. Terrell, DVM; Steven. J. Bartle, PhD; Michael. D. Apley, DVM, PhD; and Daniel. U. Thomson, DVM, PhD

Feedlot consulting veterinarians (n = 23) representing over 15.6 million feeder cattle in the United States and Canada were invited to participate in a beef cattle health and well-being recommendation survey. The objective of the study was to survey consulting feedlot veterinarians on recommended practices for cattle health and well-being. The current survey tool was built on a previous survey (Terrell *et al.*, 2011), and was reviewed by Kansas State University and industry veterinarians before distribution. Veterinarians were directed to an online survey to answer 78 questions on feeder cattle husbandry, health, and preventative medicine recommendations. Response rate was 100%. The consulting veterinarians visited feedyards in their practice an average of 1.7 times per month. Ninety-six percent of veterinarians were involved in training pen riders. All veterinarians were familiar with the Beef Quality Assurance (BQA) Feedlot Assessment Tool, and 95% used BQA concepts in employee training. Participants recommended one pen rider per 3,464 high-risk calves, and one per 6,405 low-risk calves. All veterinarians recommended an IBR vaccine for both high-risk and low-risk cattle. Banding was the most commonly recommended method of castration in cattle over 500 pounds. Ancillary therapy for BRD was recommended by half of the participants, and Vitamin C was the product most commonly recommended for such therapy. Cattle health risk was considered the most important factor for predicting morbidity. This survey provides valuable information on the current recommendations of feedlot consulting veterinarians in the United States and Canada, and offers benchmarking data for veterinarians in the industry.