# ANIMAL HEALTH II: HOST-MICROBIAL INTERACTIONS: DETECTION AND INTERVENTION

0080 Alterations in the response of pigs to Salmonella typhimurium when provided Enterobacter cloacae. J. R. Donaldson\*1, J. A. Carroll², N. C. Burdick Sanchez², J. W. Dailey², T. B. Schmidt³, T. R. Callaway⁴, and J. G. Wilson¹, ¹Mississippi State University, Mississippi State, ²USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, ³University of Nebraska, Lincoln,

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Weanling pigs are at risk of succumbing to illness due to an immature immune system and insufficient supply of available energy at the time of weaning. Recent evidence has suggested that providing pigs with Enterobacter cloacae can increase the concentration of circulating triglycerides (TAG) and thus available energy. To determine if this increase in TAG improved the response of pigs to an infection, 36 weaned pigs 30 d of age (6.7  $\pm$  0.1 kg BW) were individually housed and randomly assigned to 3 treatment groups: 1) Enterobacter cloacae (JD6301; 1 × 10<sup>10</sup> CFU); 2) an alternate form of this bacterium (JD8715;  $1 \times 10^{10}$  CFU) that secretes TAG into the surrounding environment; or a control of PBS. For each treatment, bacteria were supplemented to the water daily using a medicator water system (~1 × 106 CFU/mL). Pigs were provided water supplemented with E. cloacae for 5 d before and 3 d after in relation to being challenged with lipopolysaccharide (LPS) from Escherichia coli (25 ug/kg BW, time 0 h) and Salmonella typhimurium (1 × 10<sup>9</sup> CFU, time 6 h). Serum samples were collected every 6 h for a period of 72 h and analyzed for NEFA, TAG, and whole blood cell counts. At 24, 48, and 72 h post-challenge, gastrointestinal contents were collected and analyzed for the presence of E. cloacae and S. typhimurium. Circulating TAG increased (P = 0.05) in pigs provided JD6301 in comparison with PBS controls within 5 d of supplementation but did not increase (P = 0.33) in pigs provided JD8715. Within 18 h post-challenge with LPS (and 12) h post-challenge with S. typhimurium), an increase in NEFA (P < 0.05) and TAG (P < 0.04) was observed in pigs provided PBS in comparison with pigs provided either form of E. cloacae. Pigs provided JD6301 had a reduction (P = 0.05)in S. typhimurium populations between 24 and 72 h post-challenge. However, S. typhimurium populations in pigs provided either JD8715 or PBS did not decrease (P = 0.18) during this time period. Pigs provided JD8715 did have an increase (P =0.05) in neutrophil concentrations within 6 h post-exposure to the endoxotin. These data suggest that the oleaginous bacteria JD6301 may improve clearance of S. typhimurium from the gastrointestinal tract. Further research is needed to determine

whether this decrease is due to an improved immune response or competitive inhibition.

Key Words: pigs, probiotics, triglycerides

0081 Adhesion of Escherichia coli in piglets and association of phenotypes to known candidate genes in South African breeds. N. S. Chaora\*, Agricultural Research Council, Pretoria, South Africa.

Enterotoxigenic Escherichia coli is a major pathogenic bacterium that causes diarrhea in preweaned and postweaned piglets. The adhesion of E. coli to the brush borders of the epithelial cells of piglets is a prerequisite for effective colonization leading to diarrhea. Successful adhesion occurs in the presence of E. coli receptors that are found on the brush borders of epithelial cells. The study's objective was to compare the susceptibility of South African breeds to enterotoxigenic E. coli strains. An in vitro adhesion experiment was performed for F4, PAA, and EAST-1 E. coli strains, using intestinal brush borders from 109 pigs of 3 South African pig breeds. Large White, indigenous, and crossbred pigs that were 3- to 12-wk old were used. The results showed significant differences (P <0.05) in adhesion frequencies of receptors among the 3 breeds. Adhesion phenotypes, adhesive, weakly adhesive, and non -adhesive were found in all breeds. The F4 and PAA strains adhered in all 3 breeds. The indigenous pigs had the highest frequency of non-adhesive intestines and > 70% of the Large White pigs were adhesive to all strains. Indigenous and crossbred pigs' adhesion was higher in suckling piglets than weaners. The TFRC, MUC13, MUC4, and MUC20 genotypes were not associated with adhesion phenotypes. The South African population studied carried receptors for all strains measured. If there is an outbreak of E. coli carrying the above strains, the South African population is most likely to be affected. The indigenous pigs of the South African population studied were more resistant to F4, PAA, and EAST-1 E. coli strains, compared with Large White and crossbred pigs.

**Key Words:** adhesion, *E. coli* strains, piglets, susceptibility

# 0082 Effect of metaphylaxis on production responses and antimicrobial usage in high-risk steers.

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A trial was conducted to determine the effects of on-arrival metaphylaxis in beef cattle for controlling bovine respiratory disease (BRD) and determining subsequent effects on health and performance. Male calves in a randomized complete block design (n = 198, arrival weight = 231 kg  $\pm$  2.43) received either 3.3 mL/100 kg (6.6 mg/kg) ceftiofur crystal-

line free acid (EXC), 4.4 mL/100 kg (13.2 mg/kg) tilmicosin phosphate (MIC), or were not treated (CON). These products are commonly used in production settings. Cattle receiving metaphylaxis had 25.2% lower morbidity rates than CON (P = 0.01; 51.5% vs. 76.7%). Significant differences were not observed in morbidity rates (P = 0.14) between cattle on the MIC  $(46.4\% \pm 4.32\%)$  or EXC treatments  $(56.5\% \pm 4.32\%)$ . Of cattle requiring BRD therapy, the CON group displayed symptoms  $\sim$ 5 d earlier than cattle in the metaphylaxis group (P = 0.01). Cattle displaying BRD symptoms in the MIC group required treatment 3 d earlier than those in the EXC group (P = 0.02, 8 vs. 11 d, respectively). Metaphylaxis improved ADG (1.63 vs. 1.28 kg/d; P = 0.06) and G:F (0.29 vs. 0.22, P = 0.01)during the first 14 d compared with CON, but differences between EXC and MIC were not significant (P > 0.40) during the first 14 d. Despite differences at 14 d, no differences were observed in ADG (P = 0.20) or G:F (P = 0.18) between CON and treatment groups across the 42-d trial. Total antimicrobial usage was 6.03 vs. 6.16 g active ingredient per animal for CON vs. metaphylaxis (P = 0.88), and 5.99 vs. 6.33 for MIC vs. EXC (P = 0.74). These results suggest that both tilmicosin phosphate and ceftiofur crystalline free acid effectively reduce overall morbidity and delay onset of clinical illness in newly received beef cattle. Furthermore, this reduction in overall morbidity was achieved with minimal increase in total antimicrobial use. While overall performance outcomes were not different, animal health was improved with metaphylaxis.

**Key Words:** bovine respiratory disease, metaphylaxis

# 0083 PR-39 ameliorates *Salmonella typhimurium*-induced intestinal epithelial barrier dysfunction.

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Salmonella enterica Serovar typhimurium infection is a primary enteric pathogenic disease affecting both human and animals. PR-39 is a porcine antimicrobial peptide that shows strong antibacterial effects toward Salmonella typhimurium in vitro and multiple immunomodulation functions. Here we investigated the potential mechanisms of PR-39 in preventing Salmonella typhimurium-induced gut barrier dysfunction in mouse infection model and in polarized intestinal porcine epithelial cell line IPEC-J2. The intestinal permeability, the expression of tight junction proteins, and biodistribution of PR-39 were determined by using chamber, immunofluorescence, qRT-PCR, and in vivo fluorescence imaging technology, respectively. One-way ANOVAs were performed using a 95% confidence interval. The results revealed that PR-39 attenuated the altered ileum architecture and reduced the increased intestinal permeability in Salmonella-infected mice. These protective effects were not attributed to the antibacterial activity of PR-39 because PR-39 showed no antimicrobial activity against Salmonella typhimurium in simulated intestinal liquids or serum. Moreover, pre-treatment with PR- 39 significantly reduced the adhesion and invasion of *Salmonella typhimurium* toward polarized IPEC-J2 monolayer, and attenuated the decreased ZO-1 and claudin-1 expression caused by *Salmonella* infection. Collectively, these findings provide evidence that PR-39 could prevent *Salmonella typhimurium*-induced intestinal epithelial barrier dysfunction through an antimicrobial-independent mechanism.

**Key Words:** intestinal epithelial barrier function, PR-39, *Salmonella typhimurium* 

#### 0084 Quantification of microbial populations in organic and inorganic dairy cattle bedding materials.

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The objective of this study was to quantify differences in microbial populations of 4 different bedding types used in dairy barns: 1) deep-bedded, new sand (NS), 2) deep-bedded, recycled sand (RS), 3) deep-bedded organic solids (DBOS), and 4) shallow-bedded organic solids on top of mattresses with foam cores (SBOS). Weekly composite bedding samples were systematically collected from selected locations within randomly selected stalls in each of 4 identical pens containing 32 freestalls and ~28 lactating cows during the 49-wk study period. Microbial populations were determined by plating 10μL inoculations of duplicate sets of serial dilutions (10-1 to 10<sup>-5</sup>) on 3 selective media. Bacterial groups were quantified as: Gram-negative (total growth on MacConkey's agar), coliforms (lactose-positive colonies on MacConkey's agar), Klebsiella (red to pink colonies on MacConkey inositol-carbenicillin agar), and Streptococci spp. (total growth on Edward's modified medium agar). The relationship between bacterial populations and bedding type was analyzed in a repeated measures model using PROC MIXED (SAS 9.3). The model included effects of bedding type with sampling date repeated.  $Y_i = \alpha + \beta_1 X_{i1} + \beta_2 X_{i2} + e_i$  where  $Y_i$  is the  $\log_{10}$  CFU count for bedding sample *i* from pen  $X_{i1}$  and date  $X_{i2}$ . Bacterial counts differed among bedding materials. Fewer bacteria were isolated from NS as compared with other bedding materials, with the exception of *Klebsiella* in SBOS and Streptococci spp. in DBOS. More bacteria were isolated from DBOS compared with other bedding materials, except for Streptococci spp. in sand bedding. Distribution of bacteria varied among bedding types. In general, NS had the fewest bacteria, whereas DBOS contained the most bacteria.

Key Words: bacteria, bedding, dairy

Table 0084. Bacteria isolated from different dairy cattle bedding types ( $Log_{10}CFU/g$ )

| Bedding<br>material | Gram-<br>negative | Coliform   | Klebsiella  | Streptococci spp. |
|---------------------|-------------------|------------|-------------|-------------------|
| NS                  | 4.72ª             | 3.59a      | 2.41a       | 6.88ª             |
| RS                  | 5.25 <sup>b</sup> | $4.10^{b}$ | $3.19^{b}$  | 7.21 <sup>b</sup> |
| SBOS                | 5.81°             | $4.08^{b}$ | $2.74^{ab}$ | 8.16 <sup>c</sup> |
| DBOS                | 6.83 <sup>d</sup> | 5.70°      | 5.05°       | $7.08^{ab}$       |

 $_{a,b,c,d}$ Values with different letters in the same column differ significantly ( $P \le 0.02$ ).

0085 Prevalence of bovine mastitis pathogens in bulk tank milk. Y.-L. Bi\*1, Z. J. Cao¹, W. Sun², Y. Qin², and S. L. Li¹, ¹State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, ²Laboratorios Hipra, Gerona, Spain.

Bovine mastitis is the most significant disease of dairy herds that can cause huge economic losses in the world. The objective of this study was to assess the bacteriological quality of bulk tank milk at herd level. Bulk tank milk samples collected from 894 dairy herds in Inner Mongolia (375), Heilongiang (242), and Hebei Province (277) of China were examined for the presence of mastitis pathogens from March 2012 to May 2013. Each sample was tested using a previously validated Multiplex PCR assay for the detection of 12 pathogens at a time. In addition, a maximum of 21 samples were examined simultaneously using the Multiplex PCR assay. Contagious pathogens, including Streptococcus agalactiae (90.16%), Coagulase negative staphylococci (89.17%), Streptococcus dysgalactiae (71.14%), Arcanobacterium pyogenes (60.51%), Staphylococcus aureus (44.85%), and environmental pathogens, consisting of Coliforms (63.26%) and Escherichia coli (31.66%), were detected in the milk samples. Of the 894 bulk tank milk samples, 743 (83.11%) contained 4 or more species of bacterial pathogens. It was also observed that an increase in the frequency of isolation of bacterial pathogens was significantly associated with an increased bulk tank bacterial and somatic cell counts. Bulk tank milk with lower bulk tank bacterial and somatic cell counts had fewer species of bacteria. Herd size and farm management practices had considerable influence on the species of bacteria and bacterial and somatic cell counts in bulk tank milk. The percentage of small herds (< 50 cows) with 4 or more types of bacterial pathogens detected in milk samples was higher than that of big herds ( > 500 cows), which was 86.45% and 71.79%, respectively. Of the bulk tank milk samples, 85.20% (501/588) had 4 or more types of bacterial pathogens in winter, whereas the percentage was 88.89% (272/306) in winter. There were no differences in the species of bacteria in bulk tank milk between summer and winter. In conclusion, species of bacteria and bacterial and somatic cell counts could serve as indicators of bulk tank milk quality and we should formulate strategies to improve milk quality and reduce the incidence of mastitis in dairy herds.

Key Words: bulk tank milk, bovine, mastitis pathogens

0086 Modulation of the acute phase response in feedlot steers supplemented with Saccharomyces cerevisiae. J. O. Buntyn\*1, N. C. Burdick Sanchez², J. A. Carroll², E. Chevaux³, K. Barling⁴, S. E. Sieren⁵, S. J. Jones⁵, and T. B. Schmidt⁵, ¹Department of Animal Science, University of Nebraska, Lincoln, ²USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, ³Lallemand Animal Nutrition, Milwaukee, WI, ⁴Lallemand Animal Nutrition, Iola, TX, ⁵University of Nebraska, Lincoln.

This study was designed to determine the effect of supplementing feedlot steers with Saccharomyces cerevisiae CNCM I-1079 (SC) on the acute phase response to a lipopolysaccharide (LPS) challenge. Steers (n = 18; 266 ± 4 kg BW) were separated into 3 treatment groups (n = 6/treatment). One group was fed a standard receiving diet (Control, Cont); 1 group was fed the standard receiving diet supplemented with SC (Lallemand, Inc.) at 0.5 g/steer/d (SC-0.5), and the final group was fed the standard receiving diet supplemented with SC at 5.0 g per steer day (SC-5.0) for 29 d. On d 27, steers were fitted with indwelling jugular cannulas and rectal temperature (RT) probes that measured RT continuously at 5-min intervals, and were placed in individual stalls. On d 28, steers were challenged IV with LPS (0.5 µg/kg BW at 0 h) and blood samples were collected at 30-min intervals from -2 to 8 h and 24-h post-challenge. Serum was isolated and stored at -80°C until analyzed for cortisol and cytokine concentrations. Before the challenge, there was an effect of treatment (P < 0.001) on RT; SC-0.5 steers  $(39.50 \pm 0.03$ °C) had greater RT than Cont (39.06  $\pm$  0.04°C) and SC-5.0 (39.27  $\pm$  0.04°C) steers. Also, Cont steers had greater (P < 0.001) RT than SC-5.0 steers. Therefore, RT was further analyzed as the change from baseline. In response to LPS challenge, the change in RT was affected by treatment (P < 0.001); Cont steers had the greatest change in RT  $(0.434 \pm 0.0510^{\circ}\text{C})$  compared with SC-0.5 (-0.059  $\pm$  0.039°C) and SC-5.0 (-0.007  $\pm$  0.045°C) steers. There was a tendency (P = 0.06) for baseline cortisol concentrations to be affected by treatment; SC-5.0 steers having greater (7.8  $\pm$  0.8 ng/mL) cortisol than Cont (4.9  $\pm$  0.8 ng/mL) steers. Post-LPS challenge, there was a treatment × time interaction (P = 0.005); SC-5.0 steers had decreased (P< 0.02) cortisol concentrations than Cont steers from 4.5 to 7 h post-challenge. There was a treatment effect (P < 0.05) for all cytokines (tumor necrosis factor-α, interleukin-6, and interferon-γ). Cytokines were decreased in SC-0.5 and SC-5.0 steers compared with Cont steers following LPS challenge. While these data demonstrate that Saccharomyces cerevisiae supplementation can reduce the inflammatory response to a LPS challenge, further research is needed to determine whether or not *Saccharomyces cerevisiae* supplementation is beneficial when animals are exposed to a live pathogen.

Key Words: cattle, immune response, live yeast

0087 Performance evaluation of calves with diarrhea in different systems supplemented with yeast culture plus enzymatically hydrolyzed yeast cell wall. V. R. Rabassa\*1, B. Scherer¹, F. B. Del Pino², C. C. Brauner², F. M. Gonçalves¹, R. F. S. Raimondo¹, E. G. Xavier³, C. C. Elrod⁴, and M. Nunes Corrêa², ¹Universidade Federal de Pelotas, Pelotas, Brazil, ²Federal University of Pelotas, Brazil, ³Granjas 4 Irmãos, Rio Grande, Brazil, ⁴Vi-COR, Inc., Mason City, IA.

The aim of this study was to evaluate the performance of calves with diarrhea in different systems supplemented with yeast culture plus enzymatically hydrolyzed yeast cell wall during the neonatal period. Seventy-eight female Holstein calves were divided into 2 groups: yeast group (Celmanax, Vi-COR, Mason City, IA), n = 38, which were supplemented with 8mL/d during the first 42 d of age and a control group, n = 40, which received no supplementation. The yeast was administered daily as an oral drench and measurements of weight, heart girth, wither height, rump width, and ADG were performed weekly. The animals were also divided into 2 systems based on the types of facilities: houses (confined) and stakes (outdoor), and monitored daily for the occurrence of diarrhea. Data were analyzed by MIXED PROCEDURES of SAS. There was no difference (P > 0.05) in calf performance between groups and group × collection. However, there was an interaction (P < 0.05) between groups and system. Control calves maintained in the stakes system had greater (P < 0.05) heart girth than the yeast group  $(83.69 \pm 0.58 \text{ cm})$  and 81.65 cm $\pm$  0.68 cm, respectively) and higher (P < 0.05) ADG (0.44  $\pm$ 0.02 kg and  $0.33 \pm 0.03 \text{ kg}$ , respectively). Calves from the yeast group kept in houses had higher averages (P < 0.05)than the control group in the parameters wither height (81.43  $\pm$  0.64 cm and 79.61  $\pm$  0.54 cm), rump width (29.44  $\pm$  0.32 cm and  $28.23 \pm 0.29$  cm), and ADG  $(0.32 \pm 0.04 \text{ kg})$  and 0.21 $\pm$  0.02 kg), respectively. In conclusion, supplementation with yeast culture plus enzymatically hydrolyzed yeast cell wall can improve the growth performance of animals showing diarrhea housed in confined environments.

Key Words: dairy, health, system

## 0088 Variations in the survival of *Listeria monocytogenes* to grow in bile from porcine gallbladders.

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*Listeria monocytogenes* is a facultative intracellular, Gram-positive bacterium that can cause disease, including abortion, in sheep, goats, cattle, pigs, and poultry. These animals are also

known reservoirs for this pathogen and it is primarily acquired through ingestion of contaminated silage or soil. This bacterium's ability to survive within the gastrointestinal tract and cross the intestinal lining is directly related to the ability of the pathogen to cause disease. However, it is not clear whether there are variations in the ability of this pathogen to survive. The purpose of this study was to determine whether variations exist in the ability of different serotypes of L. monocytogenes to survive within porcine gallbladder bile and if survival affected the ability of these bacteria to invade epithelial cells in vitro. Avirulent strain HCC23 (serotype 4a) and virulent strain 10403S (serotype 1/2a) were cultured in brain-heart-infusion (BHI) broth media to mid-logarithmic ( $OD_{600} = 0.5$ ), then transferred to bile collected from pigs 30 d of age (6.7  $\pm$  0.1 kg BW). Anaerobic growth was monitored by viable plate counts on BHI agar. The influence of bile on invasion potential of L. monocytogenes was tested in colon epithelial cells (GPC-16) cultured in Eagle's Minimal Essential Media with 20% fetal bovine serum. Cell culture media was supplemented with 10% bile and HCC23 or 10403S was inoculated at a multiplicity of infection of 100:1. Cultures were incubated at 37°C, 5% CO2. At 1 h and 2 h post infection, cells were washed to remove extracellular bacteria then lysed to release intracellular bacteria. Lysates were serially diluted and plated onto BHI agar. Survival of HCC23 in bile did not change (P = 0.3), though a decrease (P = 0.05) in survival of 10403S was observed 24 h after bile exposure. Virulent strain 10403S also had a decrease (P = 0.05) in invasion potential in the presence of bile. In contrast, avirulent strain HCC23 did not decrease (P = 0.08) in invasion potential in the presence of bile. Together with previous reports of variations in the intracellular presence of L. monocytogenes in the gallbladder, these results suggest that avirulent and virulent strains respond differently to the gastrointestinal environment and this difference may influence the outcome of the infectious process.

Key Words: gallbladders, Listeria monocytogenes, pig

## 0089 Yeast probiotics vary in their potential to bind to Gram-positive or Gram-negative bacteria.

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Probiotics are widely used in the livestock industry to improve health and overall productivity. Despite the extensive amount of research performed on examining the mechanisms by which probiotics confer positive effects on hosts, their use is still highly debated and are undoubtedly under characterized. The hypothesis for this study was that variations exist in the binding potential of probiotics to Gram-negative and Gram-positive bacteria. To test this hypothesis, the binding capability of 5 different types of live yeasts or yeast cell wall products to 2 Gram-negative bacteria (*Salmonella typhimu*-

rium and Escherichia coli O157:H7) and 3 Gram-positive bacteria (Listeria monocytogenes, Clostridium perfringens, and Bifidobacterium bifidum) was determined using an adhesion assay and subsequent analysis by scanning electron microscope. The probiotics were incubated on cover slips and then challenged with the array of different pathogens. To assess each probiotic's propensity toward adhesion, extensive washings of the cover slips were done. This removal of unbound bacteria determined that any bacteria remaining resulted from direct interactions between the pathogens and probiotics, inferring antagonistic behavior of adhesion displayed by the probiotics. Though S. typhimurium bound well to all probiotics tested (> 90%, P = 0.3), E. coli O157:H7 had a preference (P = 0.003) to bind to the yeast cell wall products in comparison to live yeast. The opposite was observed for the Gram-positive bacteria tested, which exhibited an improved binding potential to live yeast (P = 0.01). A sample size of > 20 yeast cells or cell wall products was analyzed. These data suggest that combining both live yeast probiotic and yeast cell product probiotic will allow for an increase in binding to different enteric bacteria. To test the binding capability of this combination mixture to colon epithelial cells, another adhesion assay was performed and prepared for analysis by fluorescent microscope (1 fluorochrome-labeling epithelial DNA and another staining yeast actin filaments). A sample size of > 30 epithelial cells was analyzed. The data determined that no variations were observed in the ability of these probiotics to bind to epithelial cells, indicating that the antagonist activity observed is specific to the pathogen tested and not a general defect of the product. Together, these data suggest that mechanisms of action of the yeast-based probiotics are dictated by variations of direct adhesion to pathogens. Further research is warranted to determine how these variations in binding potential influence the activity of these yeast-based probiotics in vivo.

Key Words: bacteria, probiotics, yeast

0090 An analysis of Giardia lamblia and Cryptosporidium parvum in bucket calves at the University of Findlay's animal science barn. S. M. Waibel\*, F. D. McCarthy, R. M. Wood, and B. Henderson-Dean, The University of Findlay, OH.

Giardia lamblia and Cryptosporidium parvum are protozoal parasites that can cause gastroenteritis in dairy calves and are zoonotic diseases that cause intestinal enteritis in humans. The initial objective of this study was to determine the presence

of G. lamblia or C. parvum, and if environmental stressors promote shedding of G. lamblia cysts or C. parvum oocysts in male dairy calves (n = 35). The environmental stressors considered were arrival to the facility, transfer from isolation to the main barn, and processing (castration, dehorning, vaccination). Fecal samples were analyzed using rapid immunochromatographic assay. Data analysis of Group 1 (n = 17) suggested environmental stressors failed to influence shedding and isolation may not be effective at preventing the spread of disease. Subsequently, the objective of the study on Group 2 (n = 18) was to determine the effectiveness of isolation and if the same environmental stressors promote shedding of G. lamblia cysts or C. parvum oocysts. For both groups, fecal samples were collected within 24 h of arrival (IN), 24 h before isolation removal (BIR), 36 to 60 h after isolation removal (AIR), 24 h before processing (BP), and 36 to 60 h after processing (AP). Group 2 had additional fecal samples collected at the end of wk 1 (W1) and 2 (W2) in isolation. Results indicated there was no degree of significance between environmental stressors and shedding of G. lamblia or C. parvum. Isolation appeared ineffective at preventing G. lamblia or C. parvum from spreading. Results did not detect G. lamblia or C. parvum in Group 1 calves at IN. However, at BIR, calves showed an increase in measured incidence of 27%. Fecal samples from calves in Group 2 tested positive for G. lamblia or C. parvum at IN of 22% and BIR of 56%. The average for both groups was 12% at IN and 42% at BIR. Overall, 11% of calves were positive for G. lamblia and C. parvum simultaneously; 31% tested positive for C. parvum, 72% tested positive for G. lamblia, and 86% tested positive for either parasite at least once. Given the potential for infection, increased hygiene measures are recommended. The isolation procedure should be examined for plausible breeches. Potential source of infection for calves needs to be investigated.

Key Words: Cryptosporidium parvum, Giardia lamblia