# Research Summaries 1

## Duodenal Obstruction Caused by Volvulus of the Duodenal Sigmoid Flexure in Dairy Cattle

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### Introduction

Duodenal obstructions are uncommon in cattle. Recently, a new condition clinically resembling abomasal volvulus (AV) but affecting the duodenal sigmoid flexure (SF) has been recognized in dairy cattle. The purpose of this retrospective study was to characterize this condition and determine the prognosis of cattle undergoing surgery. We hypothesized that values obtained from bloodwork would be of diagnostic value.

### Materials and Methods

From December 2006 to March 2010, 25 medical records from the teaching hospital and ambulatory service were analyzed: physical examination, bloodwork, surgical findings, and patient survival. "Survival" was defined as discharge from the teaching hospital or remaining in the herd. Descriptive statistics and a student *t*-test were performed to compare biochemical parameters with cattle diagnosed with AV in our clinic during the same time period. A Fisher's exact test was used to compare the prognosis between the cattle treated at the farm or hospitalized at a teaching hospital. A *P*-value <0.05 was considered significant.

### Results

A total of 24 Holstein cows and one Holstein bull were examined because of anorexia, signs of colic, and absence of feces. On physical exam the cattle were normothermic, tachycardic, and moderately dehydrated with scant feces present in rectum. A cranial, right-sided ping with or without succussion, located dorsally at the level of intercostal spaces 10-12, was noted for 20 animals. Biochemical changes were hypokalemia, hypochloremia, and metabolic alkalosis in conjunction with hyperlactatemia and hyperglycemia. Those results, which showed more severe changes, except for the hyperglycemia, were statistically different than the results obtained from AV cattle. Changes indicating cholestasis and hepatocellular necrosis were also noted: elevated bilirubin, gamma-glutamyl transferase, and glutamate dehydrogenase. Except for the increased bilirubin, those results were not statistically different than the results obtained from AV cattle.

Of a total 25 surgeries, 16 were performed at a referral center and nine were done on the farm. Seventeen cattle had previously undergone omentopexy one day to two years before surgery to correct duodenal sigmoid flexure volvulus (DSFV). Surgical findings included: empty descending duodenum alongside a dorsally displaced, dilated cranial duodenum; distended abomasum and gall bladder; and occluding, counterclockwise DSFV. Manual reduction of DSFV was considered successful if the descending duodenum filled after cranial duodenal massage. No attempts were made at attaching the SF. All except seven surgeries were completed without performing an omentopexy or other type of abomasal fixation.

All animals received intravenous crystalloid fluids and antibiotics, while 19 received non-steroidal anti-inflammatory drug (NSAID) therapy. Eighteen of the animals were successfully managed. On necropsy of the remaining seven cows, severe duodenal edema and peritonitis implicating the bile duct were found. These animals all had evidence of duodenal necrosis or focal peritonitis at surgery. For the 18 animals that survived, 13 were hospitalized and five were treated at the farm. There was no statistical difference between hospitalized and on-farm management.

### Significance

As the duodenum courses cranially from the pylorus, it forms the SF prior to turning caudally and becoming the descending duodenum. During DSFV, the SF is displaced dorsally in a counterclockwise direction (from behind) prior to creating a volvulus in a counterclockwise direction (from top).

The prognosis of cattle with DSFV is fair whether the case is hospitalized or managed on the farm. However, it is important to realize that cattle treated on the farm need aggressive fluid therapy to recover from this condition. The fact that the duodenum is not pexyed does not seem to favor recurrence. Clinical signs of duodenal and/or abomasal obstruction with severe metabolic derangements and marked elevations in hepatic values suggest DSVF, and immediate surgery is indicated. Veterinarians must be aware of this emergent syndrome in cattle.

# Lipid Mobilization in Transition Dairy Cows Alters Endothelial Inflammatory Response

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## Introduction

A hallmark of the transition period in dairy cows is intense lipid mobilization. This mechanism of adaptation is necessary to fulfill the energy deficits experienced by cows in late gestation and early lactation. Lipid mobilization is a dynamic process involving lipolysis and lipogenesis. During the transition period, the rate of lipolysis surpasses that of lipogenesis, inducing the release of non-esterified fatty acids (NEFA) into the blood stream. As a consequence, increased NEFA concentrations disrupt systemic lipid homeostasis not only quantitatively, but also relative to composition. Previous research demonstrated that enhanced lipolysis during the transition period altered the fatty acid composition of different organs and cell populations including blood, liver, adipose tissue, and peripheral blood mononuclear cells. A common change was the increment in the concentrations of saturated fatty acids (palmitic and stearic). Although increments in plasma NEFA are linked to transition cow diseases such as ketosis and displaced abomasum, less is known about the consequences of shifts in fatty acid profiles of endothelial cells and how these changes contribute to dairy cows increased susceptibility to disease. Immune responses are highly dependent on the interactions between immune cells with the vasculature. Indeed, endothelial cells regulate the trafficking of the immune cell migration to and from tissues. We hypothesize that shifts in plasma NEFA content and composition in transition dairy cows modify endothelial cells inflammatory response by enhancing lipid mediator biosynthesis and expression of adhesion molecules.

## Materials and Methods

Bovine aortic endothelial cells (BAEC) were cultured for four and 24 hours with 0, 0.25, 0.5, and 0.75 mM concentrations of NEFA mixture complexed to bovine serum albumin. The NEFA mixture included myristic (3%), palmitic (30%), stearic acid (45%), oleic (16%), linoleic (5%), and DHA (1%) fatty acids. This complex reflected plasma NEFA fraction during the first week of lactation. To evaluate shifts in the fatty acid profile of BAEC, phospholipids were extracted and analyzed using gas chromatography. BAEC inflammatory responses were assessed at both the gene and protein level. BAEC RNA was extracted for q-PCR of IL-6, IL-8, ICAM1, TLR4, COX-1, COX-2, and 15-LOX1. Protein quantification was performed by Western blot analysis in whole cell lysates using antibodies specific to COX-1, COX-2, TLR4, and ICAM1.

## Results

Addition of NEFA-albumin complex to culture media altered the fatty acid profile of BAEC by increasing the concentration of saturated fatty acids (stearic and palmitic) in the phospholipid fraction. Changes in the NEFA content in cultured media induced a significant time and concentration dependent increase of mRNA expression of COX-2, IL-6, IL8, ICAM1, and TLR-4. Changes in gene expression were directly reflected in protein expression by a significant time and concentration dependent increment in the biosynthesis of COX-2. The expression of 15-LOX1, a major enzyme in eicosanoid biosynthesis, also increased significantly with time.

## Significance

The transition period is characterized by abrupt changes in lipid homeostasis that could enhance dairy cows' susceptibility to disease. This research demonstrated for the first time that in dairy cows, lipid mobilization augments the expression of pro-inflammatory interleukins and adhesion molecules in endothelial cells. Furthermore, quantitative and compositional alterations of plasma NEFA modify the components of inflammatory lipid mediator biosynthetic pathways at the substrate level by changing phospholipid fatty acid profile and at the enzyme level by enhancing the expression of COX-2 and 15-LOX1. Understanding the dynamics of lipid mobilization during the transition period could lead to novel nutraceutical or pharmacological interventions that modulate inflammatory responses, potentially improving the health of dairy cows during the transition period.

# Comparison between Individual and Pooled Samples of Non-esterified Fatty Acids (NEFA) and B-hydroxybutyrate (BHBA) in Transition Dairy Cows to Determine Herd Alarm Level Status

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## Introduction

As energy demands surpass dry matter intake during the transition period, elevated non-esterified fatty acids (NEFA) and B-hydroxybutyrate (BHBA) concentrations can be used as markers of the extent of negative energy balance both at the individual cow and herd level. Previous studies have demonstrated that there is a threshold above which elevated NEFA and BHBA concentrations are associated with increased risk of disease and decreased reproductive and milk production performance in individual cows. Recently, the herd alarm level, i.e., the proportion of sampled animals with metabolite concentrations above the threshold which was associated with detrimental herd-level outcomes, was determined. The objective of this study was to compare the interpretation of the proportion of individual samples above the metabolite threshold versus pooled samples to evaluate the herd alarm level status.

## Materials and Methods

Herds from a prospective cohort study which evaluated NEFA and BHBA as predictors of clinical disease were randomly selected to participate in this study. For inclusion, each herd required a minimum of 12 samples per cohort. Within each herd, two cohorts of cows were simultaneously evaluated, those 14 to three days prepartum and those three to 14 days postpartum. The metabolite NEFA was measured in cows sampled prepartum and both NEFA and BHBA were measured from cows sampled postpartum. For each herd, the metabolites were measured in 12 individual cow serum samples and in a pooled sample, derived from equal volumes (200 µL) of each serum sample. Based on our previous studies, herds were defined as being above the herd alarm level if the proportion of animals with metabolite concentration above the threshold was more than 15% ($\geq$ 2 animals from 12). The metabolite thresholds were 0.3 mEq/L for prepartum NEFA, 0.6 mEq/L for postpartum NEFA, and 12 mg/dL for BHBA. To assess whether a herd was above the herd alarm level, and therefore at increased risk for detrimental downstream outcomes, the proportion of sampled animals above the threshold and the pooled concentration were compared. Two methods were used for this comparison: 1) the proportion of animals above the threshold was used as the reference test, and the sensitivity and specificity of the pooled concentration was evaluated; 2) the Kappa statistic was evaluated to test the agreement beyond chance between the proportion and the pooled concentration. All analyses were performed using SAS v. 9.1 (SAS Inst., Inc., Cary, NC).

## Results

Sensitivity of the pooled metabolite concentration as a single test was 58% (95% Confidence Intervals: 28 to 85) for prepartum NEFA; 55% (95% CI 24 to 83) for postpartum NEFA; and 36% (95% CI 11 to 69) for BHBA. Specificity of the pooled sample was 100% (95% CI: 17 to 100, 19 to 100, and 19 to 100) for prepartum NEFA, postpartum NEFA and BHBA, respectively. The Kappa statistics were: 0.2 (95% CI: -0.1 to 0.5), 0.3 (95% CI: -0.07 to 0.6), and 0.2 (95% CI: -0.07 to 0.4) for prepartum NEFA, postpartum NEFA, and BHBA, respectively. The McNemar's *P*-values for the Kappa statistics were 0.02, 0.02, and 0.008, respectively.

## Significance

These data demonstrate that pooled samples have low sensitivity and there is only poor to fair agreement between this and the proportion of animals above the threshold test. Although pooled samples may seem desirable due to lower laboratory cost, the low sensitivity and lack of agreement between pooled samples and the proportion can lead to an increased number of false negatives. An increased number of false negatives may result in missed opportunities for herds looking to improve transition cow negative energy balance.

# Effect of Paste or Wrap Oxytetracycline Treatment on Papillomatous Digital Dermatitis

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## Introduction

Papillomatous digital dermatitis (PDD) is a contagious and painful foot lesion causing lameness in cattle. One of the common treatments for PDD is oxytetracycline powder held onto the hoof with a bandage wrap. The objective of this study was to determine if application of oxytetracycline in a topical paste without bandaging would be as effective as the wrap for the treatment of PDD.

## Materials and Methods

Mature lactating Holstein cows diagnosed with PDD during routine trimming were randomly assigned to one of three treatments: oxytetracycline in a paste, oxytetracycline powder under a wrap, or a negative control. The paste treatment consisted of oxytetracycline 1000 mixed with glycol and vinegar to aid in adhesion, while the wrap treatment consisted of an equivalent amount of oxytetracycline 1000 powder held against the lesion with a wrap, which was removed three days post-treatment. Examination of the affected hooves was carried out at day 0 (Exam=1); days 3-7 post-treatment (Exam=2); and days 8-12 post-treatment (Exam=3). Data were analyzed using a generalized linear model with a binary outcome (lesion active or lesion healed). Lesions were considered active if the cow reacted to pressure from an algometer and tissue was still pink and/or inflamed, and $P$-values $<0.05$ were considered significant.

## Results

A total of 173 cows have been enrolled in the trial. Of these, 46 and 54 cows were re-examined at days 3-7 and 8-12 post-treatment, respectively. Both *exam* and *treatment* were significant ($P<0.05$) in the final model. Based on exams at days 8-12, 0%, 47.4%, and 57.1% of lesions were recorded as healed for no treatment, paste, and wrap, respectively. Cows receiving the paste treatment had 7.4 (1.49,37.03) times greater odds of recovering from digital dermatitis over the study period than the no-treatment cows ($P=0.01$). Similarly, cows receiving the wrap treatment had 16.6 (3.25,84.60) times greater odds ($P=0.0007$) of recovering from digital dermatitis over the study period than cows receiving no treatment. There was no statistically significant difference between the paste and wrap treatments ($P=0.13$). There was also no significant difference in reoccurrence rates for the three treatments in the three months following treatment ($P>0.05$), with rates of 38.9%, 50.0%, and 55.9% for no treatment, paste, and wrap, respectively.

## Significance

Oxytetracycline is effective for the treatment of PDD, and the use of it in a paste form rather than a powder alone could eliminate the need for bandage application and subsequent removal. From an industry perspective, this is useful as it eliminates the need of the producer to remove a bandage three to five days post-treatment, and could make treating PDD less labor intensive.

# The Effect of Postparturient Uterine Douching on the Risk for Clinical Metritis in Lactating Holstein Dairy Cows

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## Introduction

Clinical metritis in the lactating dairy cow leads to decreased reproductive efficiency, decreased milk production, and predisposes the cow to other metabolic diseases. Postparturient uterine douching was historically used in humans as a prophylaxis and treatment for postpartum uterine disease. Some producers and veterinarians have advocated the use of postpartum uterine douching in dairy cows to help prevent metritis and improve uterine health, although there is little scientific evidence for its efficacy. Metritis represents a serious concern for modern dairy cattle, with a median lactational incidence of approximately 10%, and many herds have an incidence of 20–30%.[1] The average cost of a case of metritis was estimated by Overton using stochastic simulation to be approximately $350.[2,3] This represents tremendous potential in lost income to the dairy due to a greater risk of culling, loss of milk production, decreased reproductive efficiency, and increased treatment costs. The objective of this study was to determine whether cows that undergo a uterine douche shortly after calving are at decreased risk for clinical metritis.

## Materials and Methods

The study was conducted on a single 12,000 cow dairy in Idaho. The sample size was calculated based on detecting an improvement in first service conception risk of six percentage points or a six percentage point difference in risk of experiencing metritis, while allowing for a 12% loss to follow-up. Immediately following parturition, 3,050 cows were randomized via computer generated random numbers into one of two groups. Group 1 (Douche) received the standard fresh cow processing treatments and a uterine douche consisting of 2 L of hypertonic saline mixed with 30 mL of 10% povidone iodine delivered per vagina with an oral calf feeder. Group 2 (No Douche) received the standard fresh cow processing treatments but no uterine douche. Following processing, cows were managed as per ordinary farm routine. A trial code was created in the on-farm record system to facilitate tracking of the study animals.

## Results

A total of 1,903 cows and 1,106 heifers were included in the final analysis. Forty-one cows with a diagnosis of abortion were excluded. Clinical metritis (CM) was diagnosed by on-farm personnel in 30.4%, 5.4%, and 7.3% of lactation 1, lactation 2, and lactation 3+ cows, respectively. Retained placenta, dystocia, and twins were all significantly associated ($P < 0.05$) with an increased risk of metritis, but postpartum uterine douching had no effect on metritis risk. The incidence of metritis was 15.1% in both the treatment and control groups ($P = 0.95$).

## Significance

Reducing the risk of acute puerperal metritis by six percentage points would represent an overall savings of at least $10 per cow in the lactating herd and provide at least a 3:1 return on investment when compared to uterine douching.[2,3] This level of risk reduction was the basis for our sample size calculation, but we found no evidence of a benefit to prophylactic or metaphylactic douching with 30 mL of 10% povidone iodine solution mixed with 2 L of hypertonic saline in this study population. However, given the large negative impact of metritis on cow health, performance, and profitability, additional management approaches including nutritional interventions, improved housing and cow comfort, accurate diagnoses and treatment, and other pharmaceutical interventions warrant further clinical investigation.

## References

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# Effect of Nutritional Plane on Health, Performance, and Muscle Metabolism in Neonatal Dairy Calves

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## Introduction

Neonatal dairy calf maintenance energy requirements are 1.75 Mcal per day at thermoneutral temperatures. Conventional milk replacer feeding programs (e.g. 2 quarts of reconstituted solids twice per day) provide approximately 2.2 Mcal per day. Considering the abundance of environmental and pathogenic challenges faced by neonatal calves, these conventional programs provide little energy for maintaining body temperature, mounting immune responses, and growing at expected rates of 1 to 2 pounds per day and weight loss often occurs for the initial week of life. The objective of this study was to evaluate the effect of conventional nutritional plane versus a higher nutritional plane on the health status, muscle development, and initial weight loss in neonatal dairy calves.

## Materials and Methods

A randomized, controlled, and double-blinded trial was performed using Holstein calves obtained from a large commercial dairy. All births were attended by study personnel, calves fed 4L of heat-treated colostrum within one hour of birth, and then transported to individual stalls within an isolation facility. Calves were randomly assigned to a higher plane of nutrition (HPN) or conventional nutrition (CN) group and maintained for a 28-feeding (14-day) study period. Twenty-three calves were enrolled, with three lost to follow up. HPN was defined as 0.30 Mcal per kilogram of metabolic body weight (MBW) as a function of birth weight using a 28% protein, 20% fat milk replacer (n=10). CN was defined as 0.13 Mcal per kilogram MBW using a 20% protein, 20% fat milk replacer (n=10). Fecal and health scores, weight gain, dry matter intake, packed cell volume (PCV), and total protein (TP) were measured throughout the study period. Serial percutaneous muscle biopsies were performed and samples were evaluated for Atrogin-1 as a specific marker for skeletal muscle metabolism. Data were analyzed by non-parametric methods.

## Results

Initial body weight, hydration status, and passive transfer of antibodies were not different between treatment groups ($P>0.4$). Packed cell volume decreased significantly within both treatment groups between day 1 and day 2 ($P<0.05$) but did not change over time past day 2 ($P>0.3$). The number of feedings associated with severe diarrhea (FS=3) were not different between HPN and CN groups (median 5.5 feedings v. median 4.5 feedings, respectively; $P=0.36$). The HPN calves had a 3.5 times higher risk of not finishing a meal compared to the CN calves (95% CI: 1.1-11; $P=0.07$). A median of 3.3 lb (1.5 kg) of weight loss developed in the CN calves ($P=0.005$) while 2.75 lb (1.25 kg) of weight gain developed within the HPN calves ($P=0.008$) during the first week of life. HPN calves had better average daily gain than CN calves (median 445g/d v. -60g/d, respectively, $P<0.0001$). Feed efficiency (average daily gain: dry matter intake) was better for the HPN calves than the CN calves (median 524g/kg v. -139g/kg, respectively) ($P<0.0001$). Atrogin-1 concentration was higher in HPN calves than CN calves, although significance was not established (median 2.2 v. 1.1; $P=0.13$).

## Significance

Despite an increased risk of not finishing a meal, calves fed the HPN diet demonstrated greater skeletal muscle metabolism, growth, and feed efficiency than the calves fed the CN diet. In addition, significant weight loss within the first week of life did not occur for the calves fed the HPN diet. Reduction of initial weight loss may have beneficial long term effects on calf health and performance, and warrants further investigation.

# Association of Coagulase Negative *Staphylococcal* Species and Milk Somatic Cell Count of Cows from the Canadian National Cohort of Dairy Farms

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## Introduction

*Staphylococci* are a diverse group of gram-positive cocci that can infect the cow's mammary gland. For the purposes of diagnosing intramammary infection (IMI), *staphylococci* are classified based on their ability to clot plasma (coagulase test) as either coagulase-positive or coagulase-negative. The most commonly isolated coagulase-positive *staphylococci*, *S. aureus*, is considered a major mastitis pathogen. In contrast, coagulase-negative *staphylococci* (CNS) have historically been classified as minor mastitis pathogens and are rarely, if ever, further differentiated when diagnosing an IMI. The assumption has been that, as a group, CNS cause minor elevations in milk somatic cell count (SCC). However, there has been recent interest in the role of CNS in mammary gland inflammation. The aim of this study was to evaluate the relationship between CNS species and mammary quarter SCC.

## Materials and Methods

Coagulase-negative *staphylococcal* isolates were obtained from the Canadian Bovine Mastitis Research Network (CBMRN) culture collection (n = 1495). Isolates were harvested from mammary quarters of cows with subclinical intramammary infections before and after the dry period or during lactation. Data associated with each sample was obtained from the CBMRN database. The isolates presented here were speciated by rpoB gene sequencing. To date, 938 isolates from 696 mammary quarters have been evaluated. The quarter infections were from samples collected prior to dry-off (289), post-calving (159), and mid-lactation (249). Geometric mean SCC were calculated when quarters had the same CNS isolated at more than one time point in mid-lactation and late-lactation samples (n=159), and these geometric mean SCC's were used in calculating median SCC's. For samples taken post-calving, SCC's from samples taken the first week after calving were considered separately. Somatic cell count was compared between CNS species within stage of lactation using a one-way analysis of variance (ANOVA) with post-hoc pair-wise comparisons (*P*<0.05).

## Results

Twenty species of CNS were identified and significant differences in SCC were noted between species in both first week post-calving and pre-dry groups, but not in the mid-lactation group. *Staphylococcus* chromogenes, *S. simulans*, *S. xylosus*, *S. haemolyticus*, *S. cohnii*, and *S. epidermidis* were the most commonly isolated species, accounting for more than 90% of total isolates. Of the commonly isolated species, *S. epidermidis* and *S. simulans* were significantly more inflammatory (median of SCC's 682,000 and 288,000 cells/mL, respectively) in late-lactation quarters sampled, while *S. haemolyticus*, *S. epidermidis* and *S. simulans* were significantly more inflammatory in samples taken in the first week of lactation (median SCC's of 4,020,000, 4,907,000, and 2,797,000 cells/mL, respectively). The remaining commonly isolated species were associated with SCC's less than 200,000 in all sampling groups with the exception of the early lactation group. *Staphylococcus hyicus* was an uncommon isolate in this study (n=7), but was associated with high median SCC's (347,000 and 1,575,000 in mid- and late-lactation groups, respectively).

## Significance

In common with several recent studies, *S. chromogenes*, *S. simulans*, and *S. xylosus* were among the most commonly isolated CNS species. Compared with previous studies, *S. hyicus* was uncommon in this study. This might be explained by regional or herd differences in prevalence of some CNS species or may reflect differences in speciation techniques or inclusion criteria. Though *S. hyicus* was uncommon, it was significantly more inflammatory than most of the commonly isolated species and, therefore, determining its prevalence among CNS isolates seems warranted. Overall, our data concur with the historical assertion that, on average, most CNS infections cause only mild to moderate increases in milk SCC. However, the data also demonstrate differences between species with regard to the degree of elevation in SCC that warrant further investigation.

# Variation in Daily Shedding Pattern of *Staphylococcus aureus* in Naturally Occurring Intramammary Infections

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## Introduction

The cost of mastitis has been calculated at 6% of the value of production; at 2007 prices that is upwards of 2.1 billion dollars. It is estimated that 70-80% of this loss is due to subclinical intramammary infections (IMI) caused by organisms such as *Staphylococcus aureus* (SA). The control of SA is contingent on accurate diagnosis of IMI, yet currently there exists no standard for the diagnosis of SA IMI. As a result, comparisons between published works are difficult. In addition, the shedding of SA from infected quarters has been described as "intermittent", resulting in recommendations for the diagnosis of SA IMI that are cumbersome and cost-prohibitive in veterinary practice and field research. The goal of this study was to describe shedding patterns of naturally occurring SA IMI over an extended period of time and to provide a reasonable foundation upon which to determine appropriate diagnostic criteria for SA IMI. The effect of PFGE pulsotype on shedding was also examined.

## Materials and Methods

Milk samples were collected according to NMC guidelines from five multiparous cows (seven quarters) and two primiparous cows (two quarters). Milk samples were collected for 21 consecutive days, three times throughout the lactation (63 days total), frozen immediately and kept frozen for up to 22 days, and thawed at room temperature for microbiological examination. The initial culture procedure employed standard NMC guidelines. Colony counts were recorded up to 100. A SA IMI was defined as a quarter culture-positive with ≥1 cfu of SA/0.01 mL within the first three days of the first 21-day sample period. Individual quarter samples with ≥1 cfu of SA were considered positive. The milk samples were kept frozen and thawed again at a later date, and re-examined using an entire plate per sample recording cfu up to 1,000 cfu/0.01 mL. A representative isolate from each quarter was submitted for pulse field gel electrophoresis (PFGE), with PFGE clusters evaluated at 80% similarity. Initial exploratory analysis was done using STATA (v.10). Longitudinal shedding patterns of SA were examined with locally weighted regression (lowess, STATA v. 10). The effect of PFGE pulsotype on the amount of shedding was examined using a linear mixed model with heterogeneous autoregressive correlation structure (PROC MIXED, SAS 9.2).

## Results

A total of 397 milk samples from SA-infected quarters were collected for microbiological examination and 388 (97.7%) of them were culture-positive (≥ 1 cfu/0.01 mL) for SA. A total of 393 milk samples from nine quarters (seven cows) were included in the analysis. Only four of the original seven cows remained for three sample periods, for a total of 63 days. Two quarters (one cow) were followed for 42 days, and three quarters (two cows) were followed for 21 days. Results from the second plating (counted up to 1000 cfu) were used in the analysis. On the second culture 97.5% of the samples (383 of 393) were culture-positive (≥ 1 cfu). Examination of the shedding pattern revealed the consistent presence of SA, although at varied levels, in addition to different levels of shedding between quarters in the same cow. The longitudinal shedding patterns of SA over the 21-day sample periods revealed no predominant cyclic shedding patterns. Accounting for the effect of sample day, samples collected from quarters infected with SA in pulsotype I had a ln(cfu) 1.5 times greater than those in pulsotype II (*P*=0.007).

## Significance

Contrary to previous reports describing the "intermittent" shedding of SA, our study found that, although daily shedding was variable between and within cows, SA was consistently recovered from quarters infected with SA. While strain type may have an effect on the overall amount of bacterial shedding, our study would support that a single sample, culture-positive with one cfu, is sufficient to diagnose a SA IMI. Future studies examining naturally infected quarters over longer periods may further aid in developing more coherent prerequisites of infections.

# Variation in Weekly Shedding Pattern of *Staphylococcus aureus* in Naturally Occurring Intramammary Infections

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## Introduction

Despite improvements in management, prevention, and treatment, mastitis accounts for 26% of morbidity on US dairies, making it the most prevalent and costly disease of dairy cattle. In total, it is estimated that 70-80% of this loss is due to subclinical intramammary infections (IMI) such as *Staphylococcus aureus* (SA). The most prevalent contagious mastitis pathogen in the United States, SA was detected in 43% of bulk tanks examined. The control of SA is contingent on accurate diagnosis of IMI, yet there remains no definitive standard for the diagnosis of a SA IMI. Studies following cows experimentally infected with the Newbould SA strain concluded that SA was shed in a cyclical pattern and that consecutive samples were necessary to accurately diagnosis a SA IMI. Since then, new technologies, such as pulse field gel electrophoresis (PFGE) used to compare strain relatedness, have brought into question the usefulness of research based on experimental SA infections. The goal of this study was to evaluate bacterial shedding patterns of SA, specifically the influence of clonal relatedness of SA on the shedding patterns of bacteria in naturally occurring SA infections.

## Materials and Methods

Foremilk samples were collected weekly (according to NMC guidelines) from quarters in 22 lactating cows (29 quarters) at two dairies identified with SA IMI in the previous lactation or within the first seven days of the current lactation, for 26 to 44 weeks. Milk samples that were culture-negative for SA using a 0.01 mL inoculum from quarters that had been confirmed SA-positive were thawed a second time and cultured using a larger volume of milk (0.1 mL). A sample was considered culture-positive for SA with ≥1 cfu/0.01 mL. Longtitudinal shedding patterns of SA over an eight-week window (bandwidth=0.18) were examined using the lowess smoother application (STATA). The effect of strain type on ln (cfu) was examined using a linear mixed model (SAS).

## Results

Using the 0.01 mL inoculum, 914 of 1,070 samples (85%) were culture-positive ($\geq$ 1 cfu/0.1 mL). Using a 0.1 mL inoculum, 1,011 (95%) of the samples were culture-positive ($\geq$ 1 cfu/0.1 mL). There was no significant difference in the detection of SA between PFGE pulsotypes when using either the 0.01 mL inoculum ($P$=0.076) or the 0.1 mL inoculum ($P$=0.1). Of the 59 culture-negative samples using 0.1 mL, three quarters (in two heifers) accounted for 68%. While there appears to be a large difference in the amount of shedding from quarters infected with different pulsotypes, there was no significant difference ($P$=0.1) in the amount of shedding between quarters infected with isolates grouped in pulsotype 1 or 2. There was no consistent pattern of shedding identified between or within cows, with great variation noted between quarters within the same cow. There was a significant difference in linear score and test-day linear score between quarters infected with isolates in PFGE pulsotype 1 and 2.

## Significance

Contrary to previous reports of intermittent shedding, while the level of shedding in these naturally occurring infections varied, 95% of the samples were culture-positive using a larger, 0.1 mL, inoculum. Results demonstrated a remarkably consistent ability to detect SA infections when a larger inoculum was used. Given the dramatic differences in shedding within cows, future research examining whether such variation is primarily due to the biology of SA and the individual cow or due primarily to methodology will aid in developing best management practices to control SA IMI.

# Efficacy of Intramammary Extended Therapy Using Ceftiofur (Spectramast® LC) against Clinical Mastitis of Holstein Cows

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## Introduction

Little research has focused on extended therapy for cows with clinical mastitis during lactation. Ceftiofur should be effective against a wide range of mastitis pathogens including gram-positive and gram-negative bacteria. It could be considered as a reasonable choice for treatment of clinical mastitis. The objective of the present study was to determine if extended therapy would increase cure rates of a ceftiofur treatment for non-acute clinical mastitis, for all bacteria and more specifically for *Staphylococcus aureus* and streptococci. The hypothesis was that extended therapy would enhance the cure rate of ceftiofur for non-acute clinical mastitis.

## Materials and Methods

Holstein dairy cows (n = 241) from 22 dairy herds from Quebec and Ontario, Canada were included. For each case of non-acute clinical mastitis, 125 mg of ceftiofur hydrochloride (Spectramast® LC) was administered via intramammary infusion for two or eight consecutive days. Allocation to treatment groups was randomized. A clinical cure was defined as milk appearing normal 21 days after the last treatment. A bacteriological cure was defined as a treated infected mammary quarter that was bacteriologically negative for the presence of previously identified bacteria at 7, 14, and 21 days after the last treatment. Mixed logistic regression was used for statistical analysis, the fixed factor was the treatment, the random factor was the herd and co-factors were days-in-milk, severity of mastitis, and the quarter. Results were considered significant if $P < 0.05$.

## Results

The clinical cure rate was 89% (n=98/110 for each group) for both treatment regimens for all bacteria. The bacteriological cure rates for the two- and the eight-day regimen were 32% (n=15/47) and 61% (n=25/41), respectively, for all bacteria, 64% (n=9/14) and 82% (n=9/11), respectively, for streptococci, and 0% (n=0/20) and 47% (n=9/19), respectively, for *Staphylococcus aureus*. There were 16 new intramammary infections: 10 in the two-day regimen and six in the eight-day regimen. There were 11 recurrences of mastitis: eight in the two-day regimen and three in the eight-day regimen. The clinical cure rates were not different between groups. The extended therapy group had significantly better bacteriological cure rate for all bacteria ($P = 0.007$) and for *Staphylococcus aureus* ($P = 0.001$) but not for streptococci ($P = 0.50$).

## Significance

The extended therapy appears to be a reasonable choice for the treatment of non-acute clinical mastitis, especially if *Staphylococcus aureus* is the suspected pathogen. More analysis is needed to determine if it is an economical choice.

# Relationship between Serum Total Protein and Serum IgG in Holstein Calves Fed Either a Lacteal- or Plasma-derived Colostrum Replacer

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## Introduction

Bovine neonates have a naïve immune system and must initially rely on the absorption and passive transfer of maternally derived immunoglobulins (Ig) ingested in colostrum. Acceptable passive transfer (APT) is defined as a serum IgG ≥ 10 mg/mL between 24-48 hours of age. A serum total protein (STP) cutpoint of 5.0 or 5.2 g/dl is commonly used to predict APT for calves fed lacteal-derived whole colostrum (Calloway *et al*, 2002). However, many producers now feed powdered commercial colostrum replacers (CR). Some CR products contain lacteal-derived (LD) Ig while others contain Ig derived from spray-dried plasma (PD). Research is needed to determine the best STP cutpoint to predict APT for calves fed either LD or PD CR products. The objectives of this study were to 1) describe levels of passive transfer and 2) determine the relationship between STP and IgG for neonatal calves fed a commercially available LD CR or PD CR product, respectively.

## Materials and Methods

In June through August of 2009, heifer calves born at the Transition Management Facility (TMF; Emerald, WI) were enrolled into the study. Calves were removed from the dam within 30-60 minutes of birth and before suckling, weighed, then randomly assigned to be fed either a LD CR containing 150g IgG (n = 36; Land O' Lakes Colostrum Replacement, Land O' Lakes, Inc., St. Paul, MN) or a PD CR containing 130g Ig, (n=38; Colostrx® 130 Colostrum Replacer, AgriLabs, Inc., St. Joseph, MO) via an esophageal feeding tube, within two hours of birth. After the first colostrum feeding, calves were fed 1.9L of a commercial milk replacer formula twice daily until 24 hrs of age. Venous blood samples were collected from the calves at birth and 24 hrs later. The serum was separated and frozen, serum was submitted to Prairie Diagnostic Services Laboratory (University of Saskatchewan, Saskatoon, Canada), where STP (g/dl) and IgG (mg/mL) concentrations were measured using a digital refractometer, and an RID method, respectively.

Descriptive statistics were used to calculate STP, IgG, apparent efficiency of absorption (AEA, %), and APT rates in calves fed each respective CR. Univariate linear regression (Proc MIXED, SAS version 9.2) was used to describe the relationship between STP (dependent variable) and IgG (explanatory variable). The resulting regression equation was used to estimate the STP value that would be predicted if IgG was 10 mg/mL.

## Results

The mean (+/- SD) STP at 24 hrs of age for the LD CR and PD CR treatment groups were 5.4 +/- 3.8 g/dl and 4.7 +/- 1.9 g/dl, respectively. Serum IgG concentrations at 24 hrs were significantly higher in calves fed LD CR (14.7 +/- 2.9 mg/mL) versus PD CR (9.6 +/- 1.8 mg/mL). The AEA (%) was greater for calves fed LD CR (38.2 +/- 6.3%) versus calves fed PD CR (28.4 +/- 5.0%). The proportion of calves with APT was 94.4% and 36.8% in LD and PD CR treatment groups, respectively. The estimated predictive STP cut-point (95% CLs) for APT was 5.0 (4.7, 5.3) g/dl for calves fed the LD CR, and 4.8 (4.4, 5.1) g/dl for calves fed the PD CR. Using these two TP cutpoints, calves were grouped into 2x2 tables (a = APT, b = FPT, c = CR TP>cutpoint, d = CR TP<cutpoint).

## Significance

Calves fed 150 g of IgG in a LD CR experienced significantly better rates of APT and higher STP and IgG measures as compared to calves fed 130 g of Ig in a PD CR. Two potential factors contributing to these differences could include a higher total mass (g) of IgG consumed plus greater AEA % of IgG for calves in the LD CR group. A serum TP cutpoint of 5.0 g/dl or 4.8 g/dl was most predictive of APT (serum IgG of 10 mg/mL) in calves fed either an LD or PD CR product, respectively. Producers measuring TP to monitor passive transfer rates in CR-fed calves should select different TP cutpoints, depending on what type of CR product (LD or PD) is fed.

# Comparison of Feeding Lacteal- and Plasma-derived Commercial Colostrum Replacers on Fecal Scores and Pathogen Shedding in Heifer Dairy Calves

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## Introduction

Failure of passive transfer (FPT), defined as serum IgG <10 mg/mL at 24-48 hours of age, is an important problem on dairy farms. If an adequate supply of high-quality colostrum is not available, producers may feed a colostrum replacer (CR) product. However, studies are lacking to compare the effects of different CR products on passive transfer and calf health during the preweaning period. The objectives of this study were to describe the effect of feeding two different commercially available CR products, one lacteal-derived (LD CR) and one plasma-derived (PD CR) on 1) serum IgG levels during the first nine weeks, 2) the risk for development of scours, and 3) the risk for fecal shedding of common enteric pathogens in the first 14 days of age.

## Materials and Methods

The study was conducted between June and August, 2009, at a commercial transition cow facility (TMF, Emerald, WI). Newborn heifer calves meeting study eligibility criteria were randomly assigned to be fed either an LD CR containing 150 g IgG (n=36 calves, Land O' Lakes Colostrum Replacement, Land O' Lakes, Inc., St. Paul, MN) or a PD CR, 130 g Ig, (n=38 calves, Colostrx® 130 Colostrum Replacer, AgriLabs, Inc. St. Joseph, MO) via an esophageal feeding tube within two hours of birth. After the first colostrum feeding, calves were fed 1.9 L of a commercial milk replacer formula twice daily until 24 hours of age, then 2.8 L of pasteurized milk twice daily until weaning at nine weeks of age. Venous blood samples were collected from the calves at birth, 24 hours, and at three, six, and nine weeks of age for determination of serum IgG levels. Subjective fecal scores ranging from 0-4 were assigned to the calves' fecal pats daily from one to 14 days of age, with scours defined as FS ≥ 2. Fecal samples were collected and analyzed using a lateral immunochromatography test kit for *Cryptosporidium parvum*, *Escherichia coli* F5 (K99) attachment factor, rotavirus, and coronavirus at seven days of age (BioX Diagnostics, Jemelle, Belgium). A two-way BioX fecal antigen test was also performed at day 14 for *C. parvum* and rotavirus.

Linear regression (Proc MIXED, SAS version 9.2) was used to evaluate the effect of treatment on IgG measures at three, six, and nine weeks and on the number of scour days. Logistic regression and (Proc GENMOD, SAS version 9.2) was used to evaluate the effect of treatment on risk for a positive FS and the risk for shedding fecal pathogens of interest in the first 14 days. Critical $P$ values were set at $P < 0.05$.

## Results

Serum IgG levels (mg/mL) were significantly higher in calves fed LD CR at 24 hours (14.7 +/- 2.9), week 3 (8.3 +/- 2.2), and week 6 (11.0 +/- 5.0), as compared to calves fed PD CR (24 hours = 9.6 +/- 1.8; week 3 = 6.9 +/- 2.7; week 6 = 8.4 +/- 4.0). There was no effect of treatment on IgG levels at week 9. A high proportion of calves exhibited FS ≥ 2 (LD = 97%; PD = 95%) during the first 14 days. There was no effect of treatment on risk or duration of scours between one and 14 days. There was no effect of treatment on risk for shedding of one or more of the fecal pathogens investigated at either seven days (LD = 19% positive; PD = 13% positive) or 14 days of age (LD = 56% positive; PD = 63% positive).

## Significance

The very high rate of scours experienced in this study was abnormal for this farm and may be attributed, at least in part, to a cooler, wetter summer than normal, making the calf hutch environment wetter than usual. Calves fed an LD CR had significantly higher concentrations of serum IgG at 24 hours, three weeks, and six weeks, as compared to calves fed a PD CR. We would expect these differences to provide calves in the PD CR group with increased protection against enteric pathogens, but this was not demonstrated in this study.

# Effect of Feeding Bovamine® Probiotic on Passive Transfer of Immunoglobulin G in Newborn Calves

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## Introduction

Early studies have suggested that bacteria in colostrum may interfere with passive absorption of immunoglobulins (Ig) in newborn calves (James *et al*, 1981), and recent studies reported that calves fed pasteurized colostrum had improved passive transfer of IgG versus calves fed fresh colostrum (Johnson *et al*, 2007; Heinrichs *et al*, 2009a). However, a recent study reported that colostrum bacteria counts were not associated with IgG absorption (Heinrichs *et al*, 2009b). Do pathogenic or non-pathogenic bacteria, living or dead, truly interfere with Ig absorption? Bovamine® (Nutrition Physiology Company, LLC, Overland Park, KS) is a direct-fed microbial (DFM or probiotic) containing a patented combination of living *Lactobacillus acidophilus* ($1 \times 10^9$ cfu/1 g dose) and *Propionibacterium freudenreichii* ($2 \times 10^9$ cfu/1 g dose). The study objective was to describe the effect of supplementing colostrum with living or dead DFM on serum total protein (STP) and IgG levels, and apparent efficiency of absorption of IgG (AEA %).

## Materials and Methods

The study was conducted from June-August, 2009 at a 6,000-cow Jersey dairy in Minnesota. Newborn calves were removed from the maternity pen within 30-45 minutes of birth and weight recorded. Male calves meeting study eligibility criteria were randomly assigned to one of three colostrum treatment groups. Calves in all three groups were fed a standardized dose of a commercially available colostrum replacer (CR) (Calf's Choice Total® Bronze, Saskatoon Colostrum Company, Saskatoon, SK) providing 200 g IgG. The control group (Ctrl, n = 27) received CR only, while the second treatment group (DFM-Live; n = 28) was given CR inoculated with a 1 g dose of live Bovamine®, and the third treatment group (DFM-Dead; n = 26) was given CR inoculated with a 1 g dose of dead Bovamine®. For the third group, the DFM had been killed by baking at 200°F (93.3°C) for 20 minutes. A 20 mL post-inoculation sample of CR was collected and frozen for each calf for bacterial culture. Colostrum was fed within two hours of delivery via esophageal tube feeder. After the colostrum feeding, calves were fed 1.9 L of milk replacer twice daily. Two-10 mL jugular blood samples were taken from each calf; one pre-feeding (T0) and one 24 hours post-feeding (T24). Serum was frozen at -4°F (-20°C), then shipped to the Prairie Diagnostic Services Laboratory (University of Saskatchewan, Saskatoon, SK) for determination of STP (g/dl) by digital refractometry (Sper Scientific, Scottsdale, AZ) and IgG (mg/mL) by RID. Analysis of variance was used to describe the relationship between treatment and the three outcomes: AEA %, serum IgG, and STP at 24 hours.

## Results

*Lactobacillus* counts were higher in CR samples from the DFM-Live treatment group (log10 = 4.7) as compared to the Ctrl group (log10 = 4.0) or the DFM-Dead group (log10 = 4.0) ($P < 0.0001$). There was no effect of treatment on mean serum IgG (mg/mL) (Ctrl = 20.0; DFM-Live = 20.1; DFM-Dead = 20.9; $P = 0.63$), STP (g/dl) (Ctrl = 5.6; DFM-Live = 5.6; DFM-Dead = 5.7; $P = 0.79$), or AEA IgG (%) (Ctrl = 29.8; DFM-Live = 28.6; DFM-Dead = 29.1, $P = 0.58$) at 24 hours post-feeding.

## Significance

Supplementation of CR with non-pathogenic bacteria, added as either a live or dead DFM, and at the levels studied, did not affect passive transfer of IgG. More research is needed to investigate the hypothesis that passive transfer of IgG may be impeded by pathogenic bacteria in colostrum. Also, studies are needed to determine if supplementing colostrum with DFM will improve calf health. Producers should still strive to feed colostrum low in concentrations of pathogenic bacteria (e.g. *E. coli*) to reduce the risk of disease in calves.

# The Effect of Injectable Butaphosphan and Cyanocobalamin on Postpartum Serum Beta-Hydroxybutyrate, Calcium, and Phosphorus Concentrations in Dairy Cattle

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## Introduction

Catosal® (Bayer, Shawnee Mission, Kansas, USA) is a sterile solution containing 10% butaphosphan and cyanocobalamin (vitamin B12), and is intended to prevent or treat deficiencies of these nutrients. Supplemental vitamin B12 may enhance gluconeogenesis through its role in methylmalonyl-CoA mutase. Phosphorus plays important roles in the hydrogen buffering system in the blood, is a critical component of nucleic acids, and is clinically important due to its role in postparturient paresis in dairy cattle. The objective of this study was to assess the effect of two 25 mL injections of Catosal® on the serum BHBA, calcium, and phosphorus concentrations of cows in the early postparturient period. (Originally published in *J Dairy Sci*, Vol 93, No. 3, 2010.)

## Materials and Methods

Cows from four herds (n=1,122) were stratified by parity and assigned by block randomization to either treatment or control groups receiving 25 mL of Catosal® or 25 mL of sterile water by subcutaneous injection (SQ) on the day of calving and one day later. Coded treatments were used to blind personnel and investigators with respect to treatment assignment. A blood sample was collected from each cow within 24 hours of calving (before treatment was given) and again between three and 10 days-in-milk and tested for serum BHBA concentration. Non-parametric approaches were used to compare group medians and paired comparisons of different time points. Comparison of the proportions of cows in each group with a serum BHBA >1,200 µmol/L during the week after calving were made using a chi-square test, and multivariable modeling of the dichotomized BHBA concentration was performed using logistic regression. All tests were performed assuming a two-sided alternative hypothesis and *P*-values < 0.05 were considered statistically significant.

## Results

When analyzing data from all parity groups, there was no difference between the median BHBA concentrations of treated and control groups pre-treatment (*P* = 0.61) or during the first week postpartum (*P* = 0.42). There was an increase in the median BHBA concentrations of both groups between the first and second measurements (*P* < 0.001). There was no difference in the proportions of cows in the treatment or placebo groups with a serum BHBA > 1,200 µmol/L during the week after calving (68/565 [12.0%] and 81/557 [14.5%], respectively; *P* = 0.22). In a multivariable analysis of the dichotomized BHBA levels, there was no significant effect of treatment, but parity, BCS, dystocia, and retained placenta were all significant predictors. The odds of having a serum BHBA > 1,200 µmol/L during the week after calving were significantly higher for cows in their third or higher lactation compared to first-lactation cows, for cows with a BCS > 4 compared to those with a BCS < 3, for cows that had experienced dystocia, and for cows that had a retained placenta. A sub-group analysis was performed for cows that were in their third or higher lactation, due to their higher risk of hyperketonemia. While there was no difference in pre-treatment BHBA concentrations (*P* = 0.67), treated mature cows had a lower median BHBA than placebo during the week after calving (*P* = 0.047) and the proportion of mature cows with a serum BHBA > 1,200 µmol/L during the week after calving was significantly lower in the treated group than in the placebo group (48/244 [19.7%] vs 66/228 [29.0%], *P* = 0.019). Treated mature cows had 40% lower odds of having a serum BHBA > 1,200 µmol/L during the week after calving than those receiving placebo, after adjusting for the other variables.

## Significance

Certain subgroups of cows were more likely to experience elevated BHBA levels postpartum; fat cows, cows with retained placenta, older cows, and cows with longer dry periods were all more likely to have hyperketonemia. The injection of Catosal® on the day of calving and one day later may decrease the prevalence of subclinical ketosis during the week after calving in mature dairy cows, but not in first- and second-lactation animals.