Description of the Spontaneous Development of Ketonemia in the Early Postpartum Period

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Introduction

Periparturient ketosis is highly prevalent in the dairy industry and has been associated with a number of health problems, especially displaced abomasum and fatty liver infiltration, as well as decreased productivity and decreased fertility. Despite the importance of ketosis, thorough descriptive studies of naturally occurring periparturient ketosis are lacking. The objective of this observational study was to provide a detailed description of the spontaneous development of ketonemia in Holstein dairy cows in their first two weeks of lactation and to describe the associations between serum beta-hydroxybutyrate (BHBA) levels and other metabolites and milk production.

Materials and Methods

A cohort of 161 Holstein dairy cows from a commercial facility was followed for the first 2 weeks of lactation. Blood samples were collected every other day between 2 and 14 days in milk (DIM), and daily milk production was measured during the same period. The serum samples were analyzed for metabolites of primary importance in the postpartum period: BHBA, non-esterified fatty acids (NEFA), glucose and total calcium concentrations. Cows were sorted by their maximum BHBA concentration during the period (peak BHBA), and were stratified in 3 groups of equal size: low, moderate and high peak BHBA. In the first step of the statistical analyses, individual profiles were described separately for each metabolite and milk production, and the average curves by DIM were plotted for each peak BHBA group. For the final step of the analysis, the timing of the peak BHBA was extracted for each individual. A repeated measures mixed model was then fitted for all metabolites and milk production to estimate the difference between the three ketonemia groups in the days leading to, and following, the timing of peak BHBA, after adjustment for parity and DIM.

Results

On average over DIM, high serum peak BHBA levels were associated with higher NEFA, lower glucose and lower total calcium concentrations. Milk production was lower for cows in the groups reaching low and high peak BHBA concentrations, compared to those reaching intermediate levels of BHBA. While the association between DIM and calcium, glucose and milk production was fairly consistent across cows, the timing of the maximum NEFA and BHBA concentrations was more variable within the time frame of observation. Because of the curve alignment issues for NEFA and BHBA, the summary curves by DIM differed noticeably in shape from the individual cow curves, especially for BHBA, and therefore may not provide a clear insight on how ketosis might develop in fresh cows. All variables were related to the extent and timing of the peak BHBA concentration. On average, large differences in NEFA concentrations among peak BHBA groups were observed in the days preceding peak BHBA, but these decreased after it. The difference in milk production between ketonemia groups seen with the curves by DIM was still present, but the timing of peak BHBA itself did not correspond very closely to a concurrent drop in production. Calcium and glucose levels started lower in the high peak BHBA group, decreased further at the time of peak BHBA, after which the difference between ketonemia groups diminished.

Significance

Even if the average concentration of the metabolites differed significantly between peak BHBA groups, there was still an important overlap of the groups at the cow level. Differences in average energy status, based on NEFA and glucose concentrations, were not enough to explain why some cows developed high ketone concentrations and others did not. The short additional drop in calcium and glucose surrounding the peak
Concentrations of Serum Non-Esterified Fatty Acid (NEFA) and Beta-Hydroxybutyrate (BHB) through the Transition Period and their Associations with Risk of Clinical Disease

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Introduction

Serum non-esterified fatty acid (NEFA) and Beta-Hydroxybutyrate (BHB) concentrations provide important insight into the metabolic health of transition dairy cows. Previous studies have linked elevated prepartum NEFA and postpartum BHB concentrations with an increased risk of developing displaced abomasum. The objective of this study was to further characterize the relationship of both prepartum and postpartum serum NEFA, and postpartum serum BHB concentrations with clinical disease in transition dairy cows across different regions of North America.

Materials and Methods

A field study was conducted using 56 commercial dairy herds across Canada and the United States. Herds sampled were divided into four geographic regions consisting of the Midwest, Northeast (including Ontario, Canada), Southeast and Western United States. Each herd in the Midwest and Northeast regions had approximately 35 cows per herd enrolled in the study, whereas in the Southeast and Western herds included approximately 60 and 80 cows per herd, respectively. A total of 2403 Holstein cows were enrolled in the study one week prior to calving. A technician visited herds weekly at approximately the same time, after the morning feeding. During each visit a coccygeal vein blood sample was collected from cows in the week before their expected calving date, and again from the same cows in weeks 1, 2, and 3 postpartum. Body condition was scored at week -1. Blood samples were kept cool, allowed to clot and serum was harvested, and stored at -20°C within 8 hours of collection. All serum was shipped to the Animal Health Laboratory at the University of Guelph for measurement of NEFA and BHB using a Hitachi 911 auto-analyzer. The incidence of retained placenta (RP), puerperal metritis, and displaced abomasum (LDA) were recorded.

Results

Results were available from 1771 cows. Considered alone and assuming equal weight on sensitivity and specificity, the optimal cut-points for prediction of LDA were, prepartum: NEFA ≥ 0.5 mEq/L; week 1 postpartum: mol/L (i.e. mNEFA ≥ 1.0; or week 1 or 2 postpartum: BHB ≥1400 subclinical ketosis (SCK)). The 23% of cows with NEFA ≥ 0.5 in week -1 were 2.8 times more likely to subsequently have LDA than cows below this cut-point. The 20.5% of cows with NEFA ≥ 1.0 in week +1 were 4.6 times more likely to develop LDA. The prevalence of SCK and relative risk for affected cows to develop LDA were 14% and 4.4 in week +1, and 16% and
3.6 in week +2. Cows that did not have elevated NEFA pre- or postpartum or SCK had the lowest risk of LDA (0.8%) whereas cows that experienced all three risk factors had a high rate of LDA (12%). Among the 53% of cows with NEFA ≥ 0.3 mEq/L in week -1, there was a significant (P = 0.005) but modest increase in risk of RP (9% vs. 6%; RR = 1.6). Neither metabolite had a predictive univariable association with development of metritis.

Significance

These data confirm the associations of NEFA and BHB with health in the transition period and support their use as tools for monitoring or investigation of transition dairy cows. However, used alone, the positive predictive value of these associations is low, which is expected given the multifactorial nature of both RP and LDA.

The Use of Rumensin® Premix in Dairy Cows: Factors Influencing its Effects on Milk Production and Milk Fat Percentage

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Introduction

Monensin premix (Rumensin® Premix, Elanco Animal Health, Canada) has been approved in Canada for use in lactating dairy cows since 2004 at a dose range of 8 to 24 ppm (7-22g per ton). Several studies have found that monensin increases milk production and decreases milk fat percentage in lactating dairy cows. Recent research has found that some dietary factors influence the monensin effect on milk production and milk fat percentage. To assist bovine practitioners in making recommendations about the use of monensin in dairy herds, there is a need for knowledge about those dietary factors influencing monensin effects. The objectives of this project were to evaluate the effects of 16 ppm (15g per ton) of monensin on milk production (PROD) and milk fat percentage (MFP), and to find dietary factors influencing those effects.

Materials and Methods

A randomized field clinical trial was conducted using 49 Holstein dairy herds in Québec (Canada) between November 2005 and May 2006. The herd was considered as the unit of interest. Herds were balanced in two groups by milk production, housing system, feeding system and size of farm. Enrolled herds were followed for a 7-month period. Monensin treatment was allocated in a crossover design for each group. Monensin was added to the lactating dairy cow rations for a consecutive 3-month period within this time frame. No other source of monensin was provided during this trial. Diet composition and diet particle size evaluation (using the Penn State Particle Separator) data were collected on each farm every two months. Milk production and milk fat percentage data were from weekly averages of daily bulk tank data. Data were analyzed in linear mixed models where PROD and MFP were considered as outcome variables.

Results

The majority of the 49 herds were fed a total mixed ration (n=30; 61%) and were housed in tie-stalls (n=42; 86%). Mean herd size was 73 cows (min: 40, max: 175). Overall monensin effect on PROD was not significant
A Meta-analysis of the Metabolic Impacts of Monensin in Lactating Dairy Cattle.

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Introduction

Monensin shifts the microbial population in the rumen towards more gram negative bacteria, consequently changing rumen volatile fatty acid concentrations towards propionate and away from acetate and butyrate. Since the late 1980’s there have been many papers published on the effects of monensin in lactating dairy cattle. Recently, approvals for use on monensin have been obtained in Canada and the United States, while the product has been available for dairy cattle in countries such as Mexico, Australia and New Zealand for many years. The impacts of monensin on energy metabolism, including effects on serum ketones, NEFA, glucose, and urea have not always been consistent. Meta-analysis is a useful tool that can be employed to both summarize effects across studies and to investigate factors explaining potential heterogeneity of response.

Materials and Methods

An intensive literature search and screening process yielded a total of 59 papers, abstracts, and trial reports containing useable data on monensin in dairy cows. Of these, 30 papers contained metabolic data. All trials included were randomized designs but were not necessarily blinded. Data from each trial contained in the papers was extracted to a database including the number of animals, mean, and standard error for each of the monensin and control groups. Other relevant data that were common to most studies such as dose, stage of lactation, dose delivery method, and diet type (pasture, forage, component-fed) were also extracted. Meta-analysis was conducted in STATA for monensin effects on blood/serum beta-hydroxybutyrate (BHB), acetoacetate, non-esterified fatty acids (NEFA), glucose, urea, cholesterol, insulin, and calcium.

Results

The results of this trial are consistent with previous studies and confirm that monensin lowers herd milk fat percentage at a dose of 16 ppm (15g per ton) in lactating dairy cows. No effect on PROD for monensin, as measured using bulk tank data, was found. Dietary factors influencing the impact of monensin on PROD and MFP were mostly related to carbohydrate and fibre levels in the diet. Those factors could be used for predicting potential effects of monensin in herds.
(P=0.0001), urea (P=0.0001), and cholesterol (P=0.08). Monensin had no effect on serum calcium or insulin. The effect size estimates for monensin on BHB, acetoacetate, and cholesterol were heterogeneous; thus random effects models were utilized for these analytes. Effect size estimates of monensin for BHB and NEFA were positively correlated, but were negatively correlated to glucose. A consistent delivery of monensin via topdress or in a controlled release capsule gave a slightly smaller but more consistent reduction in BHB. Topdress delivery and increasing dose increased glucose response. NEFA, BHB and glucose effects were modified by stage of lactation. Larger effects for BHB and NEFA were observed in the transition period than later in lactation.

Glucose response was greater in cows after calving than before calving.

**Significance**

Monensin clearly has profound effects on energy metabolism which are marked by decreases in NEFA, BHB and acetoacetate and increases in glucose and cholesterol. The effect on increasing urea is likely a reflection of reduced protein degradation in the rumen and subsequent deamination of absorbed amino acids. Maximum benefit from monensin for improved energy metabolism will be obtained by ensuring a consistent daily dose and targeting the transition dairy cow.

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**A Meta-analysis of the Production Impacts of Monensin in Lactating Dairy Cattle.**

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**Introduction**

Monensin increases propionic acid production through effects on rumen microbial populations. Recently, approvals for use on monensin have been obtained in Canada and the United States, while the product has been available for dairy cattle in many other countries such as Mexico, Australia and New Zealand for many years. The impact of monensin on production, especially on milk fat content have been inconsistent. Meta-analysis is a useful tool that can be employed to both summarize effects across studies and to investigate factors explaining potential heterogeneity of response.

**Materials and Methods**

An intensive literature search and screening process yielded a total of 59 papers, abstracts, and trial reports containing usable data on monensin in dairy cows. Of these, there were 37 papers that contained production data. All trials included were randomized controlled designs but were not necessarily blinded. Data from each trial contained in the papers were extracted to a database including the number of animals, mean, and standard error for each of the monensin and control groups. Other relevant data that was common to most studies such as dose, stage of lactation, dose delivery method, and diet type (pasture, forage, component-fed) were also extracted. Meta-analysis was conducted in STATA for monensin effects on milk yield, dry matter intake, milk production efficiency, milk components (percent and yield), body condition score, and body weight change. A subset of trials contained dietary information. This information was used to estimate ration parameters in CPM and then these parameters were screened in meta-regression to evaluate impact on monensin treatment on milk components.

**Results**

There were a total of 37 papers containing 70 trials with monensin and production outcomes. Some stud-
Evaluation and Use of an Automated Human ß-hydroxybuturate (BHBA) Test for Cowside Detection of Subclinical Ketosis in Dairy Cattle

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Introduction

Prevalence of subclinical ketosis in dairy herds ranges from 6.9% to 34%. Subclinical ketosis can lead to decreased milk production and reproductive performance, increased risk of displaced abomasum and clinical ketosis. The gold standard diagnostic test for subclinical ketosis is the measurement of BHBA in serum or plasma. Thresholds to distinguish between normal and ketotic cows range from 1000 to 1600 µmol/l of blood BHBA. Serum BHBA measurement is useful for examining individual cows and evaluating herd health. However, it is not convenient and is expensive for use as a routine cowside diagnostic test for the early detection of subclinical ketosis. Cowside diagnostic tests for ketosis (dipsticks or tablets) are based on the degree of color change. These tests can be used semi-quantitatively because the color change is more intense in the presence of higher levels of ketone bodies. Accuracy of the tests vary from test to test and from study to study. In human medicine electronic handheld blood glucose and ketone systems are used for diabetes monitoring. The objective of this study was to evaluate the precision and accuracy of an electronic BHBA measuring system (Abbot, Wiesbaden) for the use in dairy cattle.

Materials and Methods

A total of 244 dairy cows between 4 and 40 days after calving were used for the study. Blood, milk and
urine samples were collected within an interval of up to 15 minutes. Blood samples were drawn from the coccygeal vein with vacuum tubes. Milk samples were collected after milking and urine samples from the urine stream after massage of the region underneath the vulva. One droplet of whole blood was used to load the sensor of the test-strip (Precision Xtra ß-ketone) according to the directions. The values displayed on the handheld meter were recorded onto a data capture form. Also, concentrations of BHBA in milk and urine were determined both with the electronic system and chemical dipsticks (Ketostix cowside. Blood samples were centrifuged and serum was stored at -20°C. Within 8 days serum samples were analyzed for BHBA photometrically (Cobas Mias). Serum BHBA concentrations determined in the laboratory were regarded as the gold standard. Correlation coefficients (Pearson) were calculated between BHBA in serum and whole blood, milk and urine, respectively. Sensitivity and specificity of the different tests were determined.

Results

Coefficients of correlation between serum BHBA and whole blood, milk and urine determined with the electronic system were 95.2%, 71.9%, and 66.5%, respectively. Coefficients of correlation between serum BHBA and milk were 63.1% and urine determined with Ketostix determined with Ketolac and 63.9%, respectively. Based on thresholds of 1200 and 1400 mol/l BHBA sensitivity was 0.84 and 1.0 and specificity 0.93 and 0.91, respectively for the electronic BHBA measuring system. the positive and negative predictive values were 0.66 and 0.98 (1200 mol/l BHBA ) and 0.51 and 1.0 (1400 mol/l BHBA), respectively. For both milk and urine, positive and negative predictive values were considerably lower both for the dipsticks as well as for the electronic system.

Significance

An automated electronic system to determine BHBA in whole blood is a useful and practical tool to diagnose individual cases of subclinical ketosis. Sensitivity and specificity are adequate for a cowside test. The accuracy of the electronic system was higher compared to two commonly used chemical tests. Additional studies are necessary to further validate the electronic system for the use in dairy cattle.

Relationship between Keto-Test Results and Health and Reproduction Variables: a Retrospective Study using Data from Herd Health Visits in Private Practice

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Introduction

Cows in negative energy balance (NEB) can develop subclinical ketosis (SCK) and elevated Beta-hydroxybutyrate (BHB) concentrations in milk. A milk strip cow-side test (Keto-Test; KTST) can be used to measure BHB and initiate vet-client discussions about transition cow management and energy issues during regular herd health visits. The purpose of this study was to relate KTST results to first-breeding conception rate and to the incidence of some metabolic diseases.

Materials and Methods

In 22 herds followed by a single dairy practitioner in southern Quebec, milk from cows between 4 and 21 days in milk (DIM) at the time of herd health visit was tested using the KTST. Keto-Test results, as well as dis-
ease incidence, reproduction and DHI data, were compiled using the herd health monitoring software DSA over a three-year period. Results of KTST was considered positive at a cut-off value of 100 µmol/L of BHB. Variables were herd (HERD), lactation number (LN), calving season (CS), DIM at 1st breeding (DIMAI1), breeding season (BS), success at 1st breeding (PREG) and occurrence of milk fever (MF), retained placenta (RP), metritis (ME), ovarian cyst (CY), clinical mastitis (MA), lameness (LA) or displaced abomasum (DA) in the same lactation. Logistic regression was used to test the effect of KTST on PREG or on metabolic diseases. Potentially confounding variables and their interaction with KTST were retained in the complete model when P was less than 0.25. The reduced model was obtained after deleting all variables that did not qualitatively change the odds ratio (OR).

**Results**

Results of KTST: 1034 negative cows and 394 positive cows (27.5 %). The complete logistic model for PREG included KTST, HERD, LN, MF, RP, ME, CY and DA. The reduced model contained only KTST and confirmed that KTST was not associated with PREG (P= 0.74), OR = 0.96, 95% CI (0.73 - 1.26). Percent pregnant were 32.1 and 31.1 for negative and positive cows, respectively. The complete model for MA included KTST, HERD, LN, CS, MF, KTSTxMF, RP, ME, CY, and KTSTxCY. The reduced model contained only KTST and confirmed its strong association with MA (P = 0.002), OR = 1.55, 95% CI (1.17 - 2.05) The rate of MA was 17.6 vs. 24.9% for negative and positive cows, respectively. The complete model for DA included KTST, HERD, KTSTxHERD, LN, CS, RP, LA, KTSTxLA. The reduced model contained KTST, HERD, and KTSTxHERD and showed a strong association of KTST with DA (P = 0.001), OR = 3.81, 95% CI (1.70 to 8.51), 3.48 vs. 7.87% DA. Odds ratio was 1.48, 1.45, and 3.81 for herds with a low (<5%), medium (5 – 10%), and high (>10%) incidence of DA. Other disease incidences (MF, RP, ME, CY and LA) were not significantly associated with KTST results.

**Significance**

This study did not prove a relationship between KTST results and 1st breeding conception rate, contrary to what was expected. Possible reasons for that are: low cut-off chosen (only 116 cows would have been positive had a 200 µmol/L cut-off been used), missing important variables in the analysis and false negative KTST results when cows were tested at the end of the 4-21 DIM interval. Other reproductive variables (days open, 2nd, 3rd-breeding conception rates) should also be analyzed. The strong relationship between occurrence of a DA and KTST result is not surprising. It must be interpreted with caution since in some cases, DA preceded KTST whereas, in other cases, DA happened after KTST. Results suggest that the risk for clinical mastitis during the lactation is significantly increased in cows with a positive KTST result between 4-21 DIM. This finding underlines the link between metabolic status during transition/early lactation and health during the rest of lactation. Routine submission of fresh cows to Keto-Test during herd health visits is an easy to implement practice which creates a “teachable moment” to discuss fresh cow nutrition and health. Evidence of a link between fresh cow Keto-Test result and health in later lactation is an additional reason to encourage Keto-Test use.
Frequency of Pre-partum Negative Energy Balance and Post-partum Subclinical Ketosis and the Cow- and Herd-levels in North East Dairy Herds

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Introduction

Large increases in demands for energy and nutrients occur as dairy cows transition from the last weeks of pregnancy to the first weeks of lactation. If excessive, negative energy balance (NEB) in the pre-partum period and subclinical ketosis (SCK) in the post-partum period may be associated with poor reproductive and milking performance, as well as increased incidence of metabolic (e.g. DA) and infectious diseases (e.g. mastitis). Published research suggests that SCK is the most costly disorder of dairy cows in Ontario. The objective of this study was to measure the occurrence of NEB and SCK in a large sample of progressive dairy herds to determine if there is opportunity for better management of this critical time in a cow’s life.

Materials and Methods

A convenience sample was selected from northeastern USA dairy herds and visited by study personnel. A few herds were submitted by participating veterinarians. In each herd, approximately 20 pre-partum cows 2 to 14 days prior to calving and 20 different post-partum cows 3 to 21 DIM were sampled. All appeared to be of normal health. Pre-partum NEB was assessed by measuring non-esterified fatty acids (NEFA) and post-partum SCK was assessed by measuring beta-hydroxybutyrate (BHB) in serum. The cut-points used to define NEB and SCK in individual cows were NEFA > 0.4 mEq/L and BHB > 14 mg/dl, respectively. A herd was defined to have NEB or SCK if > 15% of the sampled cows were above the cut-point. Using this sampling and interpretive criteria, the within herd prevalence can be estimated to 90% confidence.

Results

A total of 94 herds were sampled with a mean and median size of 821 and 760 cows, respectively. From these herds, 1879 and 1884 cows were sampled post-partum and pre-partum, respectively (mean parity of 2.3). 22.1% (20.5 – 23.7 90% CI) of cows were in pre-partum NEB. 12.3% (11.1 – 13.6 90% CI) of cows had post-partum SCK. 3.0% of cows were above 30mg/dl BHB indicating they had clinical ketosis. 65% of the herds had more than 15% of their sampled cows in NEB, while 14% of the herds had more than 35% of their sampled cows in NEB. 37% of the herds had more than 15% of their sampled cows with SCK, while 16% of the herds had more than 25% of their cows with SCK.—

Significance

This study shows that a large number of herds in NE USA have NEB and SCK challenges. These herds were not selected because they had a suspected problem with NEB or SCK indicating that the problem is likely under diagnosed. It is also worth noting the cows that were detected with clinical ketosis, as they were also not sampled because they had apparent health concerns, indicating that some cows with clinical ketosis are under-diagnosed. If on-going work shows SCK is as costly to NE USA dairies as it is to Ontario dairies there may room for improved transition cow management.
A Comparison of Pregnancy Detection by Palpation and a Blood Test in Dairy Cattle

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Introduction

The purpose of this project was to compare the results of a blood test for bovine pregnancy with pregnancy detection by rectal palpation by veterinarians in dairy herds using artificial insemination. The blood test evaluated was a commercially-available enzyme-linked immunosorbent assay (ELISA) which tests for pregnancy-specific protein B.

Materials and Methods

The study was conducted in the Fall of 2006 and early Winter 2007 in 12 dairy herds. Blood samples were collected at the time palpations were routinely performed and submitted to BioTracking in Moscow, Idaho. Blood test results were received electronically. Palpations were performed between 35 and 280 days post-insemination by 5 experienced dairy veterinarians. Palpation results were recorded on paper records and entered into DairyComp 305 on farms or into a database prepared for the study.

Results

Out of 417 animals diagnosed open by palpation, the blood test results were: 83.2% open (n=347), 5.5% open recheck (n=23), 3.8% pregnant recheck, and 7.4% pregnant (n=31). For 569 animals diagnosed pregnant by palpation, the blood test results were 1.9% open (n=11), 1.2% open recheck (n=7), 0.7% pregnant recheck (n=4) and 96.1% pregnant (n=547). The discrepant results were further investigated to determine possible explanations for the differences. Among 11 animals that were pregnant by palpation, but open on the blood test, 6 were subsequently recorded as in heat, checked open or aborted, 2 were confirmed pregnant and 3 were unknown. As most of the animals in the study where enrolled in synchronization protocols, 27 of the 31 cows which were diagnosed open by palpation but pregnant by the ELISA were administered prostaglandin after palpation, making it difficult to determine the causes of the different test results. However, after adjusting for days since last heat, animals that were blood test pregnant and palpation pregnant had significantly higher ELISA optical density values than animals that were blood test pregnant and open by palpation (p<0.0001). This suggested that some of the discrepancies may have been attributable to cows that were previously pregnant and had pregnancy specific protein B concentrations that were waning but still above the cut-off value for pregnancy. Approximately 5% of animals tested with the ELISA were given a “recheck open or recheck pregnant” result, compared to 0.1% of animals tested by palpation.

Significance

There was quite high agreement between the blood test and palpation results, but neither test was perfect. The economic impact of a few days delay in receiving results and a larger number of rechecks for the blood test would need to be considered when deciding which method to use.
Evaluation of a Modified Surgical Technique to Correct Urine Pooling in Cows

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Introduction

Urovagina of cows and horses often results in infertility by inducing necrotizing endometritis. This condition is seen most commonly in old, pluriparous cows and mares with poor vaginal and vestibular conformation. Adverse changes result from numerous pregnancies or from dysfunction of the constrictor vestibuli muscle caused by damage to this muscle during parturition. Cranioventral tipping of the pelvis, the result of numerous pregnancies, or damage to the constrictor vestibuli muscle causes the cranial portion of the vagina to be positioned ventral to the external urethral orifice, leading to collection of urine in the vaginal fornix. Various surgical techniques have been used to create a mucosal extension from the urethral orifice to the labia to correct urovagina in cows, but a fistula often forms in the extension, causing the surgical remedy to fail. The objective of the trial reported here was to determine if the incidence of fistula formation could be decreased by covering submucosal tissue on the floor of the vestibule, exposed by transposing mucosal flaps to create the extension, with a mucosal graft.

Materials and Methods

Thirty-eight cows were used in this study including 16 Holstein cows, 2 to 6 years old and 22 cross-bred, beef cows, aged 5 to 10 years. Cows were randomly divided into control and experimental groups. The cows in both the control group (19 cows) and the experimental group (19 cows) received a modified McKinnon technique of urethral extension, performed with the cows standing. The cows in the experimental group also had a free sheet of mucosa, obtained from the dorsal aspect of the vestibule, grafted to submucosa on the ventral aspect of the vestibule exposed during creation of the urethral extension. The wound created by removing the mucosa on the dorsal aspect of the vestibule was left to heal by secondary intention. The graft was sutured, under tension, to exposed submucosa on the ventral aspect of the vestibule. No attempt was made to cover all the exposed submucosa on the ventral aspect of the vestibule.

Results

During palpation and visual examination of the urethral extension of all cows two weeks after surgery, we found that 10 of 19 cows (53%) in the control group and six of 19 cows (32%) in the experimental group had developed a fistula, 10 to 15 mm in diameter, in the urethral extension (P = 0.19). After injecting dye into the extension, under pressure, we found that four of the nine cows (44%) in the control group and four of the 13 cows (31%) in the experimental group that had no fistula detectable by palpation and visual examination had developed a minute fistula (≤ 1 mm in diameter) in the extension. Each fistula, whether palpable or not, was located at the cranial, dorsal aspect of the extension. The total number of cows that developed a fistula in the extension, whether palpable or not, was 14 of 19 (74%) in the control group and 10 of 19 (53%) in the experimental group. The total number of cows that developed a fistula in the extension did not differ significantly between groups (P = 0.18). The incidence of fistula formation did not differ significantly between dairy cows (12/16; 75%) and beef cows (12/22; 55%) (P = 0.20). During histological examination of the biopsy of the graft and its recipient site, obtained one week after surgery, neither inosculation nor revascularization of the graft was evident in any sample.

Significance

We concluded that application of a mucosal graft to the subcutaneous tissue exposed to the vestibule using the McKinnon technique of creating a urethral extension is of little or no benefit in preventing the formation of a fistula in the extension. We observed that digital palpation and visual examination alone are often insufficient in determining the presence of a fistula. The article is in press. Available online 24 April 2007. (Copyright © 2007 Elsevier B.V.)
Novel Vaccine Reduces Prevalence and Shedding of Escherichia coli O157:H7 in Cattle

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Introduction

Escherichia coli O157:H7 is a food-borne pathogen that causes severe hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans. The gastrointestinal tract of cattle is the main reservoir for E. coli O157:H7, making cattle feces a possible direct and indirect source of exposure for humans. According to the CDC, shiga-toxin producing E. coli O157 causes an estimated 73,000 illnesses annually in the United States, resulting in over 2,000 hospitalizations and 60 deaths (Frenzen et. al, 2005). Interventions used to reduce shedding of this microorganism will lower contamination of the environment. A recently developed vaccine that targets Gram-negative bacteria’s ability to acquire iron may be a practical intervention strategy for reducing the prevalence of this pathogenic microorganism. Therefore, the purpose of the study was to evaluate the efficacy of a vaccine containing outer membrane siderophore receptor and porin (SRP) proteins in reducing fecal prevalence and shedding of E. coli O157:H7 in cattle.

Materials and Methods

Thirty, 3 to 4 month old calves were randomly assigned to one of two treatment groups and were subcutaneously administered either the placebo or the E. coli O157:H7 SRP vaccine on days 1 and 21. Calves were moved from a local Kansas farm to a BL-2 facility one week after the second vaccination was administered and were confined to individual pens. On day 36, calves were orally inoculated with a mixture of 5 strains of E. coli O157:H7 made resistant to nalidixic acid (NaIR). Blood samples were collected weekly to monitor calves’ anti-SRP antibody titers and fecal samples were collected three times a week for the following 5 weeks to monitor fecal shedding of the NaIR E. coli O157:H7. Five weeks post challenge, calves were euthanized and gut contents were collected to further evaluate the presence of the NaIR E. coli O157:H7.

Results

Both treatment groups had similar antibody titers prior to vaccination. Two weeks after the first vaccination was administered, the average number of anti-SRP antibodies in the SRP vaccinated animals was 34% higher than the number of anti-SRP antibodies present in the placebo vaccinated animals. Vaccination with the SRP E. coli O157:H7 vaccine decreased the number of cattle shedding E. coli O157:H7 in the feces (P =0.03) and there was a significantly (P = 0.04) lower fecal concentration of NaIR E. coli O157:H7 in the SRP vaccinated cattle when compared to the placebo group. Cattle vaccinated with the SRP E. coli O157:H7 vaccine had fewer samples positive for E. coli O157:H7 at necropsy when evaluating the cecum (P = 0.06), colon (P = 0.05), and rectum (P = 0.06) compared to cattle administered the placebo.

Significance

Overall, orally inoculated calves vaccinated with the SRP vaccine had a reduction in prevalence of E. coli O157:H7 when compared to calves vaccinated with a placebo. This not only defines a new potential pre/post-harvest intervention strategy for reducing food borne illness, but also helps to increase consumers’ confidence when purchasing quality beef.
Siderophore Receptor and Porin-Based Vaccine For Pre-Harvest Control of Escherichia coli O157:H7 in Feedlot Cattle

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Introduction

Escherichia coli O157:H7, which is harbored in the intestines of cattle, is a major food borne pathogen that causes hemorrhagic enteritis and hemolytic uremic syndrome in humans. Recent data indicate that pre-harvest prevalence in groups of cattle is associated with the subsequent post-harvest contamination of carcasses. Because of this, efforts to reduce carriage of this organism in harvest-ready cattle will likely reduce the number of carcasses that are contaminated with E. coli O157:H7. A novel vaccine that contains siderophore receptors and porin proteins (SRP) was designed to block the passage of iron into the bacterial cell, essentially eliminating its nutrients and reducing further colonization of this pathogenic microorganism. Previous studies have shown that this vaccine significantly reduced fecal shedding and promoted an immune response in 4 month-old mixed breed calves that were orally inoculated with naladixic acid resistant strains of E. coli O157:H7. Further evaluation of the efficacy of this vaccine in feedlot cattle is necessary. Therefore, the purpose of this study was to examine the efficacy of the SRP vaccine on the prevalence and shedding of E. coli O157:H7 in feedlot cattle.

Materials and Methods

Approximately 2,000 cattle from four feedlots in Nebraska were selected to test the vaccine. Cattle were randomly divided into 20 pens, approximately 100 cattle per pen, and were injected subcutaneously with the E. coli O157 SRP vaccine or the placebo on days 0 and 21. Rectal fecal samples were collected on day zero, and pen floor samples were collected on days 21, 35, and 70 to determine the prevalence of E. coli O157:H7. On day 85, harvest was simulated and rectal fecal samples, recto-anal mucosal swab (RAMS) and hide swab samples were collected to determine the prevalence of E. coli O157:H7. Cattle were weighed on days 0, 21, and 85.

Results

There was no treatment by day interaction when evaluating the LS means of proportions of cattle positive for E. coli O157 in both SRP vaccinates and placebo vaccinates. The fecal prevalence of E. coli O157:H7 averaged over time was lower (P=0.04) in SRP vaccinated cattle (5.2%) than in control cattle (7.8%). Also, the prevalence on hides, feces, and on RAMS at harvest was higher (P<0.02) in placebo vaccinated cattle than SRP vaccinated cattle. There was an overall 54% reduction in prevalence of E. coli O157:H7 in the SRP vaccinated cattle when compared to the controls. The SRP vaccination had no effect on the overall average daily weight gain of cattle.

Significance

The SRP vaccine reduced fecal prevalence of E. coli O157:H7 in feedlot cattle and would be a beneficial pre-harvest control strategy to minimizing transmission of E. coli O157:H7.
Campylobacter jejuni in the Alberta Beef Industry

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Introduction

Campylobacter jejuni is a bacterium known to cause enteritis in people. In Alberta, campylobacteriosis is the most common foodborne disease and the third most common reportable disease after chicken pox and hepatitis C. Poultry are considered to be a primary source of human-pathogenic C. jejuni; however other domestic animals species may be important sources or reservoirs of this bacterium. The purpose of this project was to estimate the prevalence of Campylobacter species in feedlot cattle feces and retail ground beef in southern Alberta, and to identify C. jejuni isolates for future molecular characterization (DNA microarray).

Materials and Methods

Fresh cattle feces were collected from seven commercial feedlots (n=2800, 400 per feedlot) in Alberta. Within the same temporal frame, 1200 packages of regular or lean ground beef were purchased from 60 retail grocery stores (within three cities) in southern Alberta. The feedlot cattle feces and retail ground beef were cultured for Campylobacter using enrichment techniques. A subset of the ground beef samples were subjected to direct PCR for Campylobacter species including C. jejuni.

Results

Preliminary results indicate that while relatively high levels of Campylobacter species were present in cattle feces (76-95% positive cultures depending on feedlot), not all of the positive isolates were C. jejuni. In addition, the level of Campylobacter species in retail ground beef was extremely low (less than 1% by culture).

Significance

This project showed a high prevalence Campylobacter species in cattle feces from commercial feedlots in Alberta, and was able to also estimate the prevalence of Campylobacter jejuni, bacteria of public health importance, in those samples. The retail ground beef survey supported food safety practices in the province as a very low prevalence of Campylobacter was identified using culture methodology.