Research in Veterinary Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Reprint of Milk fever in dairy cows is preceded by activation of innate immunity and alterations in carbohydrate metabolism prior to disease occurrence☆

Guanshi Zhang[a,b], Elda Dervishi[a], Burim N. Ametaj[a,*]

[a] Department of Agricultural Food, and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada
[b] Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2M9, Canada

ARTICLE INFO

Keywords:
Dairy cow
Milk fever
Innate immunity reactants
Blood variables
Biomarkers

ABSTRACT

The objective of this study was to search for potential alterations in innate immunity reactants and carbohydrate and lipid metabolism in the blood of transition dairy cows before, during, and after clinical occurrence of milk fever (MF) and identify potential predictive biomarkers of disease. One hundred pregnant multiparous Holstein dairy cows were involved in the study starting from − 8 wks before the expected day of parturition until + 8 wks postpartum as part of a large retrospective longitudinal study. Health status, DMI, milk yield, and milk composition were monitored during the whole experimental period. Six healthy cows (CON) and 6 cows that showed clinical signs of MF were selected for blood analyses. Serum concentrations of lactate, non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), haptoglobin (Hp), and serum amyloid A (SAA) were determined. Results indicated that concentrations of serum lactate, IL-6, TNF, SAA, and Hp were greater in cows with MF than those in the CON group at different time points. Moreover, serum lactate, TNF, SAA, and Hp were greater in cows with MF starting at − 8 and − 4 wks prior to parturition. Both principal component analysis (PCA) and partial least squares - discriminant analysis (PLS-DA) showed separated clusters between MF and CON cows at − 8, − 4, and disease diagnosis weeks. Overall DMI and milk production were lower in MF-affected cows. Additionally milk fat and fat:protein ratio were greater in MF. In conclusion, cows affected by MF showed alterations in some of the innate immunity reactants and metabolites related to carbohydrate metabolism several weeks prior to appearance of clinical signs of MF. Variable importance in projection plots demonstrated that TNF and SAA in the serum were the strongest discriminators between MF cows and CON ones, which might be useful as predictive biomarkers of the disease.

1. Introduction

Milk fever (MF), also known as parturient paresis, is a common periparturient disease of dairy cows that has been suggested to be triggered by an imbalance of calcium metabolism around calving. The incidence of MF tends to vary with age and breed between 0 and 10%, but may reach 25% in some herds (DeGaris and Lean, 2008). Cows with MF are at risk for metritis and dystocia and also are more susceptible to other metabolic or infectious diseases such as mastitis, displaced abomasum, retained placenta (RP), ketosis, uterine prolapse, and high culling rate during the first 30 d postpartum (DeGaris and Lean, 2008).

Milk fever has been studied for more than 2 centuries however some aspects of the pathogenesis are still a matter of controversial discussion. The hypothesis on calcium insufficiency has gained most support in the pathogenesis of MF (Hibbs, 1950; Goff, 2008). However, calcium (Ca^{2+}) hypothesis has been challenged by other recent hypotheses including the role of potassium (K^{+}) excess on the pathogenesis of the disease and dietary cationic anionic difference (DCAD; Goff et al., 2014). A meta-analytical study demonstrated the importance of other ions apart from calcium and potassium during MF in particular magnesium, which explains a considerable percentage of the variance of milk fever response (Lean et al., 2006).

Recently, another new hypothesis has been gaining support, which suggests a potential role of Gram-negative bacterial lipopolysaccharide (LPS) or endotoxin in the pathogenesis of MF (Aiumlamai et al., 1992; Ametaj et al., 2010). Lipopolysaccharide is a cell wall component of

DOI of original article: http://dx.doi.org/10.1016/j.rvsc.2017.12.008
☆ A production error resulted in this article inadvertently being printed in a regular issue. The article is reprinted here for reader convenience and for the continuity of the special issue. For citation purposes, please use the original publication details: Research in Veterinary Science, Volume 117C, April 2018, Pages 167 – 177.
* Corresponding author.
E-mail address: burim.ametaj@ualberta.ca (B.N. Ametaj).

https://doi.org/10.1016/j.rvsc.2018.02.013
Received 14 March 2017; Received in revised form 11 December 2017; Accepted 14 December 2017

Gram-negative bacteria with immunogenic properties, which might be involved in the pathogenesis of several periparturient diseases in dairy cows like retained placenta, metritis, mastitis, and laminitis (Andersen, 2003; Ametaj et al., 2010). We have shown that cows with MF have greater concentrations of SAA in the plasma and decreased plasma calcitonin-gene related peptide (CGRP) compared to healthy cows (Ametaj et al., 2003; Zebeli et al., 2013). Enhanced concentrations of SAA suggest presence of an inflammatory insult in cows with MF, related potentially to presence of endotoxin in the blood circulation (Ametaj et al., 2010).

To date, little is known about the involvement of an inflammatory insult on the etiopathogenesis of MF. Moreover, there is lack of research tackling the etiopathogenesis of MF prior to appearance of clinical signs of disease. In this study, we hypothesized that an inflammatory insult might be present in pre-MF cows and that metabolic and immunity alterations might precede occurrence of MF during the early dry off (− 8 wks) and the transition period (− 4 wks before and + 4 wks after parturition). Therefore, the objective of this investigation was to monitor and sample cows during the dry off period and retrospectively ascertain alterations in innate immunity and carbohydrate and lipid metabolism starting at − 8 and − 4 wks prior to calving in cows that were affected by MF postpartum as well as alterations during the week of diagnosis of disease and thereafter until + 4 wks postpartum. In addition, DMI, milk production, and milk composition will be evaluated to determine the effect of disease on those variables.

2. Materials and methods

2.1. Animals and diets

This study was part of a larger project designed to study the pathomechanism as well as to identify early screening biomarkers of several periparturient diseases of dairy cows. One hundred pregnant Holstein dairy cows were used in this longitudinal study. Six pregnant multiparous (parity: MF 3.2 ± 0.7 vs. CON 3.0 ± 0.9; $P = 0.89$) Holstein dairy cows, two of which died postpartum due to severe health complications, were treated for MF (identified at wk. + 1) within 24 h after calving based on diagnosis by a veterinary practitioner. Cows diagnosed with MF had no other concurrent diseases such as mastitis, metritis, lameness, retained placenta, ketosis, and left displaced abomasum and they did not have difficult birth (dystocia). Six clinically healthy cows (CON) (free of any clinical disease) that were homologous in age, parity, and body condition score (BCS), were selected for this nested case-control study. Cows from both groups were clinically healthy prior to the expected day of parturition. All experimental procedures were approved by the University of Alberta Animal Policy and Welfare Committee for Livestock, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). The study was conducted at the Dairy Research and Technology Centre (DRTC), University of Alberta.

The experimental period lasted for 16 wks, starting at − 8 wks prepartum until + 8 wks postpartum for each cow. Dry matter intake (DMI) was calculated based on the data collected from − 8 to + 8 wks. Milk production was calculated from + 1 to + 8 wks. Serum variables were analyzed from − 8 wks to + 4 wks. Milk composition was determined on + 2, + 3, + 5, and + 7 wks relative to parturition. All cows were offered a total mixed ration (TMR) during the experimental period (Supplemental Tables 1, 2, 3). Feed was provided for ad libitum intake once daily at 0800 to allow for approximately 5% orts. Total mixed ration (TMR) was formulated to meet or exceed the nutrient requirements of a 680 kg dry and early lactating cow as per National Research Council guidelines (2001). Cows were housed in individual tie stalls bedded with sawdust and with free access to water throughout the 17 wks of experimental period. One week before calving, cows were transferred to a maternity pen and returned to their stalls on the following day of calving. Individual feed intake was recorded daily throughout the sampling period. Since the onset day of lactation, cows were milked in their stalls twice (at 0500 and 1600) per day, and individual milk yield was recorded electronically. Milk composition including milk fat, crude protein (CP), somatic cell count (SCC), lactose, milk urea nitrogen (MUN), and total solids (TS) were analyzed by mid-infrared spectroscopy (MilkoScan 605; A/S Foss Electric, Hillerød, Denmark) at the Central Milk Testing Laboratory in Edmonton, Alberta.

2.2. Monitoring of the clinical health status

All cows were monitored daily starting at − 8 wks prior to the expected date of calving and continuing up to + 8 wks postpartum for potential health disorders based on clinical symptoms of diseases by a veterinary technician and trained staff. Clinical signs observed were general appearance, appetite, alertness, rectal temperature, ease of calving, body condition score (BCS), vaginal discharges (color and consistency), udder edema, flakes in the milk, teat enlargement and secretion, gait, and pain in the legs. All periparturient diseases and veterinary treatments were recorded for each cow throughout the experimental period. Diagnosis of pregnancy was performed routinely at around 60 to 70 d after insemination by a herd veterinary practitioner based on transrectal ultrasonography. The expected date of calving was determined by adding 280 days from the day of artificial insemination (AI) and it was also supported by the information of pregnancy diagnosis.

In this study, diagnosis of MF was established by a veterinary practitioner based on clinical signs (Adams et al., 2008) and response to treatment. Oral calcium, one tube (i.e., actual calcium is 57.34 g) of calcium gel (Cal-C-Fresh, Vetoquinol Canada Inc., Lavaltrie, QC) supplementation was administered to the cow (four cows) if she was alert and still standing (stage 1 hypocalcemia) and repeated after 12 h. If the cow (two cows) was depressed, recumbent, flat out on their side or completely paralyzed (stage 2 and stage 3 cases of MF), they were administered 250–500 mL (i.e., 4.21–8.42 g calcium as gluconate salt) of Cal Dextro No. 2 (Vet Tek, Inc. Elmsford, NY) intravenously (iv), over 20 min. In addition, 500 mL (i.e., 9.57 g calcium) of calcium borogluconate 23% (Bimeda-Mtc Animal Health Inc., Cambridge, ON) was administered subcutaneously. For the two died cows, they did not respond to the medication and became weak and unable to rise. The rectum protruded due to the impaired rectal mobility. Heart rate was elevated and bloat occurred in one cow. No other complications such as ketosis, displaced abomasum or metritis were observed. To avoid relapse, recumbent cows were given additional oral calcium supplementation once they were able to swallow, followed by a repeated oral treatment about 12 h later. Four cows treated for MF fully recovered from the disease during the day of treatment, while the other two were not able to return to normal health and were culled from the herd.

Serum levels of calcium were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) based on a protocol described by Cava-Montesinos et al. (2005). Briefly, calcium quantification was conducted using a Perkin-Elmer Sciex® ElanTM 6000 quadrupole ICP-MS (PerkinElmer, Inc., Waltham, MA, USA) operating in a dual detector mode with an ICP RF power of 1300 W. The accuracy of the ICP-MS analytical protocol was periodically evaluated via the analysis of certified reference standard materials (whole rock powders) BE-N and DR-N available from the SARM laboratory at the CRPG (Centre de Recherches Pétrographiques et Géologiques, Nancy, France) (Bouatra et al., 2013).

2.3. Sample collection

Blood samples were obtained from the coccygeal vein once per week from each cow (i.e., 100 cows) on every Monday morning at 0700 shortly before feeding, starting − 8 wks before the expected day of calving. All blood samples were collected into 10-mL vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and were allowed to