The Veterinary Journal 197 (2013) 175–181

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

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

Review

Heat and ultraviolet light treatment of colostrum and hospital milk: Effects on colostrum and hospital milk characteristics and calf health and growth parameters ☆

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ARTICLE INFO

Article history:
Accepted 26 March 2013

Keywords:
Dairy calves
Colostrum
Hospital milk
Ultraviolet light
Pasteurization

ABSTRACT

The aim of this study was to evaluate the effects of different physical treatments of bovine colostrum and hospital milk on milk bacteriology, immunoglobulin G (IgG) and lactoferrin concentrations, calf serum IgG concentrations and calf health, growth and survivability. Pooled colostrum samples ($n = 297$) were heat treated (HTC; 63 °C for 60 min), exposed to ultraviolet light (UVC; 45 J/cm^2) or untreated ('raw', RC). Hospital milk ($n = 712$) was subjected to high temperature short time pasteurization (HTST; 72 °C for 15 s), ultraviolet light irradiation (UVH; 45 J/cm^2) or was untreated. Neonatal Holstein heifer calves ($n = 875$) were randomly enrolled (309 HTC, 285 UVC, 281 RC) and block randomized (by colostrum treatment) into hospital milk treatments HTST ($n = 449$) or UVH ($n = 426$). HTC was more effective than UVC and HTST was more effective than UVH in reducing bacterial counts. IgG and lactoferrin concentrations were significantly lower in HTC and UVC than in RC. Lactoferrin concentrations were significantly lower in HTST than in UVH or untreated hospital milk. There were no significant differences in serum IgG concentrations among calves fed HTC, UVC or RC. Colostrum and hospital milk treatments did not have any significant effect on calf body weight gain, survivability, or frequency of diarrhea or pneumonia.

Introduction

Colostrum provides calves with immunoglobulins (Igs), non-specific immune factors and nutrients (Weaver et al., 2000), but may also expose calves to pathogens (Stabel et al., 2004). Bacteria from contaminated colostrum may reduce the efficiency of IgG absorption (James et al., 1981; Johnson et al., 2007). High temperature short time (HTST) treatment of colostrum was associated with a decrease of 22–27% in IgG concentration regardless of the temperature regime (Stabel et al., 2004); however, when colostrum was heat treated at a lower temperature (60 °C) for 60 min, the IgG concentration did not change significantly when compared to raw colostrum (Johnson et al., 2007). A recent multi-herd study demonstrated similar results (Donahue et al., 2012). The viability of pathogens, such as *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* serovar Enteritidis, was significantly reduced or eliminated in spiked colostrum after treatment at 60 °C for 60 min (Godden et al., 2006).

Feeding calves with milk replacer can be costly and the use of pasteurized, non-saleable milk (hospital milk) is an attractive alternative. Hospital milk includes milk from mastitic cows, which can have increased bacterial contamination. Pasteurization (in batch at 63 °C for 30 min or HTST at 72 °C for 15 s) can reduce the counts of MAP in inoculated milk (Gao et al., 2002). On-farm pasteurization of milk effectively destroys MAP, *Salmonella* spp. and *Mycoplasma* spp. in spiked milk (Stabel et al., 2004).

Ultraviolet (UV) light disinfection systems are commonly used for water and waste water treatment in the USA and Europe (Lindenauer and Darby, 1994; Guo et al., 2009). UV light destroys several pathogens in drinking water (Hijnen et al., 2006), *Staphylococcus aureus* in milk and *L. monocytogenes* in raw goat's milk (Matak et al., 2005; Krishnamurthy et al., 2007), although UV irradiation of milk spiked with MAP did not result in an adequate reduction in infectivity (Donaghy et al., 2009).

The aims of this study were to determine the effects of heat and UV treatment on (1) bacterial counts and concentrations of lactoferrin and IgG in colostrum and hospital milk; (2) serum IgG con-

☆ Preliminary results were presented as an abstract at the XXVII World Buiatrics Congress, Lisbon, Portugal, 3–8 June 2012.

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http://dx.doi.org/10.1016/j.tvjl.2013.03.032

centrations in calves; and (3) calf survivability, pneumonia, diarrhea and body weight gain in the preweaning period.

Materials and methods

Farm and management

The study was conducted from February to November 2011 at a commercial dairy farm that milked 2800 Holstein cows near Ithaca, New York, USA. Immediately after parturition, calves were removed from maternity pens and placed in dry sawdust bedded pens. Colostrum from multiparous cows was administered to calves. Neonatal calves were transported twice daily from the maternity area to the calf barn, which was a greenhouse-type building, with eight rows of 40 individual pens (each 1.7 m long and 1.2 m wide) isolated by plastic panels and bedded with a deep gravel base covered with pine shavings. Calves were kept in the same pen until weaning. Water and calf starter were available ad libitum. Calves were weaned at ~45 days of age by a progressive reduction in milk over 5 days.

Study design and data collection

On the basis of a priori sample size calculations, it was estimated that, with a sample size of 270 calves per colostrum treatment group, an average daily weight gain (ADWG) of 660 g (with a standard deviation of 131 g) would permit detection of differences in daily body weight gain $\geqslant$32 g. In addition, it was estimated that a sample size of 270 calves per colostrum treatment would permit detection of a difference in frequency of diarrhea of 12% between treatment groups, considering a baseline frequency of diarrhea of 50%. Sample size calculations were based on an α value of 0.05, confidence of 0.80 and a two-tailed t test.

A randomized field trial study design was used. All heifers born from February to October 2011 were eligible for enrolment in the study. All calves received 4 L colostrum within 4 h of birth by esophageal tubing (Oral Calf Feeder Bag with Probe, Jorvet). Colostrum treatments were pre-assigned by a random table generated in Excel (Microsoft) using the random number function. To prepare equal aliquots of pooled colostrum for each of the three treatments, a minimum volume of 45 L was needed. Colostrum was harvested twice daily and stored in a refrigerator (1.7 °C) until the desired volume was reached, which was typically achieved within 36 h. At that point, the pooled colostrum was firstly homogenized and then divided into three equal aliquots of at least 15 L each; one third was untreated; one third was heat treated at 63 °C for 60 min using a batch pasteurizer (DT-10G Platinum, Dairy Tech) and the last third was treated with UV light (45 J/cm^2) (UV Pure system; GEA Farm Technologies). All treated colostrum was stored in 4 L jars and refrigerated (1.7–3.3 °C) until use.

Calves were further blocked by colostrum treatment and randomized into one of two hospital milk treatments: HTST (72 °C for 15 s) or UVH (45 J/cm^2). Hospital milk was harvested twice daily and stored in a stainless steel milk tank (600 L) mounted on a transport truck and refrigerated at 5 °C until it was transported to the pasteurization room, where it was divided into aliquots and treated according to the study protocol. Calves assigned to the HTST group received 6 L/day pasteurized hospital milk (Terminator, Goodnature Products). Calves allocated to the UVH group were fed 6 L/day hospital milk treated with UV-light (UV Pure system, GEA Farm Technologies).

All machinery was sanitized prior to and after each running cycle. Both the UV Pure system and the HTST pasteurizer are pre-set with their own clean-in-place procedure. The cleaning procedure comprises a pre-rinse cycle with warm water, a high pressure washing cycle with hot water and an alkaline detergent (TRI-PFAN, GEA Farm Technologies), and a final rinse with warm water and a low foam acid cleaner (LAC, GEA Farm Technologies). The jars and the batch pasteurizer (DT-10G Platinum, Dairy Tech) were cleaned with an initial rinse with warm water, followed by a hand-brush wash using alkaline detergent, then a final rinse with warm water and a low-foam acid cleaner.

A blood sample was collected from each calf at 3 days of age; serum was harvested after centrifugation at 2500 g for 10 min and stored at −80 °C. Birth weight, weekly measures of fecal scores and body weight were recorded for each calf until 60 days of age.

Case definitions

All health-related events during the study period were recorded. Retained placenta was defined as a condition where cows failed to release their fetal membranes within 24 h of calving. Metritis was defined as the presence of fetid, watery, red–brown uterine discharge. Pneumonia was defined when two or more of the following clinical signs were detected in a calf: cough, rectal temperature >39.5 °C, respiratory rate >40 breaths/min, increased cranioventral lung sounds or wheezes. Calves were recorded as diarrheic when the fecal consistency was watery and fetid.

Microbiological assays

Hospital milk samples were collected before and after UVH treatment and HTST pasteurization twice daily for 5 days per week (January–November 2011). Colostrum samples were collected pre-processing (after pooling) and post-processing for each day (January–September 2011). Samples (50 mL) were collected, placed on ice and transported to the laboratory to be processed daily. Samples of colostrum and waste milk were homogenized, diluted serially (10^{-1}–10^{-12}) and 20 μL aliquots were plated on standard aerobic medium (EMD Millipore) for total bacterial counts (TBCs), and on specific chromogenic media for detection and enumeration of *E. coli*, *S. aureus*, and group B *Streptococcus* spp. (CHROMagar). Plates were incubated aerobically for 24 h at 37 °C. All cultures were performed in triplicate. The number of colony forming units (CFUs)/mL was calculated from the average number of colonies (from triplicates) multiplied by the appropriate dilution factor.

ELISAs

ELISAs were used to quantify IgG in the serum of calves and colostrum (Immuno-Tek Bovine IgG ELISA Kit) and lactoferrin in colostrum and hospital milk (Bethyl Laboratories, ELISA KIT). All colostrum and hospital milk samples were thawed and homogenized, and aliquots of 1.5 mL were centrifuged at 10,000 g for 15 min at 4 °C, then the supernatant was collected for analysis.

Statistical analysis

Descriptive statistics and univariable analysis were performed using SAS. Analysis of variance (ANOVA) was used to evaluate the effects of hospital milk and colostrum treatments on the log reduction of CFU and the effects of colostrum treatment (RC, HTC or UVC) on IgG and lactoferrin concentrations using the MIXED procedure in SAS.

To analyze the effect of colostrum treatment on calf serum IgG concentrations at 3 days of life, a general linear model was fitted to the data using SAS. The outcome variable was calf serum (g/L), which was modelled as a Gaussian (normally distributed data) variable. The model assumption that the residuals were normally distributed was assessed and satisfied by visual evaluation of the distribution plot of the Studentized residuals. Other independent variables offered to the model were parity of the dam (primiparous or multiparous), location of parturition (maternity pen or pre-fresh free stall, the latter where dry cows were housed for 3 weeks before the expected calving date), calving ease of the dam (assisted or non-assisted), gestation length of the dam (days), birth weight quartile, and dam post-partum health events (metritis, displaced abomasum and retained placenta). The only calf-related risk factors offered to the models were those observed before enrolment (e.g. post-treatment colostrum bacterial CFU was not offered). However, post-parturition dam-related risk factors, such as post-partum diseases, were included in the regression models. All possible two-way interaction terms between treatments and all independent variables were evaluated in the model. Manual backward variable elimination considering main effects and two-way interactions of colostrum treatment with all the other independent variables was performed. Variables and interaction terms were retained in the model when $P \leqslant 0.05$.

The effect of treatment on calf survival was analyzed by Cox's proportional hazard model in SAS. Calves were right-censored if they were alive at the end of the data collection period (60 days). Variables offered to the models included colostrum treatment (RC, UVC or HTC), hospital milk treatment group (UVH or HTST), parity of the dam (primiparous or multiparous), calving location (maternity pen or pre-fresh free stall), calving ease of the dam (assisted or non-assisted), gestation length (days), dam post-partum health events (metritis, displaced abomasum and retained placenta) and calf birth weight quartiles (first: 22–37 kg; second: 38–41 kg; third: 39–44 kg; fourth: 45–59 kg). All possible two-way interaction terms between treatments and all independent variables were evaluated in the model, as well as the two-way interaction between colostrum and waste milk treatment. Backward variable elimination (Cantor, 1997) was undertaken considering main effects and two-way interactions of colostrum and waste treatment with all the other independent variables. Variables and interaction terms were retained in the model when $P \leqslant 0.05$.

The effects of colostrum and hospital milk treatments on diarrhea and pneumonia were evaluated by logistic regression models that were fitted in Stata (StataCorp LP); the dependent variables for these models were occurrence of diarrhea or pneumonia (no = 0 or yes = 1) over the entire observation period (60 days). The independent variables offered to this model were the same as described above for the other multivariable models. Interaction terms and variable selection methodology were evaluated as described for the Cox's Proportional Hazard model. Adjusted probabilities were calculated from the model for all categorical variables retained in the model using the adjusted probability option in Stata.

A mixed general linear model was fitted to the data using SAS software to analyze the effect of colostrum and hospital milk treatment groups on repeated measures of calf ADWG per week. Calf daily weight gain was calculated for each week by subtracting the latest body weight from the previous week's body weight and dividing it by seven; therefore, the outcome variable was a series ADWG, which