Minnesota
Dairy Health Conference

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION

May 19-20, 2010
St. Paul, Minnesota

Boehringer
Ingelheim

Elanco

Pfizer Animal Health

Virtus
NUTRITION

Heat-Treatment of Bovine Colostrum Decreases Maternal Leukocyte Viability

N. Koewler[1], S. Godden[1], M. Bandrick[1], T. Molitor[1], P. Rapnicki[1], R. Bey[1] and D. Haines[2]

[1] Department of Veterinary Population Medicine, University of Minnesota

[2] Department of Veterinary Microbiology, University of Saskatchewan

Introduction

First milking colostrum is an important part of a newborn calf's passive immunologic protection as the calf does not receive in utero transmission of immunoglobulins (Ig). It contains many important constituents including Ig, maternal leukocytes, growth factors, hormones, cytokines, nonspecific antimicrobial factors, and many nutrients. The passive transfer of these factors happens in the first 24 hours of life when the calf has what is termed as an "open gut." During this time, the neonatal intestinal epithelium is able to nonselectively absorb large, intact molecules, including Ig molecules and leukocytes, and release them into the lymphatic system and ultimately into the circulation. Maternal leukocytes are a large constituent of normal bovine colostrum having a concentration of more than 1×10^6 cells/ml, and include macrophages, T and B lymphocytes, NK cells and neutrophils. The importance of these cells as a determinant of calf health is not fully understood. It is thought that they may help to stimulate development of the calf's own humoral and cell mediated immune systems. Conversely, it may be possible that they could harbor harmful pathogens including *Mycobacterium avium* ssp. *paratuberculosis* (MAP) or Bovine Leukemia virus (BLV).

Although colostrum is essential for passive transfer, it can also be a potential source of exposure for pathogenic bacteria including MAP, BLV, *Mycoplasma* spp. and *Escherichia coli*. To reduce the risk of pathogen exposure from colostrum, researchers at the College of Veterinary Medicine, University of MN, have developed a method to heat-treat colostrum in order to kill bacteria but preserve vital Ig. Previous studies heating colostrum to 60 °C for 60 minutes reported a significant reduction in bacteria counts with no differences seen in colostral Ig concentration. However, it is currently not understood if this heat-treatment process will have a detrimental effect on colostral leukocytes. In a pilot study conducted in 2006 (unpublished), it was found that the previously described heat-treatment protocol did not affect the total number of intact maternal leukocytes, as measured using a flow cytometer method (Bentley SomaCount). However, that study did not investigate the viability of colostral leukocytes after heat-treatment. The main objective of this study was to describe the effect of heat-treatment of colostrum at 60 °C for 60 min on colostral leukocyte viability. Secondary objectives were to describe the effect of heat-treatment, when using a prototype model of a colostrum pasteurizer, on colostrum IgG concentration and bacterial counts.

Materials and Methods

The study was conducted in the summer of 2009 at a transition management facility (TMF; Emerald, WI), a commercial facility managing transition and fresh cows for two large dairies in western Wisconsin. Colostrum was collected from cows and heifers within 1 hour post-

parturition. Samples that appeared pink in coloration were excluded from the study as erythrocytes could interfere with leukocyte counts. Fresh colostrum from individual cows was transferred into 3.8 L containers, stored at 4 °C for no more than two days post-collection, and then transported on ice to the University of Minnesota for processing and analysis.

Prior to processing, the fresh colostrum was mixed and three 30 mL aliquots were collected using an aseptic technique. Two samples were frozen to be tested later for IgG concentration and bacterial counts, respectively. The third aliquot was kept at 4 °C for later WBC viability testing. Heat-treatment was then performed using a prototype of a batch-type colostrum pasteurizer. The heat-treatment protocol used followed a previously validated method (McMartin et al 2006; Godden et al 2006) for temperature and time. Briefly, 2.5 quarts of raw colostrum were placed into the pasteurizer, heated to 60 °C, and maintained at that temperature for 60 minutes. After a brief cool down period three more 30 mL aliquots of heat-treated colostrum were collected using the same aseptic technique.

After collection, one set of frozen paired (pre- and post- heat-treated) colostrum samples were tested for total IgG concentration using an RID method (Prairie Diagnostic Services Laboratory, University of Saskatchewan, Saskatoon, SK). The second paired set of frozen samples underwent routine bacterial culture techniques to measure total plate count (TPC, cfu/ml) and total coliform count (TCC, cfu/ml) (University of Minnesota Udder Health Laboratory, St. Paul, MN).

The third paired set of refrigerated colostrum samples underwent processing and cell viability assessment at the University of Minnesota. Briefly, cells were isolated by a centrifugation dilution method using a 1:3 dilution ratio and 1x Phosphate Buffered Saline (PBS) as the dilutant. Colostrum was diluted 1:3 with sterile 1x PBS and subsequently centrifuged for 10 minutes at 1500 rpms. The supernatant was discarded, the pellet was resuspended in 1x PBS and the solution was centrifuged again for 10 minutes at 1200 rpms. The latter step was repeated a final time leaving a pellet that was ultimately resuspended in 1 mL 1x PBS for viability counting. Viability assessment was conducted using a trypan blue exclusion test. Briefly, cells were diluted 1:50 in trypan blue which itself was diluted 1:5 in 1x PBS. After vortexing the solution, 10 μL were added to a hemocytometer for viability counts. Only cells that were within the four outer quadrants were counted. Viable cells excluded the dye thus were light or transparent while dead cells were dark as their degrading cellular membranes were not able to exclude the dye. Both viable and dead cells were counted in all four quadrants. The quadrant counts for viable cells were ultimately averaged then multiplied by the dilution factor and 10^4 in order to get the final cells per milliliter used in analysis.

Statistical analysis was completed using SAS statistical software to generate descriptive statistics. Analysis of variance (PROC MIXED) was used to evaluate the effect of treatment (fresh vs heat-treated) on the following outcomes: count and percentage of viable WBCs (viable cells/ml, % viable), IgG concentration (mg/ml), TPC (cfu/ml) and TCC (cfu/ml). Before analysis three parameters, WBC, TPC and TCC, were log (base 10) transformed as the original data was not normally distributed. Final significance was determined at p < 0.05.

Results and Discussion

Colostral leukocyte viability was significantly reduced in heat-treated samples (log living cells = 2.19 ± 2.63; percent viable cells = 0.79 ± 1.31%) as compared to fresh samples (log living cells = 6.31 ± 0.42; percent viable cells = 39.19 ± 23.01%; $p < 0.0001$). Since heat-treatment is known to reduce or eliminate bacterial cells, it is not surprising that the process also significantly reduced the viability of colostral leukocytes. The significance of this finding is not yet understood. If colostral WBCs are beneficial to calf health and performance, then removing these cells through the heat-treatment process could be detrimental, in spite of the fact that harmful bacteria are also removed. Conversely, if these WBCs were harboring infectious pathogens such as MAP or BLV, then using heat-treatment to kill these cells and the pathogens they harbor might be beneficial to the calf. Further research is required to investigate the significance of colostral leukocytes to calf health and performance.

As expected, log transformed colostral bacteria counts were also significantly reduced in heat-treated samples (TPC = 0 ± 0; TCC = 0 ± 0) as compared to fresh samples (TPC = 4.48 ± 1.08; TCC = 3.80 ± 1.41, $p < 0.0001$). These results are consistent with previous studies reporting that heat-treatment using the aforementioned protocol was able to significantly reduce or eliminate bacteria in colostrum.

One unexpected result, colostral IgG concentrations were significantly lower in heat-treated samples (73.8 ± 21.1 mg/ml) as compared to fresh samples (89.4 ± 24.4 mg/ml, $p = 0.022$). The mean percentage loss in a given batch was 16.7 ± 11.0%. While the end product was still of very high quality (high IgG level), these results differed from previous studies using the same heat-treatment protocol but which used different pasteurizer designs, and which reported no significant negative effect of the heat-treatment protocol on colostrum IgG concentrations.

One factor that may have contributed to the excessive IgG losses incurred in the current study could be that this study used very high quality colostrum (avg = 89.4 ± 24.4 mg/ml). Previous studies have reported that very high quality colostrum samples are more prone to IgG damage during the heat-treatment process as compared to low or moderate quality colostrum. In a separate regression analysis conducted to investigate this possibility, it was determined that the initial quality of the raw colostrum (IgG, mg/ml) was positively associated with the magnitude of IgG lost after heat-treatment (IgG loss, mg/ml), but was not associated with the percentage of IgG lost after heat-treatment (IgG loss, %).

Alternatively, the excessive IgG loss observed in some but not all heat-treated colostrum could relate to the function of the prototype model colostrum pasteurizer that was used for this study. Specifically, the irregular IgG loss suggests imprecision of the heat treatment (e.g. frequency and duration of time colostrum temperature), inadequacy of agitation during the heat-treatment process, and/or failure of other critical aspects of the heating and cooling. The reliability of the unit for performing the heat treatment without excessive immunoglobulin damage requires further investigation.

Conclusions

Heat-treatment of colostrum using a prototype model colostrum pasteurizer resulted in a significant reduction in colostral leukocyte viability, colostral IgG concentrations and colostral bacteria counts. Further research is needed to investigate the significance of colostral leukocytes to calf health and performance.

Acknowledgements

References

Godden, S. 2009. Colostrum management for dairy calves; Large animal proceedings of the North American Veterinary Conference, Orlando, Florida, USA, 17-21 January, 2009. Page 11.

Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells and H. Chester-Jones. 2006. Heat-treatment of bovine colostrum. II: Effects of heating duration on pathogen viability and immunoglobulin G. J. Dairy Sci. 89:3476-3483.

Johnson, J. L., S. M. Godden, T. Molitor, T. Ames and D. Hagman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. J. Dairy Sci. 90:5189-5198.

McMartin, S., S. Godden, L. Metzger, J. Feirtag, R. Bey, J. Stabel, S. Goyal, J. Fetrow, S. Wells and H. Chester-Jones. 2006. Heat treatment of bovine colostrum. I: Effects of temperature on viscosity and immunoglobulin G level. J. Dairy Sci. 89:2110-2118.