Effect of feeding heat-treated colostrum on risk for infection with *Mycobacterium avium* ssp. *paratuberculosis*, milk production, and longevity in Holstein dairy cows

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ABSTRACT

In summer 2007, a randomized controlled field trial was initiated on 6 large Midwest commercial dairy farms to investigate the effect of feeding heat-treated (HT) colostrum on transmission of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and on future milk production and longevity within the herd. On each farm, colostrum was collected daily from fresh cows, pooled, divided into 2 aliquots, and then 1 aliquot was heat-treated in a commercial batch pasteurizer at 60°C for 60 min. A sample from each batch of colostrum was collected for PCR testing (MAP-positive vs. MAP-negative). New-born heifer calves were removed from the dam within 30 to 60 min of birth and systematically assigned to be fed 3.8 L of either fresh (FR; n = 434) or heat-treated (HT; n = 490) colostrum within 2 h of birth. After reaching adulthood (>2 yr old), study animals were tested once annually for 3 yr (2010, 2011, 2012) for infection with MAP using serum ELISA and fecal culture. Lactation records describing milk production data and death or culling events were collected during the 3-yr testing period. Multivariable model logistic and linear regression was used to investigate the effect of feeding HT colostrum on risk for testing positive to MAP during the 3-yr testing period (positive/negative; logistic regression) and on first and second lactation milk yield (kg/cow; linear regression), respectively. Cox proportional hazards regression was used to investigate the effect of feeding HT colostrum on risk and time to removal from the herd. Fifteen percent of all study animals were fed PCR-positive colostrum. By the end of the 3-yr testing period, no difference was noted in the proportion of animals testing positive for MAP, with either serum ELISA or fecal culture, when comparing the HT group (10.5%) versus the FR group (8.1%). There was no effect of treatment on first- (HT = 11.797 kg;

FR = 11,671 kg) or second-lactation (HT = 11,013 kg; FR = 11,235 kg) milk production. The proportion of cows leaving the herd by study conclusion was not different for animals originally fed HT (68.0%) versus FR (71.7%) colostrum. Although a previous study showed that feeding HT colostrum (60°C for 60 min) produces short-term benefits, including improved passive transfer of IgG and reduced morbidity in the preweaning period, the current study found no benefit of feeding HT colostrum on long-term outcomes including risk for transmission of *Mycobacterium avium* ssp. *paratuberculosis*, milk production in the first and second lactation, and longevity within the herd.

Key words: colostrum, heat treatment, Johne's disease, *Mycobacterium avium* ssp. *paratuberculosis*, pasteurize

INTRODUCTION

Johne's disease is a chronic intestinal infection of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). At an estimated \$100 loss per cow in Johne's-positive herds, the disease is one of the most economically important infectious diseases of US dairy cattle, with losses attributed to progressive weight loss, reduced milk production and premature culling (USDA, 1997; Ott et al., 1999). It was recently estimated that MAP is present in 91.1% of US dairy herds (Lombard et al., 2013).

Whereas new infections do occur in adult animals (Espejo et al., 2012), Johne's disease-control programs primarily emphasize the adoption of management practices designed to prevent transmission to newborn calves and youngstock (Kudahl et al., 2008). Fecal-oral transmission from contaminated environments is deemed the most important source of exposure. However, infective colostrum or milk represents an additional potential source of exposure to MAP. This could arise from fecal contamination of milk or colostrum from teat skin during harvest, or from direct shedding of the organism into the mammary system of infected dams (Pithua et al., 2011). Sweeney et al., (1992) reported that 27% of

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subclinically infected cows had culture-positive supra-mammary lymph nodes and 12% had culture-positive milk. Streeter et al. (1995) reported that up to 22% of infected cows shed the organism in milk and colostrum. Whereas the importance of colostrum and milk in MAP transmission has not been well described, Johne's control programs generally include such management recommendations as avoiding the feeding of pooled colostrum, feeding colostrum from test-negative cows, use of colostrum-replacement products, and feeding commercial powdered milk replacers (Nielsen et al., 2008; Pithua et al., 2009, 2011).

Another strategy to mitigate this risk may be the pasteurization of milk and colostrum using commercially available on-farm pasteurization systems. The efficacy of pasteurization to destroy MAP in milk remains controversial: Most in-laboratory or on-farm inoculation studies simulating batch pasteurization (63°C for 30 min) or HTST pasteurization (72°C for 15 s; PMO, 2007) report that the process is completely effective in destroying this pathogen in milk (Stabel et al., 1996; Keswani and Frank, 1998; Grant et al., 1999; Stabel, 2001; Stabel et al., 2003). However, some other laboratory-based inoculation studies simulating HTST pasteurization have reported that small numbers of MAP colonies may remain viable if the organism is inoculated into milk at higher concentrations (Chiodini and Hermon-Taylor, 1993; Grant et al., 1996; Sung and Collins, 1998; Gao et al., 2002).

Developing a technique to pasteurize colostrum on farms has proven to be much more challenging. Early studies attempting to pasteurize colostrum using traditional Pasteurized Milk Ordinance (PMO, 2007) temperatures have yielded unacceptable results. Batch pasteurization of colostrum at 63°C × 30 min resulted in a 24 to 59% reduction in colostral IgG concentration, depending on batch size and quality, and resulted in a significant reduction in serum IgG concentrations in calves (Godden et al., 2003). Unpublished trials by the same investigator (S. Godden) found that HTST pasteurization (72°C × 15 s) resulted in a solid pudding-like product that was impossible to feed and plugged up or cooked onto equipment, resulting in significant cleaning challenges.

The aforementioned failures with traditional pasteurization temperatures led this team of researchers to develop a lower-temperature, longer-time approach to heat-treat (HT) colostrum, the goal being to reduce pathogen exposure while still preserving important colostral IgG and viscosity characteristics. Early laboratory inoculation studies reported that heating bovine colostrum at 60°C for 60 min did not damage colostral IgG but significantly reduced concentrations of viable MAP and eliminated other important pathogens, in-

cluding *Mycoplasma bovis*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* (McMartin et al., 2006; Godden et al., 2006). Several subsequent field studies have demonstrated that colostrum may be successfully heat-treated on farm (60°C × 60 min), in larger pooled batches or in individual 3.8-L aliquots, resulting in a significant reduction in total bacteria counts and total coliform counts while having no negative effect on colostral IgG (g/L), DM (%), true protein (%), crude fat (%), lactose (%), SNF (%), other solids (%), insulin (ng/mL), lactoferrin (mg/mL), and IGF (ng/mL; Johnson et al., 2007; Donahue et al., 2012; Godden et al., 2012b; Kryzer et al., 2015).

Multiple field studies have consistently reported that calves fed HT colostrum experience significantly improved efficiency of IgG absorption resulting in higher serum IgG concentrations as compared with calves fed fresh (FR) colostrum (Johnson et al., 2007; Godden et al., 2012b; Kryzer et al., 2015). Though the exact mechanism to explain this has not been confirmed, it is hypothesized that improved passive transfer occurs because of reduced bacterial interference with IgG absorption across the small intestine (James et al., 1981; Poulson et al., 2002; Peterson et al., 2008). Finally, in one multiherd randomized field trial involving 1,071 calves from 6 large commercial Midwest dairy herds, the hazard for being treated for scours or for being treated for any illness during the preweaning period was 1.32 (95% CI: 1.14, 1.53) and 1.25 (95% CI: 1.08, 1.44), respectively, for calves fed FR colostrum compared with calves fed HT colostrum (Godden et al., 2012b). A pathway analysis suggested that the health benefits associated with feeding HT colostrum may be attributed to improved passive transfer of IgG.

Based on the benefits to neonatal calves reported by these earlier studies, the US dairy industry has already begun the process of adopting the technique of HT colostrum on farms. The USDA-NAHMS 2007 Dairy study (USDA, 2008a) reported that 6.4% of large dairies (500 or more cows) and 0.8% of all dairy operations that hand-fed colostrum reported heat-treating colostrum before feeding. However, this is still a relatively new management tool and research is still needed to fully investigate if calves fed HT colostrum will also experience long-term health or performance benefits, including reduced risk for infection with MAP. In 2007, a multiherd randomized clinical trial was initiated using 1,071 newborn heifer and bull calves on 6 commercial dairy farms in Minnesota and Wisconsin; the effects of HT on colostrum characteristics and on calf health in the preweaning period have already been reported (Donahue et al., 2012; Godden et al., 2012a,b). The major objective of the current study was to follow heifer calves from this large field study into adulthood

to describe the effect of feeding HT colostrum on the risk for infection with MAP in the adult cow. Secondary objectives were to describe the effect of treatment on milk production and longevity within the herd. The null hypothesis was that feeding HT (versus FR) colostrum at birth would result in a reduced risk for infection with MAP, improved milk production, and improved longevity within the herd in the adult cow.

MATERIALS AND METHODS

Farm Enrollment

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee. Six large commercial dairy farms in Minnesota and Wisconsin participated. This was a convenience sample of herds based on herd proximity to the University of Minnesota (approximately a 250-km radius), willingness to adhere to study protocols, and having had at least 1 animal in the herd test positive for infection with MAP by fecal culture or serum ELISA within the previous 3 yr.

Colostrum Preparation

Enrollment of newborn calves was from June to August 2007. Procedures for colostrum preparation, calf enrollment, and calf sampling and data collection in the period from birth to weaning have been described in detail in previous manuscripts (Donahue et al., 2012; Godden et al., 2012a). Briefly, first milking colostrum was collected by farm personnel within 2 h postcalving and refrigerated at 4°C for 24 to 48 h before pooling. Each day, refrigerated colostrum was pooled into one batch, thoroughly mixed, and divided into equal aliquots. One aliquot was left untreated whereas the other was heat-treated in a commercial batch pasteurizer at 60°C for 60 min (Dairy Tech Inc., Greeley, CO). The on-farm batch pasteurizers were programmed to heat colostrum to a target temperature of 60°C for 60 min, with a maximum allowable fluctuation of 0.56°C during the 60-min holding phase. Colostrum was then automatically cooled to 15.6°C. Duplicate 50-mL aliquots of FR and HT colostrum were aseptically collected from each batch and frozen at -20°C for later PCR analysis for the presence of MAP. Fresh and HT colostrum was transferred to sanitized 3.8-L bottles and refrigerated for later warming and feeding to newborn calves enrolled in the study. Colostrum was generally fed within 48 h of batch preparation.

Calf Enrollment

Newborn calves were separated from the dam shortly after birth and before suckling. All live-born heifer

calves were eligible for enrollment on 5 farms, with both heifer and bull calves enrolled on the sixth farm. Calves were systematically assigned by birth order to receive 3.8 L of HT or FR colostrum as soon as possible after birth (mean = 48 min, with 95% of calves fed within 2 h). Five farms fed the entire 3.8 L volume of colostrum by esophageal tube feeder, whereas 1 farm fed colostrum first by nipple bottle, with any remaining unconsumed colostrum fed via an esophageal tube feeder. One farm offered a second feeding of 1.9 L of colostrum by nipple bottle approximately 12 h after the first feeding, whereas the remaining 5 farms fed only a single colostrum feeding. Farm personnel recorded calf enrollment information including dam and calf identification number, birth date and time, colostrum treatment group assigned, and colostrum batch fed. After the colostrum feeding, calves were moved to individual calf housing, either hutches (3 farms) or individual pens within a barn (3 farms), where they were managed according to the farm's routine feeding and management protocols. Bull calves left the study after weaning. Female calves from the HT and FR groups within each farm were comingled in group pens following weaning at approximately 56 d of age, and subsequently raised to adulthood. Farm staff recorded all treatment events, types of treatments administered, and mortality or culling events between birth and adulthood.

Adult Cow Sample and Data Collection

After reaching adulthood (>2 yr old), study heifers in each herd were tested once annually for MAP infection in early spring 2010, summer 2011, and summer 2012, with testing aimed to coincide approximately with the first, second, and third lactation, respectively. Three years of testing was performed because it is recognized that animals may take 3 to 5 yr or longer for this chronic disease to progress to the point where diagnostic tests produce a positive test result (Berghaus et al., 2005). At each annual testing event, a trained study technician collected a 10-mL venous blood sample from each study animal via the coccygeal vein using a 20-gauge, 1-inch needle. Approximately 10 g of feces was collected rectally using disposable plastic rectal examination gloves and stored in sterile plastic sample containers before being shipped for further processing and testing. All samples were immediately placed on ice and transported directly to the Minnesota Veterinary Diagnostic Laboratory located at the College of Veterinary Medicine, University of Minnesota (St. Paul, MN).

Historical records of death or culling events that occurred during the period between enrollment and first calving were retrieved at the first testing event

(2010) from commercially available dairy management software packages installed on each study farm (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). Lactation records including calving dates, conception dates, dry-off dates, milk production (kg/cow), and death or culling events and dates were also collected electronically at each of the 3 annual MAP-testing events in 2010, 2011, and 2012.

Laboratory Testing

PCR Analysis of Colostrum for MAP. One aliquot of FR frozen colostrum from each unique batch was transported on ice to the National Animal Disease Center (USDA, Agricultural Research Service, Ames, IA) for PCR analysis. The MAP DNA was extracted from colostrum specimens using real-time PCR as described elsewhere (Pithua et al., 2010). Sample runs with threshold cycles in the range of 15 to 30 cycles were declared positive. A previous validation study reported that detection rates achieved for the colostrum sample replicates inoculated with sonicated MAP cell suspension were 75% for 1×10^9 cells/mL, 100% between 1×10^7 and 1×10^8 cells/mL, 75% for 1×10^6 cells/mL, 0 for 1×10^4 cells/mL, and 25% between 1×10^1 and 1×10^3 cells/mL. When negative control colostrum samples were tested in the validation study, 16 of 18 (89%) samples were correctly identified as negative for MAP DNA (Pithua et al., 2010).

Serological Testing for MAP Antibodies. Serum derived from blood samples was tested for antibodies against MAP using a commercially available serological ELISA test kit (HerdChek, Idexx Laboratories Inc., Westbrook, ME). Seropositivity to MAP was indicated by optical density and, for each sample, the presence or absence of MAP antibodies was reported as a sample-to-positive (S/P) ratio. For serum samples collected in spring 2010 (lactation 1), samples with an S/P value ≥ 0.25 were classified as positive for MAP antibodies. However, the cutoff values for this assay were revised when the protocol was changed by Idexx in December 2010. Consequently, for serum samples collected in 2011 and 2012 (lactations 2 and 3), samples with S/P values < 0.60 , ≥ 0.6 but < 0.7 , or ≥ 0.70 were classified as negative, suspect, or positive for MAP antibodies, respectively. The manufacturer-reported specificity and sensitivity of this ELISA test kit on serum (versus fecal culture) is 99.3% (98.3–99.7%) and 51.4% (45.2–57.6%), respectively. For the purposes of our study, we classified samples with an S/P value ≥ 0.70 as positive.

Fecal Culture for MAP. The 72-h sedimentation fecal culture method was used, as described elsewhere, to cultivate MAP from fecal samples (Wells and Wagner, 2000). Briefly, sediments derived from the fecal

samples were plated onto Herrold egg yolk agar slants containing mycobactin J (Becton, Dickinson and Co., Franklin Lakes, NJ) and an antimicrobial (0.1 mL of 50 mg amphotericin B; X-GEN Pharmaceuticals Inc., Horseheads, NY) and incubated at 37°C for 3 wk. At approximately 6 wk after inoculation, the agar slants were examined weekly for growth of MAP. On the basis of total colony counts per gram of fecal sample tested, results were reported as negative (0 colonies/tube) or positive (1 to ≥ 100 colonies/tube).

Statistical Analysis

Sample Size Estimates. An a priori sample size calculation estimated that if 550 heifers were enrolled per group (with a predicted culling or death loss of 10, 12, and 22% before the first calving event, during the first lactation, and during the second lactation, respectively), then approximately 495, 436, and 340 animals per treatment group would be present for testing for MAP in lactations 1, 2, and 3, respectively. A final sample size of 340 cows per group in the third lactation would provide 95% confidence and 80% power to detect a predicted difference of approximately 10% MAP infection in cows fed FR colostrum vs 5% MAP infection in cows fed HT colostrum (1-tailed test).

Description of Cow Characteristics at Enrollment, Calving, and Testing for MAP. All data analyses were performed using SAS (version 9.3. SAS Institute, Cary, NC), with final significance declared at $P < 0.05$. Descriptive statistics were first generated by herd and overall, to describe the number of animals enrolled, the proportion of calves fed MAP PCR-positive colostrum, the proportion of animals testing positive for MAP either by serum ELISA or fecal culture during the first, second, and third annual testing events, milk production (kg) in the first and second lactation, and the proportion of animals culled by the study's conclusion in summer 2012.

Descriptive statistics were then generated by treatment group (FR vs. HT) to describe general characteristics of study cows including the proportion of animals fed MAP PCR-positive colostrum, the proportion with a first, second, and third calving event, lactation length (days), age at calving, the proportion with a first, second, and third annual MAP testing event, and age at each MAP testing event. Finally, descriptive statistics were generated to describe, by treatment group, the crude proportion of cows tested, which produced a positive MAP test result using serum ELISA or fecal culture at each of the 3 annual testing events, and for all 3 testing events combined.

To investigate if the 2 treatment groups were similar in their enrollment and testing characteristics, univari-

able logistic regression (GLIMMIX procedure) was used to investigate if there was a relationship between treatment group (FR vs. HT, explanatory variable) and each of the following dichotomous cow characteristics: odds of being fed MAP PCR-positive colostrum, odds of experiencing a first, second, or third lactation calving event, and odds of experiencing a first, second, or third annual MAP testing event. Similarly, univariable linear regression (MIXED procedure) was used to investigate if there was a relationship between treatment group and each of the following continuous cow characteristics: age (months) at the first, second, or third lactation calving event, lactation length (days), and age (months) at the first, second, or third annual testing event. Although we experimented with controlling for herd as a random effect, this variable was forced as a fixed effect into all models as this achieved better model fit (smaller -2 log-likelihood value). All models investigated for the presence of an interaction between treatment and herd but none were detected. Models were compared during the model-building process using the -2 log-likelihood statistic, and model fit was evaluated by plotting marginal and conditional residuals (for linear regression models) and by use of the Hosmer and Lemeshow goodness-of-fit test (for logistic regression models).

Effect of Feeding HT Colostrum on Transmission of MAP. Univariable logistic regression (GLIMMIX procedure) was first used to investigate the relationship between treatment (FR vs. HT, explanatory variable) and each of the following dichotomous outcome variables of interest: odds for a cow producing a positive MAP test result when using either serum ELISA alone, fecal culture alone, or for either test (parallel interpretation) at each of the 3 annual testing events, and for all 3 test events combined. Univariable logistic regression was also used to investigate if other cow or colostrum characteristics were associated with each of the aforementioned major outcome variables, including PCR test result for colostrum fed (PCR-positive or -negative), age (months) of study animals at the first, second, or third lactation calving event, lactation length (days), and age (months) at the first, second, or third annual testing event.

Main effects significant at $P < 0.20$ in the previously described univariable models were carried forward to offer to a multivariable model investigating the effect of treatment (FR vs. HT, forced) on the major outcome variables of interest: odds for a cow producing a positive MAP test result when using either serum ELISA alone, fecal culture alone, or for either test (parallel interpretation) at each of the 3 annual testing events, and for all 3 test events combined. A backward stepwise approach was then used to remove nonsignificant variables one

at a time, starting with the least-significant variable first (largest P -value), until only significant variables remained ($P < 0.05$). Potential confounders were investigated. To be a potential confounder, a variable had to be significantly associated with both the explanatory variable (treatment group) and the outcome variable of interest (MAP test result) for a given model. A variable was identified as a confounder and retained in the model if its exclusion from the model resulted in $>15\%$ change in the estimate of the effect of treatment on the outcome variable of interest. No confounders were identified. The variable describing herd was forced as a fixed effect into all models. All models investigated for the presence of an interaction between treatment and herd but none was detected. Models were compared during the model-building process using the -2 log-likelihood statistic and model fit was evaluated by use of the Hosmer and Lemeshow goodness-of-fit test.

Effect of Feeding HT Colostrum on Milk Production. As the third lactation was incomplete for a large proportion of cows at the time of study conclusion, the authors elected only to describe milk production for the first 2 lactations. This analysis included milk production data for any cow that initiated the lactation of interest, even if she did not complete said lactation. Descriptive statistics were first generated to describe, by treatment group, the age at freshening (months), lactation length (days milking), and total milk yield (kg/cow) for cows entering a first or second lactation, respectively.

Univariable linear regression (MIXED procedure) was used to describe the relationship between the outcome variables of interest (milk production in the first and second lactations, kg/cow) and each of the following potential explanatory variables: treatment (FR vs. HT), age at calving (months), lactation length (days), and whether or not the batch of colostrum originally fed had tested positive to MAP using PCR.

Main effects significant at $P < 0.20$ in the previously described univariable models were carried forward to offer to a multivariable model investigating the effect of treatment (FR vs. HT, forced) on the 2 major outcome variables of interest: milk production in the first and second lactations (kg/cow). A backward stepwise approach was then used to remove nonsignificant variables one at a time, starting with the least-significant variable (largest P -value), until only significant variables remained ($P < 0.05$). Potential confounders were investigated (definition previously described). No confounders were identified. The variable describing herd was forced as a fixed effect into all models. All models investigated for the presence of an interaction between treatment and herd but none was detected. Models were compared during the model building process using the

−2 log-likelihood statistic and model fit was evaluated by plotting marginal and conditional residuals.

Effect of Feeding HT Colostrum on Longevity. Descriptive statistics were generated to describe, by treatment group, the proportion of animals that left the herd, whether by culling or death, in the period between enrollment and the study's conclusion on the final test day in summer 2012, and to describe the median (range) age at removal for those animals that did leave the herd before the study's conclusion. A Cox proportional hazards regression model (PHREG procedure) was developed to investigate the effect of treatment on the hazard for removal from the herd. Animals still present in the herd at the study's completion were right censored on final MAP sample date in summer 2012. Variables describing colostrum treatment group (FR vs. HT) and herd were forced into the model as fixed effects. An additional covariate offered into the model was whether the batch of colostrum originally fed had tested positive to MAP using PCR. However, it was not significant and was subsequently removed. An interaction effect between treatment and herd was investigated but was found not to be present. The Cox model assumes a proportional hazard constant over time. This assumption was tested by creating a time dependent variable [treatment $\times$ log(time)] inside the PHREG procedure and testing this term for proportionality using the proportionality_test statement. The results of the proportionality test were not significant ($P = 0.12$), indicating the model did not violate the assumption that proportional hazards were constant over time.

RESULTS

Farm and Cow Characteristics

Mean (SD, range) herd size, rolling herd average milk production, bulk tank SCC, and dry period length for the 6 participating herds during the calf enrollment period were 1,617 (454; 1,200–2,500) milking cows, 12,891 (1,167; 11,280–14,512) kg of milk, 280,983 (99,986; 149,900–430,000) cells/mL, and 44 (3.7; 37–48) days dry, respectively. All 6 herds used freestall housing and managed predominantly Holstein cows, although some crossbreds were present. Five farms used group maternity pens whereas 1 farm used individual maternity pens. Four farms housed preweaned calves on the main farm whereas 2 farms housed calves off-site. Five farms fed pasteurized whole milk and 1 farm fed commercial powdered milk replacer (20:20 fat:protein, whey protein base, nonmedicated) until weaning.

Table 1 presents crude descriptive statistics describing animal enrollment and follow-up test results by

herd and overall. Fifteen percent (139 of 924) of all study calves were fed MAP PCR-positive colostrum, with rates varying among herds (minimum = 4% in herd 1; maximum = 46% in herd 6). By conclusion of the study in summer 2012, when animals averaged 61.1 (0.8) months of age, a total of 12% (87 of 723 tested) of all animals had tested positive for MAP 1 or more times using either serum ELISA or fecal culture. Mean (SD) first and second lactation milk production for all cows was 11,806 (4,659) and 11,321 (5,884) kg/cow, respectively. Seventy percent (644 of 924) of cows had left the herd by study conclusion in summer 2012 (Table 1).

Table 2 shows that, of the 924 heifer calves originally enrolled (FR = 434; HT = 490), 15.9 and 14.3% were fed MAP PCR-positive colostrum in the FR and HT group, respectively. For all calves a first, second, and third lactation calving event was reported for 87, 69.8, and 36.7%, respectively, with a mean (SD) calving age of 23.7 (1.7), 36.8 (3.1) and 49.5 (3.1) mo, respectively (Table 2). Seventy-eight (723 of 924), 45 (413 of 924), and 24% (219 of 924) of all study animals were tested for MAP in 2010, 2011, and 2012, respectively, with a mean (SD) testing age of 31.3 (0.8), 48.6 (0.8) and 61.1 (0.8) mo, respectively. The overall mean (SD) days milking for animals entering a first or second lactation was 325 (114) and 292 (121), respectively. None of the aforementioned enrollment, calving, or testing characteristics differed between the 2 treatment groups ($P > 0.05$ for all comparisons; Table 2).

Effect of Feeding HT Colostrum on Transmission of MAP

Logistic regression models (Tables 3 and 4) indicated no effect of treatment on risk for testing positive to MAP at any time during the study. When considering the cumulative test results over the entire 3-yr testing period, the herd-adjusted proportion of cows testing positive for MAP were as follows: (1) serum ELISA: FR = 3.4%, HT = 4.4%; (2) fecal culture: FR = 7.1%; HT = 8.6%; and (3) either serum ELISA or fecal culture using parallel interpretation: FR = 8.1%; HT = 10.5% (Table 4). The odds ratio for a calf fed FR (vs. HT) colostrum to test positive for MAP using either serum ELISA alone, fecal culture alone, or either test (parallel interpretation) during the entire 3-yr testing period was 0.78 (0.41 to 1.49; $P = 0.46$), 0.82 (0.49 to 1.35; $P = 0.42$), and 0.75 (0.46 to 1.20; $P = 0.23$), respectively, and was not different between treatment groups (Table 4).

It should be noted that, even though no significant interaction was detected between treatment and herd, the aforementioned analysis was repeated after stratifying the data by herd (results not shown). Similarly, even

Table 1. Crude (unadjusted) statistics describing animal enrollment, *Mycobacterium avium* ssp. *paratuberculosis* (MAP) test results, longevity, and milk production by herd and for all herds

Parameter	Herd					
	1	2	3	4	5	All
Heifer calves enrolled	174	180	149	115	132	924
Fed PCR-positive colostrum ¹	7 (4.0)	14 (7.8)	12 (8.1)	6 (5.2)	20 (15.2)	139 (15.0)
Cows tested for MAP ¹						
Spring 2010 (≈lactation 1)	146 (83.9)	112 (62.2)	123 (82.6)	100 (87.0)	106 (80.3)	723 (78.2)
Summer 2011 (≈lactation 2)	92 (52.9)	62 (34.4)	74 (49.7)	59 (51.3)	54 (40.9)	413 (44.7)
Summer 2012 (≈lactation 3)	54 (31.0)	39 (21.7)	32 (21.5)	32 (27.8)	25 (18.9)	219 (23.7)
Positive test result for MAP ²						
Spring 2010 test	34 (23.3)	3 (2.7)	5 (4.1)	2 (2.0)	2 (1.9)	48 (6.6)
Summer 2011 test	15 (16.3)	6 (9.7)	4 (5.4)	6 (10.2)	3 (5.6)	36 (8.7)
Summer 2012 test	10 (18.5)	3 (7.7)	5 (15.6)	6 (18.8)	4 (16.0)	29 (13.2)
All 3 tests combined	44 (30.1)	9 (8.0)	12 (9.8)	10 (10.0)	7 (6.6)	87 (12.0)
Milk production (kg/cow) ³						
First lactation	12,824 (4,100)	10,714 (5,689)	13,371 (4,031)	11,580 (4,339)	10,651 (3,764)	11,806 (4,659)
Second lactation	14,298 (5,254)	13,079 (6,792)	10,879 (5,701)	10,160 (4,599)	8,600 (5,553)	11,321 (5,884)
Animals culled or sold ⁴	119 (68.4)	135 (75.0)	87 (58.4)	78 (67.8)	88 (66.7)	644 (69.7)

¹Number (%) of cows originally enrolled.
²Number (%) of cows tested which produced a positive MAP test by serum ELISA or fecal culture.
³Mean (SD) total milk yield for all cows calving into the current lactation, including cows with incomplete lactations.
⁴Number (%) of cows originally enrolled that left the herd between enrollment and study conclusion in summer, 2012 (approximately 61 mo old).

though no association was detected between the MAP PCR test result for the batch fed (positive/negative) and subsequent risk for MAP infection in the adult, and no interaction was detected between treatment and MAP PCR status of the batch fed, the aforementioned analysis was repeated after stratifying the data by MAP PCR test result for the batch (positive/negative; results not shown). Neither of these 2 approaches to stratified analysis yielded a different statistical inference.

Effect of Feeding HT Colostrum on Milk Production

The overall mean (SD) milk production reported for all animals entering a first or second lactation was 11,806 (4,659) and 11,321 (5,884) kg/cow, respectively. The mean (SD) lactation length for all animals entering a first or second lactation was 325 (114) and 292 (121) days, respectively. After adjusting for herd, lactation length (days), and age at calving (months), no difference was noted in first-lactation milk production for cows originally fed FR (11,671 kg) versus HT (11,797 kg) colostrum ($P = 0.41$; Table 5). Similarly, after adjusting for herd and lactation length (days), no difference was noted in second-lactation milk production for cows originally fed FR (11,235 kg) versus HT (11,013 kg) colostrum ($P = 0.33$; Table 5).

Effect of Feeding HT Colostrum on Longevity

A total of 69.7% (644 of 924) heifer calves originally enrolled were reported as died or culled by conclusion of the study in summer 2012. The overall median (range) age at removal was 39.6 mo (minimum = 0.07; maximum = 62.8). The proportion of animals leaving the herd was not different for animals originally fed HT (68.0%; 333 of 490) versus FR (71.7%; 311 of 434) colostrum. After controlling for the fixed effect of herd, the final proportional hazards regression model showed no effect of treatment on the hazard for removal from the herd [hazard ratio for FR = 1.08 (0.92, 1.26); $P = 0.35$].

DISCUSSION

This is the first prospective field trial designed to investigate the effect of feeding HT colostrum on long-term outcomes including transmission of MAP, milk production, and longevity in the herd. Major strengths of our study were the large sample size, randomization, and the prospective nature of the study, following 924 calves in 6 commercial dairy herds from birth to approximately 61 mo of age. One limitation is that the study population, a convenience sample of large Midwest Holstein herds, may be different in some respects

Table 2. Crude (unadjusted) statistics describing enrollment, calving, and *Mycobacterium avium* ssp. *paratuberculosis* (MAP) testing events for study cows by colostrum treatment group

Parameter	Fresh colostrum	Heat-treated colostrum
Number enrolled at birth	434	490
Fed MAP PCR-positive colostrum ¹	69 (15.9%)	70 (14.3%)
Spring 2010 (≈lactation 1)		
Number (%) with a calving event ¹	379 (87.3%)	426 (86.9%)
Age at calving (mo) ²	23.8 (1.8; 20.9–31.6)	23.6 (1.6; 20.5–31.0)
Lactation length (days milking) ^{2,3}	328 (123; 0 to 826)	323 (105; 1 to 652)
Number (%) tested for MAP ¹	339 (78.1%)	384 (78.4%)
Age at MAP testing (mo) ²	31.3 (0.8; 29.4–32.9)	31.2 (0.8; 29.3–32.9)
Summer 2011 (≈lactation 2)		
Number (%) with a calving event ¹	288 (66.4)	342 (69.8)
Age at calving (mo) ²	36.9 (3.2; 32.4–50.7)	36.6 (2.9; 29.2–47.1)
Lactation length (days milking) ^{2,3}	289 (119; 1 to 568)	296 (123; 1 to 747)
Number (%) tested for MAP ¹	186 (42.9)	227 (46.3)
Age at MAP testing (mo) ²	48.6 (0.8; 46.7–50.4)	48.6 (0.8; 46.8–51.1)
Summer 2012 (≈lactation 3)		
Number (%) with a calving event ¹	153 (35.3)	186 (38.0)
Age at calving (mo) ²	49.6 (3.3; 43.9–60.8)	49.5 (3.0; 43.2–60.3)
Lactation length (days milking) ^{2,3}	238 (121; 0 to 467)	240 (114; 0 to 423)
Number (%) tested for MAP ¹	96 (22.1)	123 (25.1)
Age at MAP testing (mo) ²	61.1 (0.8; 59.6–62.7)	61.1 (0.8; 59.7–62.7)

¹Reported: Number (% of animals originally enrolled).²Reported: Crude mean (SD; range).³Includes all cows with a reported calving event.

as compared with typical Midwest or US dairy herds. Study herds were larger and produced more milk (1,617 cows; 12,891 kg/cow) as compared with the average Minnesota dairy farm enrolled on a DHIA testing service in 2010 (124 cows; 9,740 kg/cow; MN DHIA, 2010). However, study herds should be reasonably similar to other large Midwest dairy herds.

The current study found no effect of treatment on the risk for testing positive to MAP in adult animals. This was an unexpected result, as an earlier laboratory inoculation study showed that heat treatment of bovine colostrum in a batch pasteurizer at 60°C × 60 min either significantly reduced or eliminated MAP in

each of 4 replicate batches (Godden et al., 2006). As for the secondary study outcomes, we hypothesized that calves fed HT colostrum would have improved milk production and longevity, either because of reduced risk for infection with MAP or because of improved levels of serum IgG and associated improved health during the preweaning period (Godden et al., 2012b). As the current study observed no statistically discernable effect of treatment on risk for MAP infection, then improved milk production or longevity would also not be expected in the HT group, if associated with MAP infection. Observational studies have reported that successful passive transfer of IgG is associated with longer

Table 3. Crude (unadjusted) proportions of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) test-positive cows and results of logistic regression models comparing the risk of MAP infection in cows that originally received fresh (FR) or heat-treated (HT) colostrum at birth

Item	Treatment Group		Odds ratio, fresh ¹	P-value ²
	FR (n/N, %)	HT (n/N, %)		
Spring 2010 (≈31 mo/lactation 1)				
ELISA	7/339 (2.1)	7/384 (1.8)	1.34 (0.45–3.96)	0.60
Fecal culture	21/338 (6.2)	26/384 (6.8)	1.07 (0.56–2.01)	0.85
ELISA or fecal culture	21/339 (6.2)	27/384 (7.0)	1.01 (0.54–1.89)	0.98
Summer 2011 (≈49 mo/lactation 2)				
ELISA	9/186 (4.8)	12/227 (5.3)	0.99 (0.40–2.48)	0.99
Fecal culture	13/186 (7.0)	18/227 (7.9)	0.91 (0.43–1.94)	0.80
ELISA or fecal culture	15/186 (8.1)	21/227 (9.3)	0.90 (0.44–1.82)	0.76
Summer 2012 (≈61 mo/lactation 3)				
ELISA	4/96 (4.2)	14/123 (11.4)	0.33 (0.10–1.08)	0.07
Fecal culture	8/96 (8.3)	14/123 (11.4)	0.82 (0.32–2.1)	0.67
ELISA or fecal culture	8/96 (8.3)	21/123 (17.1)	0.47 (0.20–1.15)	0.10

¹Odds ratio for a positive test result for animals in the FR group (compared with the HT group as the referent).²All models control for herd effect.

Table 4. Results of final logistic models evaluating the odds for the cumulative risk of a *Mycobacterium avium* ssp. *paratuberculosis* (MAP)-positive test result in cows that originally received fresh (FR) or heat-treated (HT) colostrum at birth¹

MAP test	Variable	Proportion testing positive ² (95% CI)	<i>b</i> coefficient	SE(<i>b</i>)	Odds ratio, fresh ³ (95% CI)	<i>P</i> -value
Serum ELISA	FR	3.4 (1.9–6.1)	−0.245	0.329	0.78 (0.41, 1.49)	0.46
	HT	4.4 (2.6–7.3)	Referent	—	1.0	
Fecal culture	FR	7.1 (4.8–10.4)	−0.204	0.255	0.82 (0.49, 1.35)	0.42
	HT	8.6 (6.1–12.0)	Referent	—	1.0	
ELISA or culture	FR	8.1 (5.6–11.2)	−0.294	0.244	0.75 (0.46, 1.20)	0.23
	HT	10.5 (7.7–14.2)	Referent	—	1.0	

¹Models estimate effect of treatment on odds for a positive test result at either the first, second, or third lactation MAP testing event.

²Proportions adjusted for herd effect.

³Odds ratio for a positive test result for animals in the FR group (compared with the HT group as the referent).

term benefits including improved rate of gain and feed efficiency, reduced age at first calving, improved first- and second-lactation milk production, and reduced tendency for culling during the first lactation (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Faber et al., 2005). In spite of the fact that serum IgG concentrations were higher in the HT group (18.0 ± 1.5 g/L) as compared with the FR group (15.4 ± 1.5 g/L), it is possible that the current study observed no effect of treatment on milk production and longevity given that both groups still experienced very good overall levels of passive transfer of IgG. We did not consider it a major limitation that the study did not attempt to capture reported reasons for culling (e.g., signs of clinical

Johne's disease, infertility, low milk production), as it is well understood that comparing reported reasons for culling requires considerable caution because of between-producer differences in case definitions and recording methods (Fetrow et al., 2006).

Several possible explanations exist for why feeding HT colostrum did not reduce risk for MAP infection in adult cows, the first being that colostrum was simply not an important source of MAP exposure in the herds participating with this study. According to the PCR test used, apparently only 15% of all study calves were fed MAP-positive colostrum. Furthermore, even in PCR-positive batches, it is possible that that an infective dose was not attained in a sufficient number batches

Table 5. Results of final multivariable linear regression models evaluating the effect if feeding heat-treated colostrum on first and second lactation milk production (kg/cow)

Lactation model	Parameter	Adjusted mean (SE)	Estimate (SE)	<i>P</i> -value
Lactation 1	Intercept	NR ¹	−3,129.7 (1,186.1)	0.0085
	Treatment			
	Fresh	11,671 (113)	−126.9 (155.1)	0.41
	Heat-treated	11,797 (107)	Referent	
	Herd			<0.0001
	1	12,412 (184)	628.4 (258.3)	
	2	12,143 (188)	359.5 (258.0)	
	3	12,463 (193)	679.6 (260.4)	
	4	10,643 (212)	−1,140.9 (276.2)	
	5	10,959 (207)	−824.1 (266.7)	
	6	11,783 (175)	Referent	
	Lactation length (d)	NR	35.8 (0.69)	<0.0001
Lactation 2	Age at calving (mo)	NR	140.7 (47.5)	0.0032
	Intercept	NR	−492.0 (373.2)	0.19
	Treatment			
	Fresh	11,235 (168)	222.2 (227.2)	0.33
	Heat-treated	11,013 (155)	Referent	
	Herd			<0.0001
	1	13,391 (248)	2074.3 (363.1)	
	2	13,045 (287)	1728.2 (338.4)	
	3	10,612 (267)	−705.5 (374.6)	
	4	8,927 (320)	−2389.8 (416.4)	
	5	9,452 (301)	−1865.3 (397.5)	
	6	11,317 (262)	Referent	
	Lactation length (d)	NR	40.1 (0.95)	<0.0001

¹NR = not reported.

to allow investigators to detect a treatment effect, even if one was present. This hypothesis is indirectly supported by the finding was that there was no association between PCR test status of the colostrum fed (positive vs. negative) and the subsequent risk for a cow to testing positive to MAP. However, given that the PCR test used has been shown to have poor diagnostic sensitivity when low concentrations of the organism are present (Pithua et al., 2010), it is possible that the PCR test missed detecting MAP in some proportion of colostrum samples. It was unexpected and cannot be explained why herd one, which had the smallest proportion of calves fed PCR MAP-positive colostrum (4%), had the highest crude proportion of cows testing positive to MAP (30.1%), whereas herd 6, which had the greatest proportion of calves fed PCR MAP-positive colostrum (46%), had the smallest crude proportion of study cows testing positive to MAP (3.7%). The accuracy and utility of this PCR test to detect MAP in colostrum, as well as the relationship between colostrum MAP PCR test results and subsequent MAP infection risk, requires further investigation. Pre- and post-HT colostrum samples were not submitted for microbial culture for MAP due to financial constraints and because previous experience of our research group predicted that all FR colostrum samples would yield a result of overgrowth or contaminated, preventing the classification of all FR samples.

A second possible explanation for why the current study found no reduced risk for MAP infection in cows fed HT colostrum is that a protective treatment effect in study herds (assuming it existed) was overwhelmed or masked by other sources of MAP exposure, including in utero transmission or the environment. It may be that the HT of colostrum minimizes initial exposure for young calves but continued exposure to infection overwhelms this protection and allows colonization of the pathogen in the gut. Additionally, because both groups of calves were comingled after weaning, then (and assuming a protective treatment effect existed) it is possible that increased numbers of infected cattle within the control (FR) group could have shed the organism into the environment, thereby exposing the treated (HT) group and biasing study results toward the null. However, this potential effect of comingling would probably not become important until infected animals were older and began shedding MAP in feces. Unfortunately, providing separate housing for the 2 study groups after weaning was not feasible on these commercial dairy operations.

Whereas the aforementioned 2 hypotheses could well explain the study findings, we still believe that colostrum can be an important source of MAP infection.

This is supported by an earlier study of 12 Midwest dairy herds that reported a lower proportion of MAP-positive animals for cows originally fed a commercial plasma-derived colostrum replacer product (7.6%) versus calves fed fresh maternal colostrum (11.9%; hazard ratio for replacer = 0.56; $P = 0.056$; Pithua et al., 2009). Apart from potential differences in treatment efficacy, many other variables could potentially explain the different findings between these 2 studies, including differences in herds, years, management, and levels of exposure to MAP from colostrum or from other sources. Unfortunately, this earlier study did not test colostrum samples for the presence of MAP, and so direct comparisons of frequency of exposure from colostrum cannot be made between the 2 studies.

A third and final possible explanation for the current study results is that the 60×60 HT protocol may not be sufficiently effective in eliminating or reducing viable MAP to below an infective dose. The earlier laboratory-based inoculation study reported that this HT protocol reduced or eliminated viable MAP in each of 4 replicate batches when inoculated at 10^3 cfu/mL. However, it must be noted that viable MAP was still present in 1 of the 4 batches up to 90 min after treatment (Godden et al., 2006). Further evidence is lent to this hypothesis by a recent in-laboratory inoculation study conducted at the Razi Institute in Tehran (M. Noori, Razi Institute, Tehran, Iran; unpublished data): 8 replicate 4.5-L batches of colostrum were inoculated with *E. coli* (10^6 cfu/mL), *S. enteritidis* (10^6 cfu/mL), *Mycobacterium avium* ssp. *avium* (10^3 cfu/mL), and *Mycobacterium avium* ssp. *paratuberculosis* (10^3 cfu/mL) and then heat-treated in a commercial on-farm batch pasteurization system at 60°C . Similar to the earlier study by Godden et al. (2006), viable MAP was present in 1 batch at 75 min and a full 90 min was required to eliminate the organism from all 8 batches (M. Noori, Razi Institute of Tehran, Karaj, Iran, personal communication).

If either or both of the aforementioned first 2 hypotheses (i.e., inadequate MAP exposure via colostrum or overwhelming MAP exposure from other sources) represent the true explanation for why the current study did not observe a reduction in MAP in cows fed HT colostrum then this would be acceptable, although not ideal. Thus, producers could continue to use the 60×60 HT protocol for its documented short-term benefits on calf health, understanding that the longer term benefit of a reduced prevalence of Johne's disease might not be realized. However, if the real explanation for these findings is that the $60^\circ\text{C} \times 60$ min HT protocol simply fails to reduce MAP to below infective levels, then this could raise a concern in that many producers pool colostrum

before HT using a batch pasteurizer. The nature of the relationship between feeding pooled colostrum and risk for MAP transmission is not well defined. One retrospective case-control study of 93,994 cow records from 808 Danish herds reported a positive relationship, with a small but statistically significant increase in the odds ratio (1.24) of being ELISA-positive for animals raised on farms that fed pooled colostrum as compared with animals raised on farms that fed colostrum from only 1 cow (Nielsen et al., 2008). Conversely, a cross-sectional observational study of 815 US dairy operations reported a negative relationship between the practice of feeding pooled colostrum and risk for a herd reporting 1 or more clinical cases of Johne's disease in the previous year (Berghaus et al., 2005). Fully recognizing the dangers inherent when trying to draw causal inferences from observational studies, and given the equivocal findings from studies to date, we must conclude that the nature and magnitude of risk introduced by feeding pooled colostrum has not been adequately described. Still, most veterinarians and Johne's disease researchers will agree that the practice of pooling has the potential to expose more calves to infectious organisms including MAP.

If it is determined that the 60 × 60 HT protocol is not sufficiently effective at eliminating or reducing viable MAP to below infective levels, then it needs to be investigated whether the protocol can be further improved. Any modifications would require enhancing the reduction of viable MAP while still preserving the important protective colostral IgG. As the first laboratory-based study demonstrated that bovine colostrum could be held at 60°C for as long as 120 min without causing a significant loss of colostral IgG, then one obvious protocol modification to explore would be to increase the duration of heat-treatment (McMartin et al., 2006). However, before that idea can be considered for adoption, it must first be verified that the earlier laboratory-based findings can be consistently replicated under field conditions without causing harm to colostrum quality or feeding characteristics. In the meantime, given that any possible increase in risk of MAP infection introduced by pooling is likely small in magnitude (Nielsen et al., 2008), and given the short-term health benefits known to be realized by using the 60 × 60 HT protocol, we do not believe that producers should discontinue heat-treating colostrum. However, and until better information is available, producers and veterinarians will need to consider this question and make their own decision on a case-by-case basis. The potential risk introduced by pooling will not be a concern to producers using The Perfect Udder Colostrum Management System (Dairy Tech Inc.) to HT colostrum from individual cows in single use disposable 3- or 4-L bags (Kryzer et al., 2015).

CONCLUSIONS

Although an earlier phase of our research demonstrated that feeding HT colostrum (60°C for 60 min) produces short-term benefits including improved passive transfer of IgG and reduced morbidity in the pre-weaning period, the current study found no beneficial effect of feeding HT colostrum on long-term outcomes, including risk for transmission of *Mycobacterium avium* ssp. *paratuberculosis*, milk production in the first and second lactation, and longevity within the herd. Future research must investigate if this lack of effect is attributable to other factors or if the 60 × 60 HT protocol needs to be modified to improve its effectiveness in reducing viable MAP in bovine colostrum.

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