Pasteurization of Colostrum Reduces the Incidence of Paratuberculosis in Neonatal Dairy Calves

J. R. Stabel¹

USDA-ARS, National Animal Disease Center, 2300 Dayton Rd., Ames, IA 50010

ABSTRACT

In the present study, the potential benefits of feeding pasteurized colostrum were demonstrated in calves born to dams naturally infected with *Mycobacterium avium* ssp. *paratuberculosis*. Calves were separated at birth from their dams and randomly allocated into a group fed either the colostrum of their dam (DC; n = 6), followed by feeding the milk of the dam for 3 wk and then milk replacer, or into a group fed pooled pasteurized colostrum (PC; n = 5) from healthy noninfected dams, followed by milk replacer. At 6 wk of age, calves were weaned onto calf starter, housed together, and fed in a similar manner throughout the rest of the 12-mo study. Calves were necropsied at the end of the study, and 25 tissue sites were sampled from each animal and cultured for *M. avium* ssp. *paratuberculosis*. Sixteen of the 25 tissue sites were positive for calves across both treatment groups, with 14 of the 16 tissue sites positive for DC calves and 9 of the 16 tissue sites positive for PC calves. The degree of colonization within a tissue was low and variable for calves within treatment groups, and fecal shedding of *M. avium* ssp. *paratuberculosis* was minimal during the 12-mo study. As a measure of the early immune response to infection, blood obtained from calves was stimulated in vitro with *M. avium* ssp. *paratuberculosis* antigen preparations, and IFN- γ secretion was measured. Antigen-specific IFN- γ was consistently greater throughout the study in DC calves (0.95 ± 0.19) compared with PC calves (0.43 ± 0.10). Although long-term benefits are unknown, these results indicate that feeding a source of colostrum from paratuberculosis-free dams may decrease the initial exposure of neonates to *M. avium* ssp. *paratuberculosis*, perhaps decreasing dissemination of infection over time.

Key words: *Mycobacterium avium* ssp. *paratuberculosis*, colostrum, dairy calf

INTRODUCTION

Current prevalence estimates for the herd-level incidence of paratuberculosis (Johne's disease) are not available, but it is clear that this disease has gained a major foothold in the dairy industry. The most recent comprehensive estimates of herd-level prevalence in the United States were amassed in the 1996 National Animal Health Monitoring System dairy survey, from which it was suggested that approximately 22% of dairy herds had greater than 10% incidence of clinical disease (Wells and Wagner, 2000). Many producers have voluntarily focused their efforts in reducing the spread of paratuberculosis to their young stock by following guidelines such as separating the calves at birth from their dams, minimizing their exposure to manure, and not allowing shared feed and water between calves and adult animals (McKenna et al., 2006). Although primarily transmitted by the fecal-oral route, *Mycobacterium avium* ssp. *paratuberculosis* is also shed into the colostrum and milk of infected dams (Sweeney et al., 1992a; Streeter et al., 1995; Stabel and Goff, 2004a). Therefore, feeding colostrum and waste milk from infected or cows of unknown infection status may result in further spread of this disease. In recent years, producers have been willing to implement changes in management such as feeding pooled colostrum from known paratuberculosis-free cows or commercial colostrum and milk replacers to circumvent the potential transmission of this pathogen from dam to calf. Another option that has recently been adopted is the use of on-farm pasteurizer units for heat treatment of colostrum or milk. On-farm pasteurization has been shown to successfully decrease or eliminate *M. avium* ssp. *paratuberculosis* as well as other neonatal pathogens such as *Salmonella* spp. and *Mycoplasma* spp. (Butler et al., 2000; Stabel et al., 2004b; Godden et al., 2006). More importantly, an economic benefit has been demonstrated from feeding pasteurized colostrum and waste milk to calves not only by saving money on the purchase of commercial products but also on the increased profit margin per calf through reduced morbidity rates and greater gains and weaning weights (Jamaluddin et al., 1996; Godden et al., 2005). Specific effects of feeding pasteurized colostrum on the level of

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¹Corresponding author: judy.stabel@ars.usda.gov

M. avium ssp. *paratuberculosis* infection within the exposed host over time have not been reported. The present study was designed to evaluate the potential benefit of feeding pasteurized colostrum in the retardation of infection to neonatal calves born to naturally infected dams as compared with calves fed colostrum and milk of their dams.

MATERIALS AND METHODS

Animals

Neonatal Holstein dairy calves were born to cows naturally infected with *M. avium* ssp. *paratuberculosis* as part of the on-site herd at the National Animal Disease Center (NADC), Ames, Iowa. Fecal and blood samples were obtained from the dams at 21 d before calving to appropriately assess their infection status. Dams were defined as subclinical or clinically infected by analyses for IFN- γ , ELISA antibody titers, and fecal shedding. Cows were considered subclinical if they were shedding low levels of *M. avium* ssp. *paratuberculosis* in their feces (<10 cfu/slant), had positive antigen-specific IFN- γ responses, and low to negligible antibody titers and were asymptomatic. Clinically infected dams characteristically had high serum antibody titers, low antigen-specific IFN- γ responses, and were shedding high numbers of *M. avium* ssp. *paratuberculosis* (>100 cfu/slant) in their feces. All dams in the present study were categorized by the above criteria as subclinical with the exception of 2 dams that fit the criteria for clinical infection.

Colostrum samples were obtained at calving, and milk samples were obtained every 3 d after calving for 3 wk to assess shedding of *M. avium* ssp. *paratuberculosis* into the milk during the periparturient period and the potential exposure of calves in the study to this pathogen. Fecal samples were cultured on Herrold's egg yolk medium (NADC) containing 2 mg/L of mycobactin J (Allied Monitor, Fayette, MO), naladixic acid (Sigma Chemical Co., St. Louis, MO; 50 μ g/mL), and vancomycin (Sigma; 50 μ g/mL) using the double decontamination and centrifugation method (Stabel, 1997). After 12 wk of incubation at 39°C, culture slants were monitored for the presence of colonies of *M. avium* ssp. *paratuberculosis* and enumerated. Colostrum and milk samples were cultured for 8 wk using Bactec liquid medium (Becton Dickinson, Sparks, MD) for the presence of *M. avium* ssp. *paratuberculosis* (Stabel and Lambert, 2004c). Due to the difficulty in culturing colostrum and milk, these samples were also subjected to DNA extraction followed by a nested PCR for the IS900 target gene to determine the presence of *M. avium* ssp. *paratuberculosis* (Stabel and Lambert, 2004c).

Calves were randomly assigned at birth to 2 treatment groups: 1) colostrum of the dam (DC), $n = 6$, and 2) pasteurized colostrum (PC), $n = 5$, with the exclusion of the calves born to the 2 clinical dams. These calves were stratified across the 2 treatment groups to provide equal weight for potential infection status within a treatment group. Calves assigned to the DC group were allowed to suckle their dam within 8 h after birth and then were physically separated from them for the remainder of the study. Milk obtained from the dams at the daily milking was fed to the DC calves for the first 3 wk of the study and then the calves were switched to commercial milk replacer for an additional 3 wk. The PC calves were caught at birth, immediately separated from their dams without suckling, and were fed 2 bottles (2 L each) of pasteurized pooled colostrum the first day. The colostrum had previously been obtained from 3 test-negative cows and stored frozen. It was thawed, pooled, and heated at 65°C for 30 min to simulate the holder method of pasteurization. This method is the standard protocol for batch processing of waste milk and colostrum on-farm and has been shown to be effective in the destruction of *M. avium* ssp. *paratuberculosis* (Stabel, 2001). The donor cows were part of a resident herd used for periparturient studies and had previously tested negative for paratuberculosis by serology and fecal culture with no historical evidence of infection within the herd. The PC calves were then fed 2.0 L of a commercial milk replacer containing 22% crude protein and 20% crude fat (Land O Lakes, Shoreview, MN) 2 $\times$ per day for 6 wk. All calves regardless of treatment group were weaned onto texturized calf starter containing 18% crude protein and 3% crude fat (Kent Feeds, Muscatine, IA) and then were gradually switched over to a mixed pelleted ration of corn, wheat middlings, and soybean meal (Mid-State Milling, State Center, IA) and hay cubes for the remainder of the study. Upon weaning, all calves were group-housed with no separation of treatment groups for the remainder of the 12-mo study. All animals were housed in Biosafety Level 2 facilities, and all procedures performed on animals were approved by the NADC Institutional Animal Care and Use Committee.

Calf Sampling and Analyses

Fecal samples obtained monthly from calves were cultured for *M. avium* ssp. *paratuberculosis* by the double decontamination and centrifugation method (Stabel, 1997). Upon termination of the study, calves were necropsied, and 25 tissues, including duodenum, ileum, and jejunum; ileocecal valve; spiral colon; and their associated lymph nodes; transverse and descending colon; colic, hepatic, and iliac lymph nodes; spleen

and liver, were excised with sterile scalpels and scissors. It was not always possible to obtain 3 distinct lymph nodes for the sections of ileum due to the shorter length of this intestinal segment; therefore, the number of ileal-associated lymph nodes averaged 1 to 2 per animal. Tissues were placed onto a sterile cutting surface, trimmed, placed into sterile bags, and stored at -70°C until assayed. Tissues were thawed and processed for culture and PCR to assess colonization of tissues with *M. avium* ssp. *paratuberculosis*. Tissues were weighed and homogenized in 0.75% hexadecylpyridinium chloride solution by use of a stomacher for 1 min and allowed to stand overnight to decontaminate the cultures. Dilutions of individual tissue homogenates were inoculated onto Herrold's egg yolk medium (NADC) containing 2 mg/L of mycobactin J (Allied Monitor), naladixic acid (Sigma; 50 $\mu\text{g/mL}$), and vancomycin (Sigma; 50 $\mu\text{g/mL}$). After 12 wk of incubation at 39°C , culture slants were monitored for the presence of colonies and enumerated. Colony confirmation was performed by PCR for the IS900 target sequence (Stabel et al., 2003).

Blood samples were obtained on d 0 (birth) from calves and monthly thereafter for up to 12 mo. To assay for secreted IFN- γ , aliquots of heparinized whole blood (1 mL) were cultured in 24-well plates either alone (nonstimulated), with 10 $\mu\text{g/mL}$ of either concanavalin A (**ConA**; Sigma), pokeweed mitogen (**PWM**; Sigma), avium-purified protein derivative (**AvPPD**; National Veterinary Services Laboratory, Ames, IA); johnin-purified protein derivative (**JPPD**; National Veterinary Services Laboratory), or a whole-cell sonicate of *M. avium* ssp. *paratuberculosis* (strain 19698, **MPS**; NADC). The MPS was prepared by the sonication and centrifugation of *M. avium* ssp. *paratuberculosis* grown to log phase in M7H9 medium (Stabel and Whitlock, 2001). After incubation of whole blood with the stimulants for 18 h at 39°C in a 5% CO_2 humidified atmosphere, plates were centrifuged at $500 \times g$ for 15 min, and the plasma was harvested from each well. Plasma samples were frozen at -20°C until assayed for IFN- γ by ELISA using a commercial kit (Bovigam, Prionics, Lincoln, NE). Samples were analyzed in duplicate and were determined to be positive for IFN- γ production if the absorbance (**Abs**_{450nm}) of the stimulated sample (ConA, PWM, AvPPD, JPPD, MPS) was 0.1 units greater than the absorbance of the nonstimulated well for that animal at that bleeding time. Serum antibody titers were determined using a commercial ELISA kit for *M. avium* ssp. *paratuberculosis* (Parachek, Prionics). Serum IgG levels were determined in calf serum samples taken on d 0 before suckling or colostrum feeding and in samples obtained at 1 mo of age using a total Bovine IgG ELISA kit (Bethyl Laboratories, Montgomery, TX).

Statistical Analyses

Analyses were performed using PROC MIXED statistical analyses with SAS Version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of treatment group and time (months after birth), random effects of calf within treatment group by time, treatment $\times$ time interactions, and the residual error. The ELISA data took into account the covariance structure associated with repeated measures for each animal, and utilized Tukey's adjustment for multiple comparisons among the fixed effects.

RESULTS

Serologic analyses run on samples collected from dams 21 d before calving are presented in Table 1. Antigen-specific IFN- γ responses were not different between the 2 groups of dams, averaging 0.33 ± 0.16 and 0.18 ± 0.06 **Abs**_{450nm}, respectively, for the DC and PC dams. The ELISA titers for *M. avium* ssp. *paratuberculosis*-specific antibody were also similar between the 2 groups of dams. The identification of positive fecal, colostrum, and milk samples from dams was equally weighted between the 2 groups in the present study (Table 1).

The nonspecific IFN- γ responses to medium control and T-cell mitogens, ConA and PWM, are presented in Figure 1. Constitutive IFN- γ responses without stimulation of cells were similar for calves in both treatment groups and remained fairly constant throughout the 12-mo study (Figure 1A). In contrast, responses to ConA and PWM increased ($P < 0.05$) by 1 mo in calves regardless of treatment group, and highly significant ($P < 0.001$) time effects (months after birth) were noted for both mitogens throughout the study. Concentrations of ConA-stimulated IFN- γ remained steady throughout the study and did not differ between treatments. The response of cells to PWM was more robust and was also phasic, with decreased responses noted at 3 and 9 mo compared with other sampling dates.

Results for antigen-specific IFN- γ secretion after stimulation of cells with AvPPD, JPPD, or MPS demonstrated differences ($P < 0.005$) in DC calves compared with PC calves (Figure 2). Antigen-specific IFN- γ remained relatively low until 3 mo of age, regardless of treatment group or the antigen preparation used. By 5 mo of age, DC calves had greater IFN- γ responses to the antigen preparations (AvPPD, $P < 0.05$; JPPD, $P < 0.03$; MPS, $P < 0.03$) compared with PC calves. Antigen-specific IFN- γ responses continued to increase throughout the remaining months of the study in DC calves. By 7 mo, antigen-specific IFN- γ responses were observed for PC calves, albeit at a lower level than

Table 1. Serologic and fecal shedding data (mean $\pm$ SEM) for naturally infected dams randomly assigned to treatment groups and utilized as the source of neonatal calves

Measurement ¹	Treatment	
	DC	PC
IFN- γ (Abs _{450nm})	0.33 $\pm$ 0.16	0.18 $\pm$ 0.06
ELISA, S/P	0.33 $\pm$ 0.21	0.41 $\pm$ 0.37
(range of values)	(0 to 1.33)	(0.03 to 1.87)
Fecal culture positive ²	3/6	2/5
Colostrum or milk culture positive ²	4/6	3/5
Colostrum or milk PCR positive ²	3/6	4/5

¹Abs_{450nm} = absorbance at 450 nm; S/P = sample to positive control ratio as per the manufacturer's instructions.

²Values represent the number of dams for each group of neonatal calves that had positive culture and PCR results for feces, colostrum, and milk.

noted for DC calves. Responses continued to increase for PC calves throughout the remainder of the study, following a pattern similar to that noted for DC calves; however, values were attenuated. The increase in IFN- γ responses noted in calves, regardless of treatment group, throughout the 12-mo period resulted in highly significant ($P < 0.0001$) time effects for each of the antigen preparations.

Serum antibody responses specific for *M. avium* ssp. *paratuberculosis* were negligible with only 2 of the 6 DC calves having a positive antibody titer by the end of the 12-mo study. Total serum IgG was measured in calves on d 0 and at 1 mo of age (data not shown). Serum samples taken on d 0 were taken before suckling of dams and contained nondetectable levels of serum IgG. At 1 mo, calves in both groups had greater ($P < 0.01$) levels of serum IgG compared with d 0, but mean values were similar between treatment groups, averaging $2,094 \pm 412$ and $1,530 \pm 461$ mg/dL for DC and PC calves, respectively. Total IgG concentrations in the colostrum from the DC dams ranged from 4,340 to 6,450 mg/dL with an average value of $5,800 \pm 500$ mg/dL. Similarly, the IgG content of the pooled pasteurized colostrum provided to PC calves was 4,800 mg/dL.

Shedding of *M. avium* ssp. *paratuberculosis* in the feces was monitored on a monthly basis throughout the 12-mo study to assess the infection status of calves. Fecal shedding was minimal and sporadic during the study (Table 2). Only 5 calves had positive samples at any of the time points, 2 calves from the DC group and 3 calves from the PC group. Upon termination of the study, a comprehensive list of 25 tissues and lymph nodes was collected from each calf and cultured for viable *M. avium* ssp. *paratuberculosis* (Table 3). Of the 25 tissue sites sampled from each calf, no viable *M. avium* ssp. *paratuberculosis* was recovered from the spleen, liver, or the proximal ileum and its associated lymph node (data not shown). In addition, other tissues from which *M. avium* ssp. *paratuberculosis* was

not recovered were the duodenum, proximal jejunum, and other intestinal lymph nodes (Table 3). Results demonstrated colonization in 16 of the different tissue sites regardless of treatment group. Interestingly, the transcending colon and the spiral colon lymph node were most frequently recorded as positive for *M. avium* ssp. *paratuberculosis*, followed by the distal jejunum and iliac lymph nodes. In total, 14 of the 25 tissue sites were positive for the DC calves compared with 9 positive of the 25 for the PC calves. Despite the relatively high number of positive tissue sites per treatment, the tissue site at times was positive for only 1 calf in the group, resulting in a low number of total positive tissues from which viable *M. avium* ssp. *paratuberculosis* was recovered for each treatment group (Table 3). Only 19 of 150 (12.7%) total tissues and 15 of 125 (12%) total tissues recovered from DC and PC calves, respectively, were positive for *M. avium* ssp. *paratuberculosis*.

DISCUSSION

Reducing calfhod morbidity and mortality rates is a major goal of dairy producers. A calf that begins its life with a strong immune system is likely to present fewer problems to the producer over its production lifespan. The main method of ensuring a healthy calf is to be certain that it receives colostral antibodies within the first 24 h of life (Matte et al., 1982). A key management tool for the control of paratuberculosis in dairy herds is to feed them colostrum from healthy paratuberculosis-free cows, to use commercial colostrum replacers, or to pasteurize colostrum on-site before feeding to minimize the risk of exposure to pathogens, including *M. avium* ssp. *paratuberculosis* (Goodger et al., 1996; Barrington et al., 2002). Heat treatment of colostrum using both batch and high-temperature, short-time commercial pasteurizer units has been shown to be effective in reducing the number of viable *M. avium* ssp. *paratuberculosis* organisms, but little is known about

the potential benefits of feeding pasteurized colostrum on the incidence of paratuberculosis in adult animals (Stabel et al., 2004b; Godden et al., 2006).

Results of the present study suggest that feeding pasteurized colostrum may decrease the degree of severity of *M. avium* ssp. *paratuberculosis* infection in young calves. A whole-blood assay for secreted IFN- γ was utilized as a measure of exposure of the calves to the bacterial pathogen. A measurable IFN- γ response to *M. avium* ssp. *paratuberculosis* antigens is suggestive of a recall T-cell response by the host, indicating

prior exposure to the mycobacterium (Waters et al., 2003; Stabel et al., 2007). Previous studies have demonstrated a utility for antigen-specific IFN- γ responses in the detection of early *M. avium* ssp. *paratuberculosis* infection in asymptomatic animals (Stabel, 2001; Huda et al., 2004; Robbe-Austerman et al., 2006). The lower IFN- γ response in PC calves suggests that the exposure

Figure 1. Interferon- γ (Abs_{450nm}, absorbance at 450 nm) responses in calves after incubation of whole blood with (A) medium control (NS), (B) concanavalin A (ConA), or (C) pokeweed mitogen (PWM) for 18 h. Calves were fed either colostrum from their dam (DC) or pooled pasteurized colostrum (PC) from a noninfected source at birth. Data are presented as means $\pm$ SEM; treatment effect for ConA: $P < 0.03$; time effect for NS and PWM: $P < 0.0001$; time effect for ConA: $P < 0.001$.

Figure 2. Interferon- γ (Abs_{450nm}, absorbance at 450 nm) responses in calves after incubation of whole blood with antigens: (A) avium-purified protein derivative (AvPPD), (B) johnin-purified protein derivative (JPPD), or (C) a whole-cell sonicate of *Mycobacterium avium* ssp. *paratuberculosis* (MPS) for 18 h. Calves were fed either colostrum from their dam (DC) or pooled pasteurized colostrum (PC) from a noninfected source at birth. Data are presented as means $\pm$ SEM; treatment effects for AvPPD, JPPD, and MPS: $P < 0.005$; time effects for AvPPD, JPPD, and MPS: $P < 0.0001$.

to the bacterium was lower than that of DC calves. Results for the tissue culture analyses would support this hypothesis. Although neither the total number of tissues positive for *M. avium* ssp. *paratuberculosis* nor the level of colonization within the tissues differed between PC and DC calves, the number of tissue sites that were positive was significantly greater for DC calves than the PC calves. These results would indicate that the pathogen was able to disseminate more efficiently in the calves fed the colostrum of their dam.

Despite the aforementioned results, the present study does not fully define the route of exposure of these calves to *M. avium* ssp. *paratuberculosis*. It is well documented that the fetus can become infected by intrauterine exposure to *M. avium* ssp. *paratuberculosis*, accounting for 9 to 26% of cases of calfhoo infection from subclinically infected dams (Seitz et al., 1989; Sweeney et al., 1992b). A recent metaanalysis of previously published data in this area suggests that the degree of intrauterine infection is dependent upon 2 major factors: the prevalence of infection within the herd and the degree of infection within the dam, with greater levels of dam-fetus transmission occurring in clinically affected cows compared with asymptomatic cows (Whittington and Windsor, 2007). All dams in the present study were classified as subclinically infected except for 2 cows that were in the clinical stage of disease. Calves from the clinical cows were stratified equally across the treatment groups, with the remaining calves born to subclinical dams randomly distributed into the 2 groups. Therefore, bias according to the infection status of the dam was avoided, and calves had an equal likelihood of developing paratuberculosis based upon intrauterine exposure. Because of the difficulty in tracking exposure to *M. avium* ssp. *paratuberculosis* in young calves, it is relatively unclear to what extent animals that are exposed intrauterine to *M. avium* ssp. *paratuberculosis* will eventually demonstrate signs of infection. Two previous studies have reported that calves born to infected dams and maintained in a clean environment throughout their lifetime had culture-positive tissues at necropsy (Dunkin, 1935; Manning et al., 2003). Although the observations in each of these studies were based upon 1 calf, more recent evidence would suggest a high likelihood of intrauterine infection in asymptomatic animals. A retrospective analysis of longitudinal serologic test results captured over a 4-yr period in a dairy herd with an initial herd prevalence of 23% suggested that cows born to seropositive dams were up to 6.6 times more likely to seroconvert at some point in their lifetime than cows born to seronegative dams (Aly and Thurmond, 2005). Additionally, a recent study in red deer (*Cervus elaphus*) concluded that the transmission rate from asymptomatic dam to

Table 2. Fecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* for calves fed colostrum of their dam (DC) or pasteurized colostrum (PC) during the study¹

Age of calf	Treatment	
	DC	PC
3 mo	Calf 55 – 0, 1, 0, 6 Calf 16 – 1, 3, 3, 3 Neg	Calf 18 – 0, 0, 0, 1 Calf 62 – 1, 0, 0, 0
7 mo	Calf 16 – 5, 0, 0, 0	Calf 21 – 1, 0, 0, 1
8 mo	Neg	Calf 62 – 1, 0, 0, 0
9 mo	Neg	Calf 62 – 1, 0, 0, 0

¹Numbers listed for each positive calf represent the colony-forming units of *M. avium* ssp. *paratuberculosis* on each of 4 slants of Herrold's egg yolk medium set up per fecal sample. Neg = negative fecal culture for calves at that time point.

fetus was extremely high, averaging 78% (Thompson et al., 2007). Further, a high percentage of the mammary glands (69%) and mammary lymph nodes (80%) were culture positive for *M. avium* ssp., suggesting that colostrum and milk from the dams would be a potential risk factor for infection (Thompson et al., 2007). To our knowledge, the present study is the first to demonstrate in a controlled manner that intrauterine exposure to *M. avium* ssp. *paratuberculosis* will result in the infection of a significant number of calves, particularly for calves born to asymptomatic dams.

In summary, calves fed colostrum and milk from their respective dams had more robust antigen-specific IFN- γ responses over the 12-mo study period as com-

Table 3. Number of positive tissue sites after culture for viable *Mycobacterium avium* ssp. *paratuberculosis* from tissues of calves fed either the colostrum of their dam (DC) or pasteurized colostrum (PC)¹

Tissue	Treatment	
	DC	PC
Duodenum/LN ²	Neg/Neg	Neg/Pos (1)
Proximal jejunum/LN	Neg/Pos (1)	Neg/Neg
Mid-jejunum/LN	Pos/Neg (1)	Neg/Neg
Distal jejunum/LN	Pos/Pos (3)	Neg/Pos (2)
Mid-ileum/LN	Pos/Neg (1)	Neg/Neg
Distal ileum/LN	Pos/Neg (1)	Pos/Neg (1)
Ileocecal LN	Pos (1)	Neg
Ileocecal valve	Pos (1)	Pos (1)
Spiral colon/LN	Pos/Pos (3)	Neg/Pos (3)
Transverse colon	Pos (3)	Pos (2)
Descending colon	Pos (1)	Pos (1)
Colic LN	Neg	Pos (2)
Hepatic LN	Pos (1)	Neg
Iliac LN	Pos (2)	Pos (2)
Positive tissue sites	14/25	9/25

¹Neg = negative culture results; Pos = positive culture on Herrold's egg yolk medium.

²Intestinal tissues are listed in tandem with their respective lymph nodes (LN), representing 2 tissue sites. Numbers in parentheses are the number of calves positive for that tissue site or sites.

pared with calves fed the pasteurized pooled colostrum from noninfected dams. In addition, strong antigen-specific IFN- γ responses were evident 1 mo earlier for DC compared with PC calves. These results suggest that the level of exposure for DC calves may have been greater than for PC calves. At necropsy, a greater percentage of tissue sites were colonized with *M. avium* ssp. *paratuberculosis* in DC calves, suggesting a more widespread infection. The long-term benefits of feeding pasteurized colostrum to neonatal calves for the specific purpose of disease retardation have not been fully addressed, particularly for paratuberculosis. However, data from the present study suggest that feeding pasteurized colostrum is a useful management tool that may decrease initial exposure to *M. avium* ssp. *paratuberculosis*.

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