

Fecal shedding of coliform bacteria during the periparturient period in dairy cows

Lisa S. Pelan-Mattocks, MS; Marcus E. Kehrl Jr, DVM, PhD; Thomas A. Casey, PhD; Jesse P. Goff, DVM, PhD

Objective—To determine whether numbers of coliform bacteria in feces of dairy cattle changed during the periparturient period and whether fluctuations were associated with changes in dry-matter intake.

Animals—12 healthy Holstein cows.

Procedure—Fecal samples were collected on a semi-regular basis (ie, 3 to 7 times/wk) beginning 4 to 6 weeks before the anticipated parturition date and continuing through the third day (5 cows) or second week (7 cows) after parturition, and total numbers of fecal coliform bacteria were determined. Daily feed intake of 7 cows was monitored.

Results—For 11 cows, fecal coliform bacterial counts between 34 and 25 days prior to parturition were low and relatively constant ($< 10^2$ change in number of bacteria). Coliform bacteria were not detected in 4 to 8% of fecal samples from 10 cows. All cows had a 10^4 to 10^7 increase in number of colony forming units/g of feces near the time of parturition. Number of fecal coliform bacteria peaked within 7 days of parturition in 9 cows and within 12 days of parturition in 3. Number of fecal coliform bacteria was not correlated with feed intake.

Conclusions and Clinical Relevance—Cows may have large increases in fecal coliform bacteria count during the periparturient period; however, periparturient cows do not continually shed high numbers of coliform bacteria, and coliform bacteria may not always be detectable by conventional culture methods. Changes in fecal coliform bacteria count did not correlate with changes in dry-matter intake. (*Am J Vet Res* 2000;61:1636-1638)

In cows, intramammary infection (IMI) can be caused by exposure of the teat ends to opportunistic environmental pathogens, such as coliform bacteria and streptococci other than *Streptococcus agalactiae*.^{1,2} These pathogens are often found in organic bedding material, manure-covered pens and yards, heavily contaminated water, and areas with wet or damp floors.^{2,3} Although disinfection of teat ends after milking, proper maintenance of milking equipment, and intramammary treat-

ment of cows at the end of lactation are effective for reducing numbers of contagious pathogens, such methods are usually ineffective at reducing prevalence of IMI caused by environmental pathogens.⁴

On well-managed dairies with low herd somatic cell counts, clinical mastitis caused by environmental pathogens, rather than subclinical or clinical mastitis caused by contagious pathogens, is the most common type of mastitis.^{1,5,6} There is concern that prevalence of IMI caused by environmental pathogens may be increasing,^{7,8} even though detection of environmental pathogens can be difficult because IMI caused by such pathogens are often of short duration.¹ This increase in prevalence may be attributable to a decrease in the percentage of quarters infected with contagious pathogens, or may be associated with the trend toward more confinement housing, larger herd size, and shorter time on pasture.⁸ As the available housing area per cow decreases, the incidence of coliform mastitis increases.²

Among periparturient cows, the incidence of IMI and clinical mastitis caused by coliform bacteria is high. For instance, in one study,⁹ 11 of 14 episodes of *Escherichia coli* IMI that originated during the lactation period began during the first 90 days after parturition. The high incidence of bacterial and viral infections during the periparturient period suggests that cows are immunosuppressed during this time.¹⁰ Disturbances in cellular and humoral immunity in cattle during the periparturient period have been reported and suggest a marked reduction in the ability of dairy cattle to respond to infectious diseases.¹¹⁻¹⁸

Number of bacteria in bedding is directly correlated with prevalence of IMI caused by environmental pathogens.¹⁹ Because periparturient cows have a high incidence of coliform mastitis and newborn calves frequently have diarrhea secondary to infection with coliform bacteria, we wanted to determine whether fecal shedding of coliform bacteria by dairy cows increased during the periparturient period, thereby increasing numbers of coliform bacteria in the environment. Specifically, the purposes of the study reported here were to determine whether numbers of coliform bacteria in feces of dairy cattle changed during the periparturient period and whether fluctuations in numbers of coliform bacteria shed in the feces were associated with changes in dry-matter intake.

Materials and Methods

Twelve Holstein cows were included in the study. In the first part of the study, fecal samples were collected from 5 cows on a semiregular basis (ie, 3 to 7 times/wk) beginning 4 to 6 weeks before the anticipated parturition date and continuing through the third day after parturition. In the second part of the study, fecal samples were collected from the

Received Aug 6, 1997.

Accepted Apr 24, 1998.

From the Department of Microbiology, Immunology, and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011 (Pelan-Mattocks); and the Metabolic Diseases and Immunology Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA 50010 (Kehrl Jr, Casey, Goff). Dr. Kehrl Jr's present address is Pfizer Global Research and Development, Veterinary Medicine Pharmaceutical Discovery, Terre Haute Laboratories, Terre Haute, IN 47808.

The authors thank Arlen Anderson, Norm Tjelmeland, Creig Caruth, and Gene Riels for technical assistance.

Address correspondence to Dr. Kehrl Jr.

remaining 7 cows on the same semiregular basis beginning 4 to 6 weeks before the anticipated parturition date and continuing through the second week after parturition, and daily feed intake was recorded. The first part of the study was performed during the summer (June through September); the second part of the study was performed during the fall and winter (October through February). All fecal samples were collected at 7 am by means of manual extraction from the rectum. Within 1 hour after collection, samples (3 to 5 g) were diluted in PBS solution (15 mM KH₂PO₄, 8 mM Na₂HPO₄, 137 mM NaCl, 2.6 mM KCl, pH 7.4) in a ratio of 1 g of feces to 5 ml of saline solution and vortexed for 1 minute. Serial dilutions ranging from 5×10^{-5} to 5×10^{-8} were created, and 0.1-ml aliquots of each dilution were plated on MacConkey agar and sorbitol MacConkey agar[†] plates. Plates were incubated at 37 C for approximately 20 hours,^{20,21} and numbers of lactose-fermenting bacterial colonies were counted.

During the first part of the study, if a high number of coliform bacteria were detected in a fecal sample, an additional sample was collected the following day and tested for *E coli* O157:H7. During both parts of the study, cows were fed nutritionally balanced rations designed for the end of gestation and the early lactation period.²²

Data analysis—Linear regression^b was used to determine whether changes in feed intake were correlated with fluctuations in numbers of coliform bacteria in feces.

Results

Fecal shedding of coliform bacteria during the periparturient period—In all 5 cows in the first part of the study, numbers of coliform bacteria in fecal samples between 34 and 25 days prior to parturition were relatively constant (< 10² change in number of bacteria). However, in all 5 cows, number of coliform bacteria in fecal samples transiently increased by 10⁴ to 10⁷ colony-forming units (cfu)/g of feces within the 20 days prior to parturition; in 4 of the 5 cows, the number of coliform bacteria peaked within 7 days prior to parturition. No other consistent patterns in shedding of coliform bacteria in feces were evident. Coliform bacteria were not isolated from up to 7% of all samples collected from each cow prior to parturition. In addition, up to 4% of all samples from each cow contained 1 to 50 cfu/g of feces. *Escherichia coli* O157:H7 was not isolated during the study.

Association between fecal shedding and feed intake—Pattern of fecal shedding of coliform bacteria for the 7 cows in the second part of the study was similar to that for cows in the first part of the study. For 5 of the 7 cows, coliform bacteria were not isolated in up to 8% of all samples; in the 2 remaining cows, coliform bacteria were always isolated. Ninety percent of fecal samples from which coliform bacteria were not isolated had been collected between 34 and 15 days prior to parturition; the remaining 10% had been collected after parturition. In addition, up to 24% of all samples from each cow contained 1 to 50 cfu/g of feces. For 5 of the 7 cows, the number of coliform bacteria increased markedly within 7 days before or after parturition; for the other 2 cows, the number peaked within 12 days before or after calving. Feed intake was not consistently altered in the cows. Some cows had a decrease in feed intake before the number of coliform bacteria in fecal samples peaked, and others had a

decrease after number of coliform bacteria peaked. One cow never had a decrease in feed intake. When data for all cows were considered together, feed intake was not correlated with change in the number of coliform bacteria in fecal samples (overall correlation coefficient, -0.019; *P* = 0.79). When data for individual cows were analyzed separately, 2 of the 7 cows had significant negative correlations between feed intake and fecal coliform bacteria count (correlation coefficients, -0.55 and -0.9; *P* = 0.002 and < 0.001). *Escherichia coli* O157:H7 was not isolated during the study.

Discussion

All 12 cows in this study had large fluctuations in fecal coliform bacteria count (ie, a 10⁴ to 10⁷ increase in number of coliform bacteria/g of feces) within 12 days before or after calving. This is also the time when coliform IMI rates are reported to be the highest.⁸

Current dogma would suggest that coliform bacteria would always be detectable in feces from cattle. Surprisingly, in only 2 of 12 cows monitored were coliform bacteria always detected in the fecal samples obtained. Between the 2 parts of the study, coliform bacteria were not detected in 4 to 8% of the fecal samples collected from 10 cows 34 to 15 days prior to parturition. We believe this to be a conservative estimate of the percentage of fecal samples negative for coliform bacteria, because samples were collected only 3 to 4 times per week. Our data also agree with results of earlier studies²³⁻²⁶ that found that coliform bacteria, particularly *E coli*, were not regularly isolated from feces of adult cows.

Whether increased bacterial shedding in feces is related to immunosuppression of cows during the periparturient period is not clear; however, the combination of increased shedding and immunosuppression may explain the high rates of IMI during this period. It has been established that IMI rates directly correlate with number of bacteria in bedding¹⁹ and that clinical infection can result from exposure of teat ends to minimal numbers of bacteria.²⁷ For instance, mastitis could be efficiently produced in cows by experimentally infusing as few as 30 cfu of *E coli*.²⁸

There is evidence that fecal shedding of coliform bacteria may increase in adult cattle and sheep deprived of feed.^{29,30} Normally, the rumen represents a hostile environment for enteric bacteria such as *E coli*, with only 10³ to 10⁶ viable *E coli*/ml of rumen fluid. However, during periods of feed restriction, pH can exceed 7.0, and volatile fatty acid concentrations can decrease (< 50 mM),^{31,32} which could allow *E coli* to survive and grow. It has been reported that most cows have a 15 to 30% decrease in dry-matter feed intake around the time of parturition³³; therefore, we wondered whether the periparturient increase in fecal coliform bacteria shedding was associated with a decrease in feed intake. However, in the present study, number of coliform bacteria in fecal samples was not correlated with feed intake.

Other possible explanations for the transient increases in fecal coliform bacteria shedding are limited, and it seems unlikely that one factor alone could adequately explain these fluctuations. These fluctua-

tions may simply reflect fluctuations in substrate availability and use by the variety of microbial flora in the ruminant gastrointestinal tract.

Because fecal contamination is the primary avenue by which carcasses become contaminated with pathogenic microbes, the USDA Food Safety and Inspection Service (FSIS) has recently required licensed slaughter plants to test carcasses for *E coli* as an indicator of how well the plants are controlling fecal contamination. However, results of the present study suggest that reliability of using *E coli* detection as a means for monitoring fecal contamination of carcasses during slaughter may be questionable. The FSIS chose to test for *E coli*, rather than *Salmonella* spp, because it was thought that *E coli* would be a better indicator of fecal contamination. However, numerous samples in the present study did not yield any bacteria or yielded only negligible colony counts. Thus, a lack of *E coli* detection does not preclude fecal contamination.

Because *E coli* O157:H7 is reported to be a normal resident of the intestinal tract of cattle, we postulated that increased shedding of the organism by periparturient cows may represent an important source of infection for newborn calves. However, *E coli* O157:H7 was not isolated from any of the cows in this study despite substantial increases in total number of coliform bacteria in fecal samples. A recent study³⁴ estimated that herd prevalence of *E coli* O157:H7 infection among cattle was 0.3 to 0.7% and that prevalence among individual animals within those herds ranged from 1.8 to 16%. Because the present study included only 12 cows, we were not surprised that we did not isolate *E coli* O157:H7.

^aSorbitol MacConkey Agar No.3, OXOID, Unipath Ltd, England.

^bGeneral Linear Model Procedure, SAS, version 6.12, SAS Institute, Cary, NC.

References

1. Smith KL, Todhunter DA, Schoenberger PS. Environmental mastitis: cause, prevalence, prevention. *J Dairy Sci* 1985;68:1531-1553.
2. Howell D. Survey on mastitis caused by environmental bacteria. *Vet Rec* 1972;90:654-657.
3. Brander GC. Dairy herd environment and the control of mastitis. *Vet Rec* 1973;92:501-506.
4. Eberhart RJ, Buckalew JM. Evaluation of a hygiene and dry period therapy program for mastitis control. *J Dairy Sci* 1972;55:1683-1691.
5. Erskine R, Eberhart R, Spencer S. Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. *J Am Vet Med Assoc* 1988;192:761-765.
6. Hogan JS, Smith KL, Hoblet KH, et al. Field survey of clinical mastitis in low somatic cell count herds. *J Dairy Sci* 1989;72:1547-1556.
7. Eberhart RJ. Coliform mastitis. *J Am Vet Med Assoc* 1977;170:1160-1163.
8. Smith KL, Todhunter DA, Schoenberger PS. Environmental pathogens and intramammary infection during the dry period. *J Dairy Sci* 1985;68:402-417.
9. Todhunter DA, Smith KL, Hogan JS, et al. Gram-negative bacterial infections of the mammary gland in cows. *Am J Vet Res* 1991;52:184-188.
10. Hill AW. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. *Res Vet Sci* 1981;31:107-112.
11. Kehrli ME Jr, Goff JP. Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. *J Dairy Sci* 1989;72:1188-1196.
12. Nagahata H, Makino S, Takeda S et al. Assessment of neutrophil function in the dairy cow during the perinatal period. *Zentralbl Veterinarmed [B]* 1988;35:747-751.
13. Kehrli ME Jr, Nonnecke BJ, Roth JA. Alterations in bovine lymphocyte function during the periparturient period. *Am J Vet Res* 1989;50:215-220.
14. Sordillo LM, Babiuk LA. Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon gamma. *Vet Microbiol* 1991;28:189-198.
15. Cai T-Q, Weston PG, Lund LA, et al. Association between neutrophil functions and periparturient disorders in cows. *Am J Vet Res* 1994;55:934-943.
16. Detilleux JC, Kehrli ME Jr, Stabel JR, et al. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Vet Immunol Immunopathol* 1995;44:251-267.
17. Sordillo LM, Redmond MJ, Campos M, et al. Cytokine activity in bovine mammary gland secretions during the periparturient period. *Can J Vet Res* 1991;55:298-301.
18. Sordillo LM, Babiuk LA. Modulation of bovine mammary neutrophil function during the periparturient period following in vitro exposure to recombinant bovine interferon gamma. *Vet Immunol Immunopathol* 1991;27:393-402.
19. Hogan JS, Smith KL, Hoblet KH, et al. Bacterial counts in bedding materials used on nine commercial dairies. *J Dairy Sci* 1989;72:250-258.
20. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* 1986;23:869-872.
21. Cray WC, Moon HW. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl Environ Microbiol* 1995;61:1586-1590.
22. Goff JP, Horst RL. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever. *J Dairy Sci* 1991;74:3863-3871.
23. Howe K, Linton A, Osborne A. A longitudinal study of *Escherichia coli* in cows and calves with special reference to the distribution of O-antigen types and antibiotic resistance. *J Appl Bacteriol* 1976;40:331-340.
24. Larsen H, Larsen J. Occurrence and distribution of drug resistant *E coli* in faeces from cattle and swine. *Nord Vet Med* 1972;24:651-659.
25. Smith HW, Crabb WE. The faecal bacterial flora of animals and man: its development in the young. *J Pathol Bact* 1961;82:53-66.
26. Smith H. The development of the bacterial flora of the faeces of animals and man: the changes that occur during aging. *J Appl Bacteriol* 1961;24:235-239.
27. McDonald JS, Packer RA. Incidence of intramammary infections during lactation in dairy cattle repeatedly exposed to *Streptococcus agalactiae* and *Aerobacter aerogenes*. *Am J Vet Res* 1968;29:1525-1532.
28. Shuster DE, Lee EK, Kehrli ME. Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. *Am J Vet Res* 1996;57:1569-1575.
29. Rasmussen MA, Cray WC, Casey TA. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. *FEMS Microbiol Lett* 1993;114:79-84.
30. Grau FH, Brownlie LE, Smith MG. Effects of food intake on numbers of salmonellae and *Escherichia coli* in rumen and faeces of sheep. *J Appl Bacteriol* 1969;32:112-117.
31. Meiske JC, Salsbury RL, Hoefler JA. The effect of starvation and subsequent refeeding on some activities of rumen microorganisms in vitro. *J Anim Sci* 1958;17:774-781.
32. Michalowski T. Effect of feeding frequency on the diurnal changes in microbial protein, volatile fatty acids and ammonia contents of the rumen of sheep. *J Agric Sci* 1979;93:67-70.
33. Marquardt J, Horst R, Jorgensen N. Effect of parity on dry matter intake at parturition in dairy cattle. *J Dairy Sci* 1976;60:929-934.
34. Faith NG, Shere JA, Brosch R, et al. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Appl Environ Microbiol* 1996;62:1519-1525.