FOODBORNE PATHOGENS AND DISEASE
Volume 5, Number 6, 2008

DOI: 10.1089/fpd.2008.0141

A Survey of Bacteriological Quality and the Occurrence of *Salmonella* in Raw Bovine Colostrum

B.A. Houser,[1] S.C. Donaldson,[1] S.I. Kehoe,[2] A.J. Heinrichs,[3] and B.M. Jayarao[1]

Abstract

In recent years, bovine colostrum has gained popularity as a human food because it is an excellent source of bioactive proteins, which have been claimed to inhibit viral and bacterial pathogens, improve gastrointestinal health, and enhance body condition. A study was conducted to determine bacteriological quality and occurrence of *Salmonella* in colostrum collected from dairy herds ($n = 55$) in Pennsylvania. Colostrum samples were analyzed for standard plate count, preliminary incubation count, laboratory pasteurization count, *Staphylococcus aureus*, *Streptococcus agalactiae*, coagulase negative staphylococci, streptococci, coliforms, and non-coliforms. A standardized polymerase chain reaction assay was used for detection of *Salmonella* in colostrum. *Salmonella* were detected in 8 of 55 (15%) of colostrum samples. *Streptococcus agalactiae* (1000 colony-forming units [CFU]/mL) was detected in one colostrum sample. The mean standard plate count (977,539 CFU/mL), preliminary incubation count (12,094,755 CFU/mL), laboratory pasteurization count (615 CFU/mL), *Staphylococcus aureus* (306 CFU/mL), coagulase negative staphylococci (164,963 CFU/mL), streptococci (256,722 CFU/mL), coliforms (323,372 CFU/mL), and non-coliforms (111,544 CFU/mL) counts in colostrum were considerably higher than raw bulk tank milk counts reported previously from Pennsylvania. Analysis revealed that farm size did not influence the bacteriological quality of colostrum. Collection, handling, and storage of colostrum need to be addressed to improve bacteriological quality of colostrum intended not only for feeding calves but also for human consumption.

Introduction

The nutritional benefits of colostrum have been widely researched and documented. Bovine colostrum is considered an excellent source of bioactive proteins including growth factors, immunoglobulin G, lactoperoxidase, lysozyme, lactoferrin, and cytokines presumably beneficial to human health (Playford *et al.*, 2000; Thapa, 2005a, 2005b; Gapper *et al.*, 2007). Human health benefits are thought to include inhibition of viral and bacterial pathogens, improved gastrointestinal health, and enhanced body composition (Playford *et al.*, 2000; Kelly, 2003). Colostrum products have been growing in popularity due to an increased demand for functional foods and dietary supplements as an alternative to traditional drug therapy (Gapper *et al.*, 2007).

High quality raw colostrum is essential for producing quality colostrum products. Denaturation of bioactive proteins found in colostrum results in loss of physiological function and beneficial effects of the protein (Gapper *et al.*, 2007). Traditional pasteurization processes have been shown to denature many bioactive colostral proteins; therefore, in many cases other methods must be used to process colostrum for

[1]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, Pennsylvania.
[2]Department of Animal and Food Science, University of Wisconsin—River Falls, River Falls, Wisconsin.
[3]Department of Dairy and Animal Science, The Pennsylvania State University, University Park, Pennsylvania.

human consumption (Dominguez *et al.*, 1997). Previous studies have focused on handling and processing procedures to generate methods to preserve bioactive protein function in colostrum resulting in higher quality finished colostrum products (Li *et al.*, 2006). Very few studies have assessed the effects of bacteriological quality of raw colostrum on the quality of finished colostrum products.

Many species of bacteria found in dairy products produce proteases capable of cleaving and denaturing beneficial proteins (Hantsis-Zacharov and Halpern, 2007). These bacteria gain entrance into dairy products from the bovine udder and the farm environment (Jayarao, 2004). Previous studies have shown that the presence of high numbers of bacteria in raw milk may lead to inferior quality of finished milk products (Boor *et al.*, 1998; Rajmohan *et al.*, 2002; Straley *et al.*, 2006; Barbano *et al.*, 2006). The same may be true for colostrum products. Also, pathogenic bacteria relevant to human health risks have been isolated from raw milk and colostrum in the past (Jayarao and Henning, 2001; Godden *et al.*, 2006). A public health risk could result if these pathogens are capable of surviving processing of colostrum for human consumption. Therefore, it is essential that raw colostrum used to make colostrum products for human consumption has low counts of bacteria relevant to colostrum protein quality, and the raw colostrum should be free of pathogenic organisms in preprocessing stages. The objectives of this study were to determine the bacteriological quality and prevalence of *Salmonella* in colostrum collected from dairy herds in Pennsylvania.

Materials and Methods

Colostrum samples

A convenience sample of farms was chosen from the four major dairy regions within Pennsylvania. Farms surveyed within these areas were comparative to the number of operating dairy farms within the region. A total of 55 Holstein dairy herds in 15 Pennsylvania counties participated in the study including 17 small (<100 head), 10 medium (100–200 head), and 28 large (>200 head) dairy operations. One cow per farm was fully milked out, and colostrum was mixed thoroughly. Samples were transported on ice to the laboratory and processed within 24 hours.

Bacteriological analysis of colostrums

Colostrum samples were analyzed for bacteriological quality through standard plate count (SPC), preliminary incubation count (PIC), and laboratory pasteurization count (LPC) as described by Jayarao *et al.* (2004). Briefly, PIC was obtained by incubating a 4.5 mL aliquot of colostrum at 12°C for 18 hours. The LPC was carried out by incubating a 4.5 mL aliquot of colostrum at 62.8°C for 30 minutes. For bacterial enumeration, 50 μL of the SPC, LPC, and PIC aliquots were plated on plate count agar and incubated at 32°C for 48 hours.

Colostrum differential bacteriological counts were determined for *Staphylococcus aureus* (SA), *Streptococcus agalactiae* (SAG), coagulase negative staphylococci (CNS), streptococci (SS), coliforms (CC), and non-coliforms (NC) as described by Jayarao *et al.* (2004). Edward's modified agar supplemented with colistin sulfate and oxolinic acid was used to estimate numbers of SS and SAG (Sawant *et al.*, 2002). To determine CC and NC, MacConkey's agar no. 3 (Oxoid, Lenexa, KS) was used and Baird-Parker agar (Difco, Lawrence, KS) was used for CNS and SA. All agar plates were inoculated with 50 μL of colostrum and incubated at 32°C for 48 hours and enumerated.

There are no previous reports or guidelines for evaluating the bacteriological quality of colostrum. Jayarao *et al.* (2004) reported the following guidelines for evaluating bacteriological quality of raw bulk tank milk: SPC ≤ 5000 colony-forming units (CFU)/mL, PIC ≤ 10,000 CFU/mL, LPC ≤ 100 CFU/mL, CNS ≤ 500 CFU/mL, SS ≤ 500 CFU/mL, CC ≤ 50 CFU/mL, NC ≤ 200 CFU/mL; SA and SAG should not be detected. Although these guidelines are primarily for bulk tank milk, the same guidelines were applied here to draw inferences on bacteriological quality of colostrum.

Detection of Salmonella *using a polymerase chain reaction (PCR) assay*

Colostrum samples were examined for *Salmonella* using the *invA* gene–based PCR assay

described by Ferretti *et al.* (2001). Instagene Matrix (Bio-Rad, Hercules, CA) was used to isolate bacterial DNA from all colostrum samples.

Results

Bacteriological quality of colostrum

Mean colostrum SPC and PIC for all farms was 997,539 CFU/mL and 12,094,755 CFU/mL and ranged from 140 to 9,070,000 CFU/mL and from 240 to 90,700,000 CFU/mL, respectively (Table 1). LPCs for all farms ranged from 0 to 18,000 CFU/mL with a mean of 615 CFU/mL. Although small herds and large herds had the lowest and highest mean counts for all three bacteriological quality parameters, respectively, no significant difference in bacteriological quality of colostrum was observed with respect to herd size.

Differential bacteriological quality of colostrum

Mean colostrum counts for all samples were 164,963 CFU/mL for CNS, 256,722 CFU/mL for SS, 323,372 CFU/mL for CC, and 111,544 CFU/mL for NC, and they ranged from 0 to 3,980,000 CFU/mL for CNS, from 0 to 5,600,000 CFU/mL for SS, from 0 to 3,950,000 CFU/mL for CC, and from 0 to 3,000,000 CFU/mL for NC (Table 1). Small herds had the lowest mean CNS, SS, and CC counts, while medium herds had the lowest mean NC counts. Large herds had the highest mean counts for all differential bacteriological quality parameters. No significant difference in differential bacteriological quality of colostrum could be determined with respect to herd size.

SA counts ranged from 0 to 12,000 CFU/mL with a mean of 306 CFU/mL while SAG was only detected in one colostrum sample (from a large farm) at a level of 1000 CFU/mL (not included in tables). Medium herds had the lowest SA counts while small herds had the highest counts. No significant difference in differential bacteriological quality of colostrum with respect to herd size could be determined.

Only 5 (9%) out of the 55 colostrum samples analyzed in this study were determined to be of high bacteriological quality based on all nine bacterial count parameters. These five colostrum samples were from two small herds, one medium herd, and two large herds. Overall, small herds had the highest percentage (12%) of colostrum samples considered to be of high bacteriological quality as compared to medium (10%) and large (7%) herds.

Table 1. Bacteriological Quality of Colostrum Samples Among Farms of Different Sizes

			Colostrum bacterial counts (CFU/mL)							
Herd size (head)	*N*		*SPC*	*PIC*	*LPC*	*SA*	*CNS*	*SS*	*CC*	*NC*
Small (<100)	17	Mean	218,612	7,874,582	94	729	1,795	16,701	64,425	73,710
		Median	4590	50,000	10	0	1340	780	230	80
		Min	330	1400	0	0	0	0	0	0
		Max	5,100,00	83,900,000	830	12,000	10,800	152,000	700,000	740,000
Medium (100–200)	10	Mean	1,017,482	9,087,320	831	92	177,138	269,488	252,569	26,657
		Median	16,220	655,000	60	0	8840	3615	1,900	695
		Min	1280	1600	0	0	300	220	0	0
		Max	5,440,000	72,500,000	5020	420	1,032,000	1,710,000	1,620,000	232,200
Large (>200)	28	Mean	1,463,335	15,731,087	855	126	259,681	397,890	505,876	164,831
		Median	59,420	813,000	35	0	2765	4400	5285	450
		Min	140	240	0	0	120	0	0	0
		Max	9,070,000	90,700,000	18,000	2000	3,980,000	5,600,000	3,950,000	3,000,000
Total	55	Mean	997,539	12,094,755	615	306	164,963	256,722	323,372	111,544
		Median	15,300	324,000	30	0	2260	2140	600	360
		Min	140	240	0	0	0	0	0	0
		Max	9,070,000	90,700,000	18,000	12,000	3,980,000	5,600,000	3,950,000	3,000,000

SPC, standard plate count; PIC, preliminary incubation count; LPC, laboratory pasteurization count; SA, *Staphylococcus aureus*; CNS, coagulase negative staphylococci; SS, streptococci; CC, coliforms; NC, non-coliforms. Only one colostrum sample contained *Streptococcus agalactiae* and is not included in the table.

Prevalence of pathogenic organisms

Salmonella spp. were detected in 8 out of 55 (15%) colostrum samples. *Salmonella*-positive samples were from three small herds, one medium herd, and four large herds. It was noted that all eight colostrum samples positive for *Salmonella* spp. also had SPCs and PICs above 5000 CFU/mL and 10,000 CFU/mL, respectively. The five colostrum samples that were observed to be of high bacteriological quality mentioned above were all negative for *Salmonella* spp.

Discussion

Bacterial contamination of raw milk is associated with reduced quality of finished milk and milk-based products (Boor *et al.*, 1998; Rajmohan *et al.*, 2002; Straley *et al.*, 2006; Barbano *et al.*, 2006). It has been previously reported that the bacteriological quality of milk correlates to management practices including udder hygiene, teat preparation, and proper cooling of milk (Chambers, 2002). The same observations could be applied to colostrum, assuming that bacterial contamination of raw colostrum may reduce the quality of finished colostrum-based products and that bacteriological quality of raw colostrum is influenced by herd management practices.

SPC estimates the total number of aerobic bacteria present in milk. Jayarao *et al.* (2004) reported that high quality raw milk should have an SPC $\leq$ 5000 CFU/mL. In our study, 69% of colostrum samples analyzed had SPCs greater than this standard. In fact, several samples had SPCs greater than the standard by more than 3 logs. In order to protect public health, the Pasteurized Milk Ordinance (US FDA/CFSAN) enforces an SPC standard of 100,000 CFU/mL for raw milk leaving the dairy farm. Our data showed that 38% of colostrum samples analyzed had SPCs $>$ 100,000 CFU/mL (Table 2). This suggests that a large proportion of colostrum is not fit for human consumption due to tremendous bacterial loads. High SPC of raw colostrum may be due to improper cooling and storage after collection. Kehoe *et al.* (2007) reported that 7% of producers surveyed stored colostrum in open-topped containers and 2% of producers stored raw colostrum at room temperature.

The PIC indicates the number of psychrotrophic bacteria in raw milk. Psychotrophic bacteria are able to grow during refrigeration and may release proteolytic enzymes and are active at cold temperatures, which may reduce quality through the breakdown of beneficial lacteal proteins (Hantsis-Zacharov and Halpern, 2007). PICs should not exceed 10,000 CFU/mL in high quality milk (Jayarao *et al.*, 2004). Eighty-two percent of colostrum samples analyzed in this study had PICs $>$10,000 CFU/mL (Table 2) and 22% of total colostrum samples exceeded the standard PIC by more than 3 logs. These results suggest that there is a high probability of lacteal protein denaturation in a large portion of raw bovine colostrum, even if stored at refrigeration temperatures, before it is processed into products for human consumption. Kehoe *et al.* (2007) reported that 22% of surveyed producers store raw colostrum at refrigeration temperatures.

The LPC determines the number of thermoduric bacteria present. Thermoduric bacteria are

Table 2. Stratification of Herd Size and Colostrum Bacteriological Quality

		Bacteriological quality parameters (% of total no. of herds)					
		SPC		*PIC*		*LPC*	
Herd size (head)	*N*	*>5000 CFU/mL*	*<5000 CFU/mL*	*>10,000 CFU/mL*	*<10,000 CFU/mL*	*>100 CFU/mL*	*<100 CFU/mL*
Small (<100)	17	47	53	71	29	24	76
Medium (100–200)	10	90	10	90	10	40	60
Large (>200)	28	75	25	86	14	32	68
Total	55	69	31	82	18	31	69

SPC, standard plate count; PIC, preliminary incubation count; LPC, laboratory pasteurization count.

Table 3. Stratification of Herd Size and Colostrum Differential Bacteriological Quality

		Differential bacteriological quality parameters (% of total no. of herds)[a]									
		SA		*CNS*		*SS*		*CC*		*NC*	
Herd size (head)	*N*	*>0 CFU/mL*	*0 CFU/mL*	*>500 CFU/mL*	*<500 CFU/mL*	*>500 CFU/mL*	*<500 CFU/mL*	*>50 CFU/mL*	*<50 CFU/mL*	*>200 CFU/mL*	*<200 CFU/mL*
Small (<100)	17	47	53	65	35	59	41	65	35	47	53
Medium (100–200)	10	40	60	80	20	80	20	70	30	60	40
Large (>200)	28	36	61	79	21	75	25	79	21	54	46
Total	55	42	58	74	26	71	29	73	27	53	47

[a]SA, *Staphylococcus aureus*; CNS, coagulase negative staphylococci; SS, streptococci; CC, coliforms; NC, non-coliforms.

able to survive pasteurization, thus contaminating finished lacteal products. For high quality milk, the LPC should be ≤100 CFU/mL (Jayarao *et al.*, 2004). In this study, 69% of colostrum samples had an LPC ≤ 100 CFU/mL (Table 2) suggesting thermoduric bacteria will not likely contribute to a decrease in quality of pasteurized colostrum products.

High quality milk should have CNS and SS counts that do not exceed 500 CFU/mL (Jayarao *et al.*, 2004). In the current study 74% and 71% of all colostrum samples had CNS and SS counts exceeding the standard value, respectively (Table 3). In fact, CNS and SS counts for several colostrum samples were more than 4 log greater than the standard value. SAG and SA are both mastitis pathogens. These organisms should not be detected in high quality milk. In the present study nearly 42% of colostrum samples were SA contaminated and one colostrum sample (from a large herd) tested positive for SAG suggesting mastitis pathogens are a source of raw colostrum contamination and may lead to decreased quality finished colostrum products.

Gram-negative bacteria account for a large proportion of psychrotrophs found in raw milk products (Cousin, 1982). *Pseudomonas* spp. are able to produce heat stable proteases that survive milk product processing (Rajmohan *et al.*, 2002; Barbano *et al.*, 2006). The presence of these species in raw colostrum may lead to reduced function of bioactive proteins in finished colostrum-based products thereby reducing quality. Jayarao *et al.* (2004) reported that CC and NC counts should not exceed 50 CFU/mL and 200 CFU/mL, respectively, in high quality raw milk. In our study, 73% and 53% of all colostrum samples exceeded CC and NC standard counts, respectively (Table 3). These results suggest that it is probable that lacteal protein degradation may continue in postprocessing colostrum products made from low bacteriological quality raw colostrum.

Salmonella spp. have been isolated from raw milk in the past (Jayarao and Henning, 2001). When analyzing colostrum samples for pathogenic organisms, *Salmonella* spp. were detected in 15% of colostrum samples. The frequency of *Salmonella* spp. observed here is greater than that observed in a previous study by Jayarao and Henning (2001) who reported a frequency of *Salmonella* spp. in raw bulk tank milk of only 6.1%. Our findings are alarming and suggest that colostrum products that are not properly pasteurized could be a source of *Salmonella* exposure and may be a public health threat. A previous study by Kehoe *et al.* (2007) reported that herd size influenced colostrum management practices as well as colostrum quality. No correlation between bacteriological counts in raw colostrum and herd size was observed in our study.

Conclusions

The U.S. Food and Drug Administration has accepted the safety of colostrum products for human consumption based on no adverse health effect findings. As long as colostrum-based product manufacturers do not make health claims, they can market colostrum-based products for human consumption as foods, thus evading quality standards and strict product regulation (Gapper *et al.*, 2007). Bacteriological quality of raw colostrum may highly influence the bioactive protein quality of finished

colostrum-based products marketed for human consumption. In our study only 9% of raw colostrum samples met all criteria needed to be considered high quality and 16% of samples tested positive for *Salmonella* spp. Collection, handling, and storage of colostrum need to be addressed to improve bacteriological quality of colostrum intended not only for feeding calves but also for human consumption.

Acknowledgments

This research was a component of NC-1042; Management Systems to Improve the Economic and Environmental Sustainability of Dairy Enterprises. Partial funding was provided by the Pennsylvania Department of Agriculture, Animal Health Commission.

Disclosure Statement

No competing financial interests exist.

References

Barbano DM, Ma Y, and Santos MV. Influence of raw milk quality on fluid milk shelf life. J Dairy Sci 2006;**89(E. Suppl.):**E15–E19.

Boor KJ, Brown DP, Murphy SC, *et al.* Microbiological and chemical quality of raw milk in New York State. J Dairy Sci 1998;**81:**1743–1748.

Chambers JV. The microbiology of raw milk. In: *Dairy Microbiology Handbook,* 3rd ed. Robinson RK (ed). New York: John Wiley & Sons, Inc., 2002, pp. 39–90.

Cousin MA. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J Food Prot 1982;**45:**172–207.

Dominguez E, Perez MD, and Calvo M. Effect of heat treatment on the antigen-binding activity of anti-peroxidase immunoglobulins in bovine colostrum. J Dairy Sci 1997;**80:**3182–3187.

Ferretti R, Mannazzu I, Cocolin L, *et al.* Twelve-hour PCR-based method for detection of *Salmonella* spp. in food. Appl Environ Microbiol 2001;**67:**977–978.

Gapper LW, Copestake DEJ, Otter DE, *et al.* Analysis of bovine immunoglobulin G in milk, colostrum, and dietary supplements: a review. Anal Bioanal Chem 2007;**389:**93–109.

Godden S, McMartin S, Feirtag J, *et al.* Heat-treatment of bovine colostrum. II: Effects of heating duration on pathogen viability and immunoglobulin G. J Dairy Sci 2006;**89:**3476–3483.

Hantsis-Zacharov E and Halpern M. Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Appl Environ Microbiol 2007;**73:**7162–7168.

Jayarao BM and Henning DR. Prevalence of foodborne pathogens in bulk tank milk. J Dairy Sci 2001;**84:**2157–2162.

Jayarao BM, Pillai SR, Sawant AA, *et al.* Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J Dairy Sci 2004;**87:**3561–3573.

Kehoe SI, Jayarao BM, and Heinrichs AJ. A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. J Dairy Sci 2007;**90:**4108–4116.

Kelly GS. Bovine colostrums: a review of clinical uses. Alternative Medicine Review 2003;**8:**378–394.

Li S, Zhang HQ, Balasubramaniam VM, *et al.* Comparison of effects of high-pressure processing and heat treatment on immunoactivity of bovine milk immunoglobulin G in enriched soymilk under equivalent microbial inactivation levels. J Agric Food Chem 2006;**54:**739–746.

Playford RJ, Macdonald CE, and Johnson WS. Colostrum and milk-derived growth factors for the treatment of gastrointestinal disorders. Am J Clin Nutr 2000;**72:**5–14.

Rajmohan S, Dodd CER, and Waites MW. Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. J Appl Microbiol 2002;**93:**205–213.

Sawant AA, Pillai SR, and Jayarao BM. Evaluation of five selective media for isolation of catalase negative gram-positive cocci from raw milk. J Dairy Sci 2002;**85:**1127–1132.

Straley BA, Donaldson SC, Sidhu MS, *et al.* Probable causes of reduced shelf life of pasteurized fluid milk. Proceedings of the 45th National Mastitis Council, Tampa , FL, 2006. pp. 300–301.

Thapa BR. Health factors in colostrum. Indian J Pediatr 2005a;**72:**579–581.

Thapa BR. Therapeutic potentials of bovine colostrums. Indian J Pediatr 2005b;**72:**849–852.

[US FDA/CFSAN] United States Food and Drug Administration/Center for Food Safety and Nutrition. 2004. Grade "A" Pasteurized Milk Ordinance, 2003 Revision. p. 27.

Address reprint requests to:
B.M. Jayarao, M.V.Sc., Ph.D. M.P.H
Department of Veterinary and Biomedical Sciences
The Pennsylvania State University
111 Henning Building
University Park, PA 16802

E-mail: bmj3@psu.edu