Review

Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications

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ABSTRACT

Milk and products derived from milk of dairy cows can harbor a variety of microorganisms and can be important sources of foodborne pathogens. The presence of foodborne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Most milk is pasteurized, so why should the dairy industry be concerned about the microbial quality of bulk tank milk? There are several valid reasons, including (1) outbreaks of disease in humans have been traced to the consumption of unpasteurized milk and have also been traced back to pasteurized milk, (2) unpasteurized milk is consumed directly by dairy producers, farm employees, and their families, neighbors, and raw milk advocates, (3) unpasteurized milk is consumed directly by a large segment of the population via consumption of several types of cheeses manufactured from unpasteurized milk, (4) entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms, and subsequent contamination of processed milk products and exposure of consumers to pathogenic bacteria, (5) pasteurization may not destroy all foodborne pathogens in milk, and (6) inadequate or faulty pasteurization will not destroy all foodborne pathogens. Furthermore, pathogens such as *Listeria monocytogenes* can survive and thrive in post-pasteurization processing environments, thus leading to recontamination of dairy products. These pathways pose a risk to the consumer from direct exposure to foodborne pathogens present in unpasteurized dairy products as well as dairy products that become re-contaminated after pasteurization. The purpose of this communication is to review literature published on the prevalence of bacterial foodborne pathogens in milk and in the dairy environment, and to discuss public health and food safety issues associated with foodborne pathogens found in the dairy environment. Information presented supports the model in which the presence of pathogens depends on ingestion of contaminated feed followed by amplification in bovine hosts and fecal dissemination in the farm environment. The final outcome of this cycle is a constantly maintained reservoir of foodborne pathogens that can reach humans by direct contact, ingestion of raw contaminated milk or cheese, or contamination during the processing of milk products. Isolation of bacterial pathogens with similar biotypes from dairy farms and from outbreaks of human disease substantiates this hypothesis.

INTRODUCTION

MORE THAN 200 known diseases are transmitted through food by a variety of

agents that include bacteria, fungi, viruses, and parasites. According to public health and food safety experts, each year millions of illnesses in the United States and throughout the world can

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be traced to foodborne pathogens. While the food supply in the United States is one of the safest in the world, the Centers for Disease Control and Prevention (CDC, 2003, 2004) estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5,000 die each year from foodborne illness. The risk of foodborne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness today. Consequently, preventing illness and death associated with foodborne pathogens remains a major public health challenge. Furthermore, food safety is a global issue, and an increase in import and export of food products could lead to introduction and establishment of new diseases in geographical areas that have never experienced the foodborne pathogens.

The purpose of this communication is to review literature published on the prevalence of foodborne pathogens, primarily *Campylobacter jejuni*, Shiga toxin-producing *E. coli* (STEC), *Listeria monocytogenes*, and *Salmonella*, in milk and in the dairy environment, and to discuss public health and food safety issues associated with foodborne pathogens found in the dairy environment.

PREVALENCE OF FOODBORNE PATHOGENS IN MILK

Several surveys have detected foodborne pathogens in bulk tank milk (Davidson, 1989; Doyle and Roman, 1982; Farber et al., 1988; Fedio and Jackson, 1990; Hassan et al., 2000; Jayarao

and Henning, 2001; Liewen and Plautz, 1988; Lovett et al., 1983, 1987; McEwen et al., 1988; McManus and Lanier, 1987; Muraoka et al., 2003; Murinda et al., 2002a,b, 2004a,b; Rohrbach et al., 1992; Slade et al., 1988; Steele et al., 1997; Van Kessel et al., 2004; Waak et al., 2002). Results of those studies have shown clearly that the prevalence of foodborne pathogens, including *C. jejuni*, STEC, *L. monocytogenes*, and *Salmonella* spp. in milk, varies considerably (Tables 1 and 2).

The prevalence of foodborne pathogens in milk is influenced by numerous factors such as farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographical location, and season. However, in spite of the variation, all of these surveys demonstrated quite clearly that milk can be a major source of foodborne pathogens of human health significance.

Rohrbach et al. (1992) reported that the frequency of isolation of foodborne pathogens from 292 bulk tank milk samples from dairies in east Tennessee and southwest Virginia was 12.3% for *C. jejuni*, 8.9% for *Salmonella* species, 4.1% for *L. monocytogenes*, and 15.1% for *Yersinia enterocolitica*. One or more foodborne pathogens were isolated from 32.5% of bulk tank milk samples evaluated. One of the four foodborne pathogens was isolated from 73 of 95 positive samples, and 22 samples contained two or more foodborne pathogens. Grade classification of the dairy, milking facilities, barn type, milking hygiene, reported incidence of clinical mastitis among cows, or the number of

TABLE 1. SURVEYS ON THE ISOLATION OF *CAMPYLOBACTER JEJUNI* AND SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* FROM BULK TANK MILK

Foodborne pathogen	Isolation rate (%)	Reference
<i>Campylobacter jejuni</i>	0.9	Doyle and Roman (1982)
	1.5	Lovett et al. (1983)
	0.4	McManus and Lanier (1987)
	1.2	Davidson et al. (1989)
	12.3	Rohrbach et al. (1992)
	0.5	Steele et al. (1997)
	9.2	Jayarao and Henning (2001)
Shiga toxin-producing <i>Escherichia coli</i>	0.9	Steele et al. (1997)
	3.8	Jayarao and Henning (2001)
	0.8	Murinda et al. (2002b)

TABLE 2. SURVEYS ON THE ISOLATION OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA* SPP. FROM BULK TANK MILK

Foodborne pathogen	Isolation rate (%)	Reference
<i>Listeria monocytogenes</i>	4.2	Lovett et al. (1987)
	1.3	Farber et al. (1988)
	5.4	Slade et al. (1988)
	4.0	Liewen and Plautz (1988)
	1.6	Davidson et al. (1989)
	1.9	Fedio and Jackson (1990)
	4.1	Rohrbach et al. (1992)
	2.7	Steele et al. (1997)
	4.6	Jayarao and Henning (2001)
	12.6	Hassan et al. (2000)
	1.0	Waak et al. (2002)
	4.9 to 7.0	Muraoka et al. (2003)
<i>Salmonella</i> spp.	6.5	Van Kessel et al. (2004)
	4.7	McManus and Lanier (1987)
	2.9	McEwen et al. (1988)
	8.9	Rohrbach et al. (1992)
	0.2	Steele et al. (1997)
	6.1	Jayarao and Henning (2001)
	1.5	Hassan et al. (2000)
	2.2	Murinda et al. (2002a)
	2.6	Van Kessel et al. (2004)

cows per farm were not significantly associated with the isolation of foodborne pathogens in bulk tank milk. Of 84 dairy producers who used teat disinfection and antibiotic dry cow therapy that were classified as having good milking hygiene, 29 (35%) had bulk tank milk contaminated with foodborne pathogens compared to 12 of 29 (31%) dairies with poor milking hygiene (odds ratio 1.2, $p = 0.7$). Almost 35% of dairy producers who participated, indicated that they consumed raw milk produced on their farm, and 25% of the bulk tank milks from these farms were contaminated with one or more foodborne pathogens (Rohrbach et al., 1992).

In a similar study, bulk tank milk from 131 dairy herds in eastern South Dakota and western Minnesota was examined for the presence of foodborne pathogens (Jayarao and Henning, 2001). Thirty-five of 131 (26.7%) bulk tank milk samples contained one or more species of pathogenic bacteria. *Campylobacter jejuni*, STEC, *L. monocytogenes*, *Salmonella* spp., and *Y. enterocolitica* were detected in 9.2%, 3.8%, 4.6%, 6.1%, and 6.1% of bulk tank milk samples, respectively. Isolates of *Salmonella* belonged to "O" serogroups D ($n = 4$), B ($n = 2$), C ($n = 1$), and

E ($n = 1$). All six isolates of *L. monocytogenes* were identified as O antigen type 1. Four of five isolates of *E. coli* encoded for the Shiga-toxin 2 gene, while one strain encoded for the Shiga-toxin 1 gene. *Escherichia coli* O157:H7 was not isolated from bulk tank milk samples. Manufacturing grade raw milk producers were at a higher risk (odds ratio 4.98; confidence interval, 1.96–12.22) of having one or more foodborne pathogens in their bulk tank milk than were Grade A milk producers. Twenty-one of 79 (26.6%) dairy producers who consumed raw milk produced on their farm had one or more pathogenic bacteria in their bulk tank milk.

More recently, Van Kessel et al. (2004) reported results on the prevalence of foodborne pathogens in bulk tank milk samples obtained as part of the National Animal Health Monitoring System Dairy 2002 Survey. The objective of this study was to determine the national prevalence of *Salmonella*, *L. monocytogenes*, and fecal coliforms in bulk tank milk in the United States. Bulk tank milk samples ($n = 861$) were collected from farms in 21 states. Coliforms were detected in 95% (818 of 860) of samples, and the average somatic cell count (SCC) was 295,000 cells/ml. Twenty-two samples (2.6%)

were culture-positive for *Salmonella*, and nine serotypes were identified: Montevideo ($n = 7$), Newport ($n = 4$), Muenster ($n = 2$), Meleagridis ($n = 2$), Cerro ($n = 2$), 44:Z36 (Z38) ($n = 2$), Dublin ($n = 1$), Anatum ($n = 1$), and 9, 12:nonmotile ($n = 1$). *Listeria monocytogenes* serotypes 1/2a, 1/2b, 3b, 4b, and 4c were isolated from 56 (6.5%) of samples; 93% were serotypes 1/2a, 1/2b, and 4b, the most common human clinical isolates. There were no apparent relationships between SCC or specific plate count and incidence of *Salmonella* or *L. monocytogenes*.

Another pathogen that is found frequently in bulk tank milk and is a significant cause of mastitis in dairy cows throughout the world is *Staphylococcus aureus*. The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *S. aureus*. Enterotoxins produced by enterotoxigenic strains of *S. aureus* are classified according to serotypes into A–H groups and toxic shock syndrome toxin (TSST). The frequency of enterotoxigenicity amongst staphylococcal strains is highly variable (Genigeorgis, 1989; Mossel and Van Netten, 1990). Studies on *S. aureus* isolated from cows showed enterotoxigenicity ranging from 0 to 56.5% (Bennet et al., 1986; Castro et al. 1986; Kenny et al., 1993; Masud et al., 1993; Ruzickova, 1994). TSST was detected in *S. aureus* isolated in milk from cows with clinical and subclinical mastitis, and in farm bulk tank milk (Takeuchi et al., 1998). Enterotoxigenic *S. aureus* was isolated from raw milk samples at a milk cooperative in Kenya (Ombui et al., 1992) and in bulk milk collected from dairy farms in Trinidad (Adesiyun et al., 1998). It was inferred from these studies that bulk tank milk was a potential source of enterotoxigenic *S. aureus* in milk and milk products, and may constitute a health hazard to consumers. Enterotoxigenic strains of *S. aureus* have been reported to cause a number of diseases or food poisoning outbreaks because of ingestion of contaminated dairy products or milk (Adesiyun et al., 1998; Asao et al., 2003; Genigeorgis, 1989). The most recent large-scale outbreak occurred during June 2000 in Japan caused by consumption of low-fat milk produced from skim milk powder contaminated with *S. aureus* enterotoxin A (Asao et al., 2003).

The prevalence of *C. jejuni* in bulk tank milk has been reported to range from <1% to slightly more than 12% (Table 1). *Campylobacter jejuni* is the most frequently identified cause of acute infectious diarrhea in developed countries, including the United States (Friedman, 2000). About 2.4–4 million cases of campylobacteriosis are associated with 120 deaths each year (Mead et al., 1999). Foodborne illness caused by *Campylobacter* is characterized by sporadic cases of chronic gastritis, enterocolitis, and septicemia. Foodborne infections by *Campylobacter* can result in sequelae like *Campylobacter*-associated Guillian-Barré syndrome (Nachamkin, 2000). Humans get infected through ingestion of contaminated non-pasteurized milk, milk not properly pasteurized, untreated water, and raw or improperly cooked poultry (Evans et al., 1996; Fashey et al., 1995; Hanninen et al., 1998; Hutchinson et al., 1995). *Campylobacter jejuni* are excreted through feces and animal secretions (Waterman et al., 1984), and dairy cattle get infected through ingestion of water and feeds contaminated with manure. *Campylobacter jejuni* is an infrequent cause of mastitis in dairy cows, and it can be shed in milk of asymptomatic cows (Gundmundson and Chirino-Trejo, 1992). Direct milk excretion of *C. jejuni* by clinically healthy cows has been described and implicated in the etiology of human enteritis following consumption of contaminated milk (Orr et al., 1995). Large-scale outbreaks due to *Campylobacter* have been associated with drinking unpasteurized milk or contaminated water. Cow manure is a principal reservoir for *Campylobacter* and farm practices using manure as fertilizer on cropland are considered a risk factor for occurrence of *Campylobacter* foodborne disease.

Far less is known about the prevalence of Shiga toxin-producing *E. coli* (STEC) in milk than the other major foodborne pathogens. STEC are of immense economic and public health significance. STEC O157:H7 is characterized by low infectious doses, typically 1–100 colony-forming units (Paton and Paton, 1998). STEC are highly pathogenic in humans, where they cause serious acute illness and long-term sequelae (Karmali, 1989, 2004; Nataro and Kaper, 1998; Paton and Paton, 1998). Manifestations of illnesses caused by STEC that are linked to production of Shiga toxins include,

non-bloody diarrhea, diarrhea-associated hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (Karmali, 1989). HUS cases in North America are predominantly caused by *E. coli* O157:H7. Enterohemorrhagic *E. coli* (EHEC) strains constitute a subtype of STEC serotypes that have been firmly associated with bloody diarrhea and HUS. EHEC are generally more pathogenic than other STEC because they possess a fuller complement of virulence determinants that encode production of intimin and enterohemolysin (Karmali, 2004). Serotypes O26, O111, O103, and especially O157 have been the predominantly isolated EHEC (Nataro and Kaper, 1998).

Of the few reports published (Jayarao and Henning, 2001; Murinda et al., 2002b; Steele et al., 1997), the prevalence of STEC in bulk tank milk has been reported to occur in 0.8–3.8% of samples evaluated (Table 1). Murinda et al. (2002b) reported the detection of *E. coli* O157:H7 from eight of 30 (26.7%) dairy farms at different sampling times. Two of 268 (0.75%) bulk tank milk samples and eight of 415 (1.93%) culled dairy cow fecal samples tested positive for *E. coli* O157:H7. Murinda et al. (2004b) isolated O26, O111, O103, and O157:H7 EHEC serotypes from dairy cows and/or the dairy farm environment. It appears that these serotypes could be of epidemiological significance in the United States. However, since there are presently no well-defined serotype-specific methods for their routine isolation, the epidemiological significance of non-O157:H7 STEC or EHEC remains undefined.

Reports on the prevalence of STEC associated with mastitis are rare. Stephan and Kuhn (1999) and Barrow and Hill (1989) indicated a prevalence of STEC in *E. coli* mastitis of 2.75% (four of 145) and 0.5% (one of 237), respectively. Conversely, Cullor (1997) indicated the absence of Stx-producing *E. coli* from 500 cases of coliform mastitis that included *E. coli*. A recent study by Murinda et al. (2004a) demonstrated that, of 105 *E. coli* mastitis isolates evaluated, all were stx-negative, suggesting alternative virulence characteristics are involved in mastitis; only four of these isolates were positive for *eaeA* gene sequences. Thus, it would appear that STEC are rarely associated as a causative agent of bovine mastitis.

The prevalence of *L. monocytogenes* in bulk tank milk has been reported to range from 1% to >12% (Table 2). *Listeria monocytogenes* causes septicemia and meningitis in humans. Pregnant women are particularly susceptible since *L. monocytogenes* infection may result in spontaneous abortions or stillbirth of the fetus. *Listeria monocytogenes* has been isolated from mammals, birds, fish, crustaceans and insects. In addition, *L. monocytogenes* are widespread in nature and live naturally in plants and soil environments. It can grow in a wide range of temperature and pH. This adaptability enables *L. monocytogenes* to grow in refrigerated raw milk and in low quality silage with a pH of >4.5. At high bacterial concentrations, *L. monocytogenes* can survive minimum HTST pasteurization (Bunning et al., 1988). *Listeria monocytogenes* can cause mastitis in cows, and it can be shed in milk of asymptomatic cows (Bourry et al., 1995; Jensen et al., 1996; Winter et al., 2004). Human contamination occurs through consumption of raw milk, or products manufactured with raw milk or ingestion of processed food cross-contaminated with pathogens present in the food processing plant environment (Gravani, 1999). In cattle, *L. monocytogenes* can cause neurological disease, abortion, or asymptomatic infections. Healthy, but infected animals, shed *Listeria* in feces and fecal contamination of pastures or vegetables has been implicated as a source of contamination for humans and ruminants. Therefore, spreading untreated manure onto pastures and cropland is regarded as a risk factor for *L. monocytogenes* foodborne disease.

The prevalence of *Salmonella* species in bulk tank milk has been reported to range from <1% to almost 9% (Table 2). *Salmonella* species have been linked with illness in animals and humans, and are one of the most commonly reported causes of human foodborne disease (Mead et al., 1999). *Salmonella* live in the intestinal tract of various animal species, including cattle, and therefore represent a major reservoir for human foodborne disease. Humans are infected, primarily via fecal contamination of food products or water; however, direct contact with sick animals or humans are additional sources of contamination, especially for farm families. A high percentage of human

salmonellosis occurs through consumption of raw milk or dairy products manufactured with raw milk (CDC, 2002, 2003).

Several *Salmonella* serotypes have been isolated from clinically ill cattle. *Salmonella enterica* serotype Typhimurium definitive type 104 (DT 104) is of particular concern to animal and public health agencies due to its multiple antimicrobial resistance (Besser et al., 2000). In-line milk filters from farms located in central, east, north, and west regions of New York State were evaluated to determine the prevalence of *Salmonella* in New York dairy herds. Six of 404 (1.5%) milk filters were positive for *Salmonella* spp. From these isolates, one was confirmed as *S. Typhimurium* DT 104 (Hassan et al., 2000). In another study conducted on 12 dairy farms from Minnesota, Michigan, New York, and Wisconsin where fecal, bulk tank milk, and environmental samples were analyzed for *Salmonella*, it was found that 1.1% of bulk tank milk ($n = 91$) and 12.6% of in-line milk filter samples ($n = 87$) were positive for *Salmonella* spp. (Warnick et al., 2003).

More recently, there has been an increase in infections caused by *Salmonella enterica* serotype Newport (CDC, 2002; Weir et al., 2004). These infections can be severe. An increasing number of *S. Newport* isolates have been shown to be resistant to 9 or more antimicrobial agents (Zhao et al., 2003). Risk factors that may be associated with *S. Newport* infection in humans include direct exposure to dairy farms, ingestion of raw milk and cheese made from unpasteurized raw milk, and consumption of raw or undercooked ground beef (Gupta et al., 2003). Results of the recent National Animal Health Monitoring System Dairy 2002 Survey reported by Van Kessel et al. (2004) indicated that 2.6% of bulk tank milk samples were culture-positive for *Salmonella*, and four of 22 isolates were *S. Newport*.

PREVALENCE OF FOODBORNE PATHOGENS IN THE DAIRY ENVIRONMENT

Dairy farms are an important reservoir of foodborne pathogens. The presence of foodborne pathogens in milk is due to direct contact with

contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal (Figs. 1 and 2). Most foodborne pathogens inhabit the ruminant intestinal tract, and therefore, dairy cattle are considered a major reservoir of *Salmonella*, *Campylobacter*, and STEC. *Listeria* species are widespread in nature and live naturally in plants and soil environments. Epidemiological studies have shown that cattle probably become infected through consumption of water and feedstuffs contaminated with feces and other cattle secretions/excretions. Presence of foodborne pathogens in bulk tank milk seems to be directly linked to fecal contamination that occurs primarily during the harvesting of raw milk, however, some foodborne pathogens can cause mastitis in which case the organism can be directly excreted into milk. Introduction of raw milk contaminated with foodborne pathogens into processing plants and their persistence in biofilms represents an important risk of post-pasteurization contamination that could lead to exposure of the consumer to pathogenic bacteria. (Arizcun et al., 1998; Roberts and Wiedmann, 2003; Wong, 1998).

The microaerophilic and thermophilic nature of *C. jejuni* hampers growth of this organism in feed and in nature. In spite of the fastidious requirements of *C. jejuni*, they are very versatile and metabolically active organisms capable of exploiting a variety of environments, especially the intestinal tract of mammals and birds. Enu-

FIG. 1. Maintenance and recycling of foodborne pathogens on the dairy farm. Foodborne pathogens originate from direct contact with contaminated sources, primarily feces, in the dairy farm environment. The primary source of foodborne pathogens in milk appears to be directly linked to fecal contamination that occurs during the milking process.

FIG. 2. Cycling of foodborne and veterinary pathogens in the dairy farm environment and their transfer to milk. (A) Amplification of the pathogen in the cow. (B) Dissemination in the immediate environment of the cow via feces. (C) Accumulation of feces on the dairy. (D) Spreading cow manure onto croplands. (E) Crops become contaminated with pathogens. (F) Contaminated feed consumed by cows. (G) Milk can become contaminated with pathogens during milking. (H) Pathogens enter bulk tank milk. (I, J) Unpasteurized milk, cheese, and other dairy products made from unpasteurized milk consumed by humans.

meration studies have shown that a critical amplification stage in the *Campylobacter* cell cycle occurs in the intestines of asymptomatic animals. Once cells are excreted to the environment, they must utilize survival strategies until ingested by a susceptible host. Thus, the intestinal tract and feces of susceptible animals (carriers) are considered the major reservoir of this foodborne pathogen. A similar process was described for *L. monocytogenes*. Data reported by Nightingale et al. (2004) supports the model in which the presence of the pathogen depends on the ingestion of contaminated feed followed by amplification in bovine hosts and fecal dissemination in the farm environment. A similar

series of events seems to occur with STEC. The influence of the diet (grains vs. forage) on the shedding of STEC in feces suggests that an amplification stage also occurs in the gastrointestinal (GI) tract of ruminants. The terminal rectum of the GI tract is an important site where this pathogen showed specific tropism (Naylor et al., 2003). *Escherichia coli* O157:H7 was detected in the terminal rectum regardless of whether animals were experimentally or naturally infected. The pathogen was detected in feces up to 4 weeks after experimental inoculation or 22 days in those that cohabited with infected animals. These findings lead authors to propose the existence of "super-shedders"

and colonization of the terminal rectum was a pre-condition for this status (Naylor et al., 2003). Taken together, colonization in the bovine GI tract and amplification of *C. jejuni*, *L. monocytogenes* and *E. coli* O157:H7 appear to be required stages in the cell cycles. Shedding of foodborne pathogens in feces and distribution in the environment where cows live ensures animal re-infection and persistence of the pathogen on the farm. This coupled with infection of other mammals, birds, and insects that live on the farm demonstrate that production units are a major reservoirs for foodborne pathogens.

A recent study by Murinda et al. (2004b) was conducted to investigate the major habitats of pathogens on dairy farms that could act as reservoirs and transient carriers of *Salmonella* spp., *L. monocytogenes*, *C. jejuni*, and O157 and non-O157 STEC. *Campylobacter jejuni*, *Salmonella* spp. and *L. monocytogenes*, sorbitol-negative (SN)–STEC O157:H7 and sorbitol-positive (SP)–STEC, respectively, were isolated from 5.1%, 3.8%, 6.5%, 0.7%, and 17.3% of samples evaluated. Diverse serotypes of SP-STEC including O157:H7, O26:H11, O111, and O103 were isolated. None of the five pathogen groups studied were isolated from bulk tank milk. Most foodborne pathogens (44.2%) were isolated directly from fecal samples. Bovine fecal samples, lagoon water, bedding, bird droppings and rats constituted areas of major concern on dairy farms. Although in-line milk filters from two farms tested positive for *Salmonella* or *L. monocytogenes*, none of the pathogens were detected in the corresponding bulk tank milk samples, probably due to a dilution effect. In another study conducted on 12 dairy farms from Minnesota, Michigan, New York, and Wisconsin (Warnick et al., 2003), *Salmonella* spp. were isolated from the sick cow pen floor, calving pen floor, milking cow feed bunk, lagoon or other manure storage areas, and cow water tank or drinking cups. From 4049 fecal samples tested, 9.3% were *Salmonella*-positive. The percentage of positive samples per farm ranged from 0.3 to 28%. From 811 environmental samples, 12.9% were positive for *Salmonella* spp. with a range from 0% to 40% per farm.

STEC O157:H7 serotype has been isolated frequently from cattle feces, and most human

EHEC O157:H7 infections originate, either directly or indirectly, from this source (Besser et al., 1997, 2001). Several investigations aimed at the identification of possible intervention strategies to control the prevalence of *E. coli* O157:H7 on farms have linked production practices (critical points) with persistence of this foodborne pathogen in cattle and generation of reservoirs in the farm environment (Elder et al., 2000; Garber et al., 1995; Hancock et al., 1997; Shere et al., 1998; Zhao et al., 1995). Among these, diet (Buchko et al., 2000; Cray et al., 1998; Diez-Gonzalez et al., 1998; Herriot et al., 1998), age of cattle (Cray and Moon, 1995; Garber et al., 1995), management of manure and fecal slurry, contaminated animal drinking water (Faith et al., 1996; LeJune et al. 2001; Shere et al., 1998), and management of pre- and post-weaned calves (Faith et al., 1996; Garber et al., 1995; Shere et al., 1998) have been identified as risk factors for infection and shedding of *E. coli* O157:H7 by cattle.

Especially important is the use of manure as a fertilizer or contaminated water to irrigate field crops. Contaminated manure and irrigation water were probable vehicles for the pathogen in many human disease outbreaks. Supporting data was obtained from a study where the occurrence and persistence of *E. coli* O157:H7 was determined on lettuce and parsley grown in soil fertilized with contaminated poultry or bovine manure composts or treated with contaminated irrigation water. Results from this study indicated that *E. coli* O157:H7 could persist for 154–217 days in soils fertilized with contaminated composts. After seedlings were planted, *E. coli* O157:H7 could be detected on lettuce and parsley for up to 77 and 177 days, respectively. In addition, *E. coli* O157:H7 persisted in soil for more than 5 months after application of contaminated compost or irrigation water, regardless of source or crop type (Islam et al., 2004).

FOOD SAFETY AND PUBLIC HEALTH IMPLICATIONS

Pasteurization is regarded as an effective method for eliminating foodborne pathogens and other bacteria from milk. However, the in-

creasing number of reports on detection of foodborne pathogens in pasteurized fluid milk and ready-to-eat dairy products clearly indicates that pasteurization alone is not the final solution for the control of milkborne pathogens. In addition, consumption of raw milk has been recognized as a major cause of foodborne diseases. Although the true incidence of milkborne disease in the United States is unknown, there are reports that link consumption of contaminated raw milk, inadequately pasteurized milk, or consumption of dairy products adulterated with contaminated raw milk to cases of human foodborne disease (Evans et al., 1996; Fahey et al., 1995; Fleming et al., 1985). For example, a high proportion of human infections caused by *C. jejuni* occurred through ingestion of untreated water, non-pasteurized milk, and inadequately pasteurized milk contaminated with this foodborne pathogen (Evans et al., 1996; Fahey et al., 1995). Raw milk is also used to produce hard cheeses that are aged for more than 60 days such as Cheddar, Colby, Parmigiano, and Provolone. Eleven foodborne disease outbreaks associated with cheese were reported in the United States from 1958 to 1991 (CDC, 1996; Johnson et al., 1990). Causative factors in cheese-related disease outbreaks were post-pasteurization contamination, faulty pasteurization equipment or procedures, and use of raw unpasteurized milk (Johnson et al., 1990).

Outbreaks of human salmonellosis have also been linked to ingestion of raw milk contaminated with *Salmonella* (CDC, 2003). The 2002–2003 multistate (Illinois, Indiana, Ohio, and Tennessee) outbreak of *Salmonella* Serotype Typhimurium infections was linked to an Ohio farm comprised of a working dairy, restaurant, snack bar, and petting zoo with goats, cows, calves, lambs, and pigs. The dairy was the only place in Ohio that legally sold raw milk in jugs, and served both milk and milk shakes made with raw milk to customers.

Although numerous studies have documented that foodborne pathogens of public health significance have been isolated from bulk tank milk and are capable of causing disease in humans, people continue to consume raw milk. Many farm families consume raw milk simply because it is a traditional practice and it is less expensive to take milk from the

bulk tank than buying pasteurized retail milk. Additionally, some believe that raw milk has a higher nutritional value than pasteurized milk (Hegarty et al., 2002). A study by Headrick et al. (1997) showed that people with less than a high school education were more likely to consume raw milk than those who had completed high school, suggesting that level of education may influence a person's choice to consume raw milk.

Rohrbach et al. (1992) reported that 68 of 195 (34.9%) dairy producers in East Tennessee and Southwest Virginia consumed raw bulk tank milk produced on their farm. Of the bulk tanks from which raw milk was consumed by dairy producers, 25% (17 of 68) contained one or more species of *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica* and *Salmonella* (Rohrbach et al., 1992). On farms where producers did not consume raw milk, 32% (40 of 127) of bulk tanks were contaminated with one or more of the above zoonotic pathogens, and bulk tank milk quality was not significantly different from those dairy producers who reported that they consumed raw milk from the bulk tank (25%, 17 of 68). The prevalence of consumption of bulk tank milk containing one or more potential human pathogenic bacteria among all dairy producers was 17/195 or 8.7%. Jayarao and Henning (2001) reported that 79 of 131 (60%) dairy producers in eastern South Dakota and western Minnesota consumed raw bulk tank milk produced on their farm. Of the 79 dairy producers who consumed raw milk, 21 (26.6) contained one or more species of *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica*, STEC, and *Salmonella*. There was no significant difference in the incidence of pathogenic bacteria in raw milk of dairy producers who did and did not consume raw milk. Thus, based on the limited information available, it would appear that numerous persons in the rural community consume raw unpasteurized bulk tank milk. Using data on incidence of raw milk consumption of 60% by dairy producers from the survey conducted by Jayarao and Henning (2001), and assuming that 25% of bulk tanks contain one or more potential human pathogens based on data reported by Rohrbach et al. (1992) and Jayarao and Henning (2001), the prevalence of consumption of contaminated bulk tank milk

would be 15%. Thus, risk exposure of people in the rural community to potential pathogenic bacteria capable of causing disease in humans via consumption of raw unpasteurized milk can be very high. Data clearly demonstrate that consumption of raw milk increases the chances of direct contact with foodborne pathogens or toxins produced by foodborne pathogens (i.e., *S. aureus* enterotoxins) and is therefore a risk factor of human foodborne disease. Educational efforts should be aimed at making the rural population aware of the health risks associated with consumption of raw unpasteurized milk.

In addition to direct consumption of contaminated raw milk, introduction of raw milk contaminated with foodborne pathogens into dairy processing plants represents an important risk of contamination of milk products that could lead to exposure of consumers to pathogenic bacteria. Although milk pasteurization is regarded as an effective method to eliminate foodborne pathogens, some dairy products do not undergo pasteurization (i.e., specialty cheeses). Furthermore, pathogens such as *L. monocytogenes* survive and thrive in post-pasteurization processing environments thus leading to recontamination of dairy products. These two significant exposure pathways pose a risk to the consumer from direct exposure to foodborne pathogens in unpasteurized dairy products as well as dairy products that are re-contaminated in the post-pasteurization processing environment. The increasing number of incidences in which foodborne pathogens are detected in fluid milk and ready-to-eat dairy products clearly indicate that pasteurization is not the ultimate tool to control milkborne pathogens. It is likely that fecal and foodborne pathogen contamination occurs during the harvesting of raw milk (i.e., milking, collection, and storage) and the farm environment likely plays a major role in the presence of foodborne pathogens in bulk tank milk. Reducing the potential for contamination during harvesting of milk should result in a reduction of foodborne pathogens in raw milk.

Introduction of *L. monocytogenes* into food processing plants results in reservoirs that are difficult to eradicate. Biofilms are a constant issue in food processing environments. *Listeria monocytogenes* survived for extended periods

on stainless steel and rubber, materials commonly used in food-processing equipment. Some components in the rubber inhibited growth of the organism, but also affected the efficacy of sanitizers on biofilm inactivation. These conditions led to the persistence of low numbers of *L. monocytogenes* on equipment surfaces making eradication difficult (Wong, 1998). The level of contamination of milk processing plants was investigated by several research groups. In a survey conducted in 39 frozen milk plants in California, *L. monocytogenes* was the only species recovered from five (12.8%) plants. Observations of this study indicated that the type of product received and type of pasteurization did not influence recovery of *Listeria* from a plant. However, a significant association with size of the plant and recovery of *Listeria* was observed. Also, it was observed that the level of sanitation and extent of environmental contamination control program (ECCP) were not associated with recovery of *Listeria* from a plant. The rate of recovery of *Listeria* from plants with above average sanitation and excellent or moderate ECCP was 6.81%, whereas the recovery rate from plants with below average sanitation and slight or no ECCP was 27.5%. Interestingly, the highest recovery rates of *Listeria* in frozen milk product plants were in batch flavoring, freezing and ingredient blending, and package filling areas (Walker et al., 1991). In a study conducted in 21 dairy processing plants in Vermont, 80 of 378 sites (21.2%) were identified as *Listeria*-positive, and of these 35 (43.8%) were positive for *L. monocytogenes* (Pritchard et al., 1995).

Mycobacterium avium subsp. *paratuberculosis* (MPTB), the etiologic agent of Johne's disease in dairy and beef cattle and sheep, is considered by some to be an emerging foodborne pathogen. This concern is based on reports that have shown that Crohn's disease in human patients bears a clinical resemblance to Johne's disease. Current evidence neither supports or refutes a mycobacterial cause of Crohn's disease, but evidence does suggest that either a subset of human patients are presumably infected with MPTB or that the ulcerated tissues of Crohn's disease patients selectively harbor MPTB acquired as an environmental contaminant or opportunistic pathogen (Bannantine et

al., 2004; Harris and Barletta, 2001; Sechi et al., 2001). Milk from dairy cows with Johne's disease could be a potential vector in the transmission of MPTB to humans based on evidence demonstrating that the pathogen can be shed and isolated from raw milk and that MPTB may survive pasteurization (Grant et al. 2002a,b; Millar et al., 1996).

While the role of MPTB is firmly established in Johne's disease in cattle and sheep, its role in Crohn's disease is less clear (Bannantine et al., 2004). The need for a definitive answer to this important question has been recognized by the scientific community and is currently being investigated by several research groups. The biology of MPTB makes its manipulation in the laboratory and development of rapid and accurate diagnostic methods difficult. New detection methods are needed to definitively answer questions surrounding MPTB as a foodborne pathogen and if this pathogen is a contributing factor in Crohn's disease. Recent advances in the study of the MPTB genome and immunology have led to the identification of specific DNA sequences and protein antigens that may allow reliable detection of MPTB (Bannantine et al., 2004). Improving the detection of MPTB would help to answer fundamental questions on the epidemiology and pathogenesis of MPTB including its role in foodborne illness.

CONCLUSION

Milk can harbor a variety of microorganisms and can be an important source of foodborne pathogens. The presence of foodborne pathogens in milk can be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. The dairy industry should be concerned about dairy food safety because (1) outbreaks of disease in humans have been traced to the consumption of unpasteurized milk and have also been traced back to pasteurized milk, (2) unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbors, and raw milk advocates, (3) unpasteurized milk is consumed directly by a large segment of the population via

consumption of several types of cheeses manufactured from unpasteurized milk, (4) entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms, and subsequent contamination of processed milk products and exposure of consumers to pathogenic bacteria, (5) pasteurization may not destroy all foodborne pathogens in milk, and (6) faulty pasteurization will not destroy all foodborne pathogens.

Information presented in this review supports the model in which the presence of pathogens depends on ingestion of contaminated feed followed by amplification in bovine hosts and fecal dissemination in the farm environment (Figs. 1 and 2). The final outcome of this cycle is a constantly maintained reservoir of foodborne pathogens that can reach the human population by direct contact, ingestion of raw contaminated food (raw milk or cheese made with raw milk), or contamination during the processing of milk. Isolation of bacterial pathogens with similar biotypes from dairy farms and from outbreaks of human disease substantiates this hypothesis.

The challenges to providing a safe and nutritious food supply are complex because all aspects of food production—from farm to fork—need to be considered. Given the considerable national/international demand and expectations for food safety and the formidable challenges of producing and maintaining a safe food supply, food safety research and educational programs has taken on a new urgency. As the system of food production and distribution changes, the food safety system needs to change with it. A strong science-based approach that addresses all the complex issues involved in continuing to improve food safety and public health is necessary to prevent foodborne illnesses. Not only must research be conducted to solve complex food safety issues, but also results of that research must be communicated effectively to producers and consumers. Research and educational efforts identifying potential on-farm risk factors will better enable dairy producers to reduce/prevent foodborne pathogen contamination of dairy products leaving the farm. Identification of on-farm reservoirs could aid with implementation of

farm-specific pathogen reduction programs. Foodborne pathogens, mastitis, milk quality and dairy food safety are indeed all interrelated. A safe, abundant and nutritious milk and meat supply should be the goal of every dairy producer in the world.

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