

Salmonella, Listeria, E. Coli and Mycobacterium paratuberculosis in Milk Is there Cause for Concern?

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Introduction

Throughout the world food safety and quality is a topic of public concern. Well-publicized and widespread foodborne disease outbreaks have created an awareness of potential threats to human health from food products.^{1,2,3} Much of the concern about foodborne disease is related to the changing nature of outbreaks.⁴ Traditionally, outbreaks of foodborne disease were acute and highly local in nature, often following a social event in a community. The more recent events have been the result of low-level contamination of widely distributed commercial food products. Instead of a local food handling error, recent large epidemics have generally been the result of an error in the industrial chain of food production.

Milk-borne illnesses have been recognized since the beginning of the dairy industry.⁵ Milk is a highly nutritious food that is ideally suited for growth of pathogenic and spoilage organisms. Prior to widespread adoption of pasteurization, bacterial infections such as diphtheria, scarlet fever and tuberculosis were often linked to consumption of raw milk products. Routine pasteurization has been highly effective in ensuring the safety of dairy products. Even though dairy products are consumed on a daily basis in the United States, milk, ice cream and cheese have been identified as the vehicle for less than 1.5% of all foodborne illness cases investigated by the Center for Disease control.⁶ In cases involving dairy products, errors in the pasteurization process or the addition of non-pasteurized eggs to dairy products have frequently been identified as the route of contamination.^{7,8} Regardless of the rarity of milk associated foodborne disease, the importance of dairy products in human diets requires systems that ensure dairy product safety. The objective of this paper is to review the risks of selected potential human pathogens in our milk supply.

Salmonella (including Salmonella DT104)

The Disease in Cattle. Various serotypes of Salmonella are well established as a cause of disease in both juvenile and adult dairy cattle.⁹ The most common clinical signs are fever and diarrhea. Abortion due to bacteremia or endotoxemia can occur. In adult dairy cattle, clinical signs often occur during periods of reduced immune function such as the peri-parturient period. The risk period for calves varies depending upon serotype (usually <14 days of age for S. typhimurium, 1 week to 6 months of age for S. dublin). The disease is spread through fecal-oral transmission and is maintained within cattle populations by 1) carrier animals; 2) infected calves; 3) environmental contamination. Contaminated feed is a source of infection. In the US, it is estimated that 5-20% of dairy cow feed is contaminated with Salmonella.¹⁰ Multiple drug resistant Salmonella DT104 has been isolated from a commercial feed sample in a feed survey in the northwestern United States.¹²

The National Animal Health Monitoring System (NAHMS) Dairy '96 study reported fecal shedding of Salmonella in 5.4% of dairy cows sampled. Fecal shedding was higher in cows that were due to be culled within 7 days and in cull cows sampled at markets.¹¹ Based upon a single sample from 91 dairy operations and 97 cull dairy cow markets across 19 states, the study estimated that 27.5% of dairy operations and 66.7% of markets contained at least one cow shedding Salmonella.

Zoonotic Potential. Salmonella has been recognized as a foodborne pathogen for well over 100 years.⁵ Human infections with Salmonella are quite common with an estimated case rate of 13.8 cases/100,000 people.¹² The vast majority of people infected with Salmonella experience mild to moderate, self-limiting gastroenteritis. A very small number of individuals (often immune suppressed) experience severe and occasionally fatal outcomes. An emerging strain of Salmonella has attracted attention because of multiple drug resistance.¹² This strain of Salmonella was first detected in humans in 1984 and in cattle in 1988.^{13,14} Symptoms in humans include severe gastroenteritis and one study of this pathogen reported a case-fatality rate of 3%.¹⁵ The reported case-fatality rate for other types of Salmonella infections in humans is approximately 0.1%.

Source of Human Infections. Eggs are the primary source of Salmonella infections.¹⁶ Of 293 outbreaks with known sources reported in the United States between 1985 and 1996, 233 (79%) were related to eggs or egg-containing foods.¹⁷ Improperly cooked meat and poultry products and direct contact with infected animals have been the primary source of DT104 infections.¹⁵ Since 1967, authorities in the United States have documented 4 large outbreaks involving the consumption of improperly pasteurized or raw milk contaminated with either *S. typhimurium* or *S. dublin*.⁵ The infective dose of Salmonella varies depending upon serotype and the immune status of the individual, but it is currently believed that infections can be established for some serotypes with very low doses (<100 cells).¹⁸

Critical Milk Safety Issues

Are Salmonella spp. shed in milk? Salmonella are an infrequent cause of mastitis in dairy cows but several species of Salmonella have been documented to colonize udders and shed at levels of up to 2000 organism/ml.¹⁹ Salmonella has been detected in raw milk in farm bulk tanks in several studies (Table 1).²⁰

Is raw milk a risk? Ingestion of raw milk or raw milk products are a well-documented cause of Salmonella infections.⁵ Raw milk products including non-fat dry milk and ice-cream have also been the vehicle for outbreaks of Salmonella.

Does pasteurization effectively kill it? Standard methods of pasteurization (both vat pasteurization and high-temperature, short time pasteurization) are very effective in destroying Salmonella.¹⁸ In outbreaks that have been originally attributed to ingestion of pasteurized milk inadequate pasteurization and post-pasteurization contamination have been documented.⁵

Is cheese a risk? Over 90% of all Wisconsin origin cheese is made from pasteurized or heat-treated milk.^a Cheese made from pasteurized milk can be considered safe. Raw milk cheeses have been implicated in several Salmonella outbreaks.⁵ The low dose of Salmonella potentially required to establish infection suggests that raw milk cheeses could pose a public health hazard, especially for people with compromised immune systems.

Listeria

The Disease in Cattle. *Listeria monocytogenes* presents as three distinct clinical syndromes in ruminants.²¹ Neurologic disease is the most clinically recognizable form and is often referred to as “circling disease.” Affected animals exhibit fever, anorexia, head tilt, circling, decreased consciousness and/or other neurologic signs. Additional clinical syndromes include septicemia (often resulting in death) in calves and abortion in pregnant females. The organism is present in chopped forages and multiplies when the pH is >5.0. Infection occurs when digestive tract mucous membranes are injured (from sharp forages etc.) and the organism can ascend nerves to infect the brain stem. The disease generally occurs sporadically in adult animals and is amenable to antibiotic therapy.

Zoonotic Potential. Healthy people rarely develop serious illness from *Listeria* but susceptible populations (pregnant women, newborns or people with impaired immune function) can develop serious illness.²² Listeriosis during pregnancy can cause flu-like symptoms with fever and chills and may lead to premature birth or loss of the fetus. Meningitis, septicemia and gastrointestinal symptoms may be seen in other individuals. The mortality from listeric meningitis may be as high as 70%. The time of onset of the disease can range from a few days to several weeks. In 1987, the Center for Disease Control estimated that there were at least 1,600 cases with 415 deaths per year in the US.²³

Source of Human Infections. Consumption of raw milk, raw-milk cheeses, coleslaw and hotdogs have been the source of most outbreaks of human listeriosis in the last 2 decades.^{24,23} Since August 1998, at least 50 illnesses caused by a rare strain of *L. monocytogenes* have been reported to the CDC by 11 states.²² As of January 1999, 6 adults had died and 2 pregnant women had reported spontaneous abortions. The vehicle for transmission was hot dogs and deli meats produced by one manufacturer.

Critical Milk Safety Issues

Is it shed in milk? *L. monocytogenes* has been reported to cause mastitis and can be shed in milk and feces. Shedding in milk can occur from both clinically affected and asymptomatic animals. The organism has been consistently recovered from raw milk in farm bulk tank surveys (Table 1). *Listeria* organisms can multiply in raw milk at a wide range of temperatures, including refrigeration.

Is raw milk a risk? Worldwide, dairy products have been responsible for 5 major outbreaks involving about 600 people since 1949.⁵ Raw milk products (soft or Mexican style cheeses) have been implicated in most outbreaks.

Does pasteurization effectively kill it? *L. monocytogenes* is more heat tolerant than many other pathogens, but current pasteurization methods are considered to

^a J.R. Bishop, 1999. Center. Dairy Research, University of WI, personal communication

be effective. *L. monocytogenes* can be a post-pasteurization contaminant of ice cream.⁵ The U.S. has a “zero tolerance” policy for *L. monocytogenes* in ready to eat food products including dairy products. If the organism is identified in a product, a complete recall of the product is issued. In 1994-1995, *Listeria* accounted for 13 of 18 dairy related recalls. No foodborne disease outbreaks were linked with these recalls.

Is cheese a risk? Cheese produced from pasteurized milk should not be a public health hazard. Ingestion of raw milk cheeses have been a documented source of human listeriosis and should be avoided. People with compromised immune systems and women of childbearing age are at especially high risk of contracting listeriosis from this route.

E. Coli (including O157:H7)

The Disease in Cattle. Most strains of *Escherichia coli* are normal inhabitants of the gastrointestinal tract of humans and animals. Several distinctive disease conditions of cattle can be attributed to *E. coli*.²¹ Septicemic colibacillosis occurs in neonatal calves and is associated with failure of passive transfer. Clinical signs include dehydration and endotoxic shock. Enterotoxigenic *E. coli* (ETEC) may cause secretory diarrhea in calves up to 21 days of age and enteropathogenic *E. coli* (EPEC) often cause malabsorption diarrheas in calves concurrently affected with viral diarrhea. In mature lactating dairy cattle, *E. coli* are a well-recognized cause of clinical mastitis. Recently, verotoxin producing *E. coli* strains (such as *E. coli* O157:H7) have been identified. *E. coli* O157:H7 has only rarely been associated with disease in cattle. The NAHMS Dairy '96 survey identified *E. coli* O157:H7 in fecal specimens from 0.9% of milk cows and 2.8% of milk cows due to be culled within 7 days.¹¹ The prevalence on farms was higher with at least 1 culture positive cow found in 24.2% and 30.9% of farms and markets respectively. In a western study, seven of 205 (3.4%) cull dairy cows on tested farms were positive for *E. coli* O157:H7.²⁵ *E. coli* was found in 30.1% of 209 samples of cattle feed.²⁶ While none of the samples were positive for O157:H7, fecal contamination of feedstuffs is a likely possibility for environmental contamination.

Zoonotic Potential. The classification of coliform diseases in humans is similar to cattle. Enteropathogenic and enterotoxigenic *E. coli* have been identified as causes of human diarrhea for at least 50 years.⁵ The most common clinical syndrome is diarrhea, nausea, abdominal cramping and fever. In contrast, enterohemorrhagic *E. coli* caused by O157:H7 is noteworthy because of the seriousness of the syndrome. Hemorrhagic colitis with severe abdominal pain may progress to hemolytic uremic syndrome (HUS) resulting in acute renal failure. Case fatality rates may approach 10%.⁵ In 1998, the CDC estimated that the incidence rate of human illness caused by O157:H7 was 2.8 per 100,000 population.²⁷

Source of Human Infections. Fecal contamination of drinking water or contamination during food preparation are the most common source of EPEC and ETEC caused outbreaks. Human illness caused by these pathogens is not common in developed countries with good hygienic practices. The vast majority of *E. coli*

O157:H7 outbreaks in humans have been related to undercooked hamburger.^{2,5} Consumption of raw milk and raw milk cheese have been documented vehicles for several outbreaks.⁵ Secondary transmission among humans has also been documented.

Critical Milk Safety Issues.

Is it shed in milk? *E. coli* are a well-recognized cause of mastitis in dairy cattle. However, to date, *E. coli* O157:H7 has not been recognized as a cause of mastitis. A study that examined >500 isolates of milk obtained from coliform mastitis cases was not able to find O157:H7 in any of the samples.²⁸ *E. coli* O157:H7 has been recovered from raw milk in bulk tank surveys (Table 1). Fecal contamination during milking is the likely source.

Is raw milk a risk? Raw milk is an infrequent vehicle of foodborne illness caused by ETEC or EPEC strains of *E. coli*. Fecal contamination of milk during the milking process is a possible source of raw milk contamination. Consumption of raw milk contaminated with O157:H7 on dairy farms has been a documented vehicle for HUS in children.²⁹

Does pasteurization effectively kill it? All strains of *E. coli* (including O157:H7) are readily killed by pasteurization.

Is cheese a risk? In the U.S., two outbreaks of gastroenteritis have been related to cheese. In both instances, post-pasteurization contamination was documented.⁵ The most recent episode occurred in 1985. Homemade cheese from non-pasteurized goat's milk was recently implicated in an outbreak in Scotland.

Mycobacterium paratuberculosis

The Disease in Cattle. *Mycobacterium paratuberculosis* is the causative agent for a disease of ruminants commonly referred to as "Johne's Disease." The clinical symptoms of Johne's disease were first described in 1895.³⁰ Cattle become infected with *M. paratuberculosis* as calves but do not usually develop clinical signs until reaching 2 to 5 years of age. The risk of establishing infection decreases dramatically as animals age. Calves less than 4 months of age are considered most susceptible.²¹ The primary route of transmission is fecal-oral but in-utero transmission may rarely occur. An additional route of transmission is ingestion of infected colostrum or milk, however the percent of infected cows that shed in colostrum and/or milk is unknown. Most infected cattle never develop clinical signs but may shed *M. paratuberculosis* in their feces. Animals that progress to clinical signs typically present with chronic weight loss, diarrhea and hypoproteinemia. Clinically infected animals shed large numbers of organisms in their feces. The disease is believed to occur in ruminants throughout the world. In the U.S., herd level estimates of prevalence vary widely depending upon testing and sampling procedures. The NAHMS Dairy '96 study estimated that 22% of herds may be infected with the *M. Paratuberculosis*.³¹

Zoonotic Potential. *M. paratuberculosis* is **not** a proven pathogen in humans. However, Crohn's disease (a type of inflammatory bowel disease) in humans presents with a clinical syndrome similar to Johne's disease in ruminants. Several species of *Mycobacterium* (including *M. paratuberculosis*) have been isolated from intestinal biopsies in a small number of patients with Crohn's disease.^{32,33}

The issue is confusing because a number of studies have been **unable** to demonstrate *M. paratuberculosis* DNA in tissue of patients with Crohn's. ^{34,35,36,37} There are numerous theories regarding the etiology of Crohn's disease. ³⁸ The Crohn's and Colitis Foundation of America states that there is a strong genetic component to the disease. A person with an affected relative has a 10 fold higher risk than that of the general population. ³⁸ Environmental factors are also important with people living in developed countries incurring higher risk. Reputable Crohn's advocacy organizations from around the world make similar statements. ^{39,40} In summary, at this time, a causal relationship between Crohn's and Johne's disease has not been confirmed. ⁴¹

Critical Milk Safety Issues

Is it shed in milk? *Mycobacterium paratuberculosis* organisms have been recovered in low numbers in milk samples and colostrum obtained from clinically and subclinically infected cows. ^{42,43} The only study that reported concentration recovered an extremely low concentration of the organism (Table 2). ⁴⁴

Does pasteurization effectively kill it? This question became a controversial and high profile topic when a study demonstrated *M. paratuberculosis* DNA (not viable organisms) in retail milk in England. ⁴⁵ This controversial study spurred a number of researchers to investigate the issue. All of the studies have used milk inoculated with relatively high numbers of *M. paratuberculosis* bacteria. Various methods of pasteurization have been used. ^{46,47,48} Current results suggest that commercial methods of pasteurization used in the dairy industry are effective in inactivation of this organism. ⁴⁹

Summary

Milk is rarely but occasionally linked to outbreaks of foodborne disease in humans. Several disease causing pathogens in cows (*Salmonella spp.*, *Listeria monocytogenes* and *E. coli*) can contaminate raw milk. For over 50 years, the process of pasteurization has proven highly effective in protecting public health. However, the threat to human health from ingestion of **unpasteurized** milk should not be underestimated. In 1986, 2 Wisconsin farm children developed HUS from drinking unpasteurized milk on a farm. ²⁹ In May 1999, a group of children in Scotland were exposed to *E. coli* O157:H7 at school from eating raw milk cheese from goats milk from a grandparents farm. To ensure personal health, farm families and visitors to farms should not drink unpasteurized milk. Additionally, raw milk products are dangerous for people with suppressed immune systems. This population includes, children, pregnant women, many elderly people, people taking immune suppressing drugs (such as chemotherapy) and people with immune deficiency diseases.

Many of the human pathogens that affect cows are shed in feces. Care should be taken during the milking process to reduce the possibility of fecal contamination of raw milk. Hands should always be washed prior to milking and ideally throughout the milking process. Farm managers should work with their local veterinarians to develop herd management plans that ensure the health of their dairy cattle.

Table 1. Recovery of pathogens from unpasteurized bulk tank milk.

Pathogen	States or Province	No. Samples	% Bulk Tanks Positive	Ref.
Salmonella	WI, MI, IL	678 tanks	4.70%	20
	Ontario	1,721 tanks	0.17%	50
	SD, MN	131 tanks	6.10%	51
	Tennessee & VA	292	8.90%	52
Listeria	Ontario	1,721 tanks	2.73%	50
	SD, MN	131 tanks	4.60%	51
	Tennessee & VA	292	4.10%	52
E. Coli (O157:H7)	Ontario	1,721 tanks	0.87%	50
	WI	115 tanks	10.00%	53
M.paratuberculosis	Not Documented	None	Not Documented	

Table 2: Key Issues Regarding Human Health Risk

	Salmonella	Listeria	E. Coli O157:H7	M.paratuberculosis
Zoonotic Potential	Yes	Yes	Yes	Not proven
Min. documented Infective Dose (Human)	<100 cells	<1000 organisms	Unknown – probably low	Not applicable
Maximum documented Level Shed in Milk	2,000/ml	10,000cfu/ml	Not documented	2-8 cfu/ml
Documented milk borne transmission	Yes	Yes	Yes – very rare	No
Raw milk risk	Yes	Yes	Yes	Not Applicable
Killed by pasteurization	Yes	Yes	Yes	Currently believed to be
Usual route of human infection	Eggs & undercooked meats	Raw milk & undercooked meats, cold cuts	Undercooked meat	Not applicable

References

- ¹ Anonymous. Centers for Disease Control and Prevention. 1985. Listeriosis outbreak associated with Mexican-Style cheese – California. *Morbidity and Mortality Weekly Report*. 34:357-359.
- ² Anonymous. Centers for Disease Control and Prevention. 1993. Update: Multistate Outbreak of *Escherichia coli* O157:H7 infections from hamburgers – Western United States, 1992-1993. *Morbidity and Mortality Weekly Report*. 42:258-263.
- ³ Anonymous. Centers for Disease Control and Prevention. 1998. Multistate outbreak of listeriosis – United States, *Morbidity and Mortality Weekly Report*. 42:1085.
- ⁴ Tauxe, R.V., 1997. Emerging Foodborne Diseases: an evolving public health challenge. *Emerging Infectious Diseases* [serial online] 3:1-14, Online available: www.cdc.gov/cnidod/eid/index.htm.
- ⁵ Ryser, E.T. 1998. Public Health Concerns. Pages 263-404 in *Applied Dairy Microbiology*. E.H. Marth and J. L. Steele, ed. Marcel Dekker, Inc. NY.
- ⁶ Bean, N.H., Goulding, J.S., Lao, C., Angulo, F.J.. 1996 Surveillance for foodborne-disease outbreaks—United States, 1988-1992. *Morbidity and Mortality Weekly Report* 45(SS-5):1-73.
- ⁷ Anonymous, Centers for Disease Control and Prevention. 1994. Outbreak of *Salmonella enteritidis* Associated with Nationally distributed ice cream products – Minnesota, South Dakota, and Wisconsin.

- ⁸ Ryan C.A., Nickels, M.K. 1987. Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. *J Am Med Assoc.* 11;258(22):3269-74.
- ⁹ Smith, B.P. 1990. Salmonellosis. Pages 818-822 in *Large Animal Internal Medicine*. B.P. Smith ed. C.V. Mosby Co. St. Louis MO
- ¹⁰ Hancock, D., Dargatz, D. 1995. Implementation of HACCP on the farm. Hazard Analysis and Critical Control Point (HACCP) Symposium, 75th Conference of Research Workers in Animal Diseases, Chicago, IL.
- ¹¹ Wells, S., Fedorka-Cray, P.J., Besser, T., et al. 1998. E. coli O157 and Salmonella – status on U. S. dairy operations. Available online at <http://www.aphis.usda.gov/vs/ceah/cahm/>
- ¹² Akkina, J. E., Hogue, A. T., Angulo, F. J., Johnson, R., et al. 1999. Epidemiologic aspects, control, and importance of multiple-drug resistant Salmonella Typhimurium DT104 in the United States.
- ¹³ Anonymous, Centers for Disease Control and Prevention. Minutes of the Salmonella serotype Typhimurium definitive type 104 (DT104) workshop. Atlanta: Center for Disease Control and Prevention. May 1, 1997.
- ¹⁴ Hollingsworth, J., Kaplan, B. 1997. Federal agencies collaborate to control dangerous new Salmonella strain. *J Am Vet Med Assoc.* 210;1712,1716.
- ¹⁵ Wall P.G., Morgan D., Lamden, K., et al. 1994. A case control study of infection with an epidemic strain of multiresistant Salmonella typhimurium DT104 in England and Wales. *Commun Dis Rep CDR Rev* 4:R130-135.
- ¹⁶ St. Louis, M. E., Morse, D. L., Potter, M. E., et al. 1988. The emergence of grade A eggs as a major source of Salmonella enteritidis infections—new implications for control of Salmonellosis. *J Am Med Assoc* 259;2103-2107.
- ¹⁷ Angulo, F.J., Swerdlow, D. L. 1998. Salmonella enteritidis infections in the United States. *J Am Vet Med Assoc.* 12;213:1729-1731.
- ¹⁸ D'aost, J.Y. 1989. Salmonella. Pages 327-445 in *Foodborne Bacterial Pathogens*. M.P. Doyle ed. Marcel Dekker Inc., New York.
- ¹⁹ Fontaine, R. E., Cohen, M. L., Martin, W. T., Vernon, T.M. 1980. Epidemic salmonellosis from cheddar cheese—surveillance and prevention. *Am J Epidem* 111:247.
- ²⁰ McManus, C., Lanier, J.M. 1987. Salmonella, Campylobacter jejuni, and Yersinia enterocolitica in raw milk. *J Food Prot* 50:51.
- ²¹ Rebhun, W.C., 1995. Diseases of Dairy Cattle. Pages 410-413. Williams and Wilkins. Media PA
- ²² Anonymous, Centers for Disease Control and Prevention. 1999. Update: Multistate outbreak of listeriosis – United States, 1998-1999. *Morbidity and Mortality Weekly Report*. 47:1117-1118.
- ²³ Anonymous, 1992. Listeria monocytogenes. In *Foodborne Pathogenic Microorganisms and natural toxins handbook*. Center for Food Safety and Applied Nutrition. Available online at <http://vm.cfsan.fda.gov/~mow/chap6.html>
- ²⁴ Lovett, J. 1989. Listeria monocytogenes. Pages 283-310 in *Foodborne Bacterial Pathogens*. M.P. Doyle ed. Marcel Dekker Inc., New York.
- ²⁵ Rice, D.,H, Ebel, E. D., Hancock, D. D., Besser, T. E. et al. 1997. Escherichia coli O157 in cull dairy cows on farm and at slaughter. *J Food Prot.* 60:1386-1387.
- ²⁶ Lynn, T.V., Hancock, D. D., Besser, T. E., Harrison, J.H., et al. 1998. The occurrence and replication of Escherichia coli in Cattle Feeds. *J Dairy Sci.* 81:1101-1108.
- ²⁷ Anonymous. Centers for Disease Control and Prevention. 1999. Incidence of foodborne illnesses: preliminary data from the foodborne diseases active surveillance network (FoodNet) – United States, 1998. *Morbidity and Mortality Weekly Report*. 48:189-194.
- ²⁸ Cullor, J.S. 1997. Mastitis and dairy environment pathogens of public health concern. . Page 20-32 in *Proc. National Mastitis Council*, Albuquerque, NM, Feb 16-19, 1997. Natl. Mast. Council Madison, WI.
- ²⁹ Martin, M.L., Shipman, L.D., Wells, J. G., Potter, M.E., et al. 1986. Isolation of Escherichia coli O157:H7 from dairy cattle associated with two cases of haemolytic uremic syndrome. *Lancet* 2:1043.
- ³⁰ Johne, H. A., Frothingham, J. 1895. Ein eigenthuemlicher fall von tuberculose beim rind. *Dtsch. Z. Tiermed. Pathol.* 21:438-454.

- ³¹ NAHMS. 1997. Johne's Disease on U.S. Dairy Operations. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO.
- ³² Chiodini, R. J., 1989. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. *Clin. Microbio. Rev.* 2:90-117.
- ³³ Sanderson, J.D., Moss, M.T., Tizard, M.L.V., Hermon-Taylor. 1992. *Mycobacterium paratuberculosis* DNA in Crohn's disease tissues. *Gut* 33:890-896.
- ³⁴ Frank, R.S., and Cook, S.M., 1996. Analysis of paraffin sections of Crohn's disease for *Mycobacterium paratuberculosis* using polymerase chain reaction. *Mod. Pathol.* 9:32-35.
- ³⁵ Rosenberg, W.M.C., Bell, J. I., Jewell, D.P., 1991. *Mycobacterium paratuberculosis* DNA cannot be detected in Crohn's disease tissues. *Gastroenterology* 100:A611.
- ³⁶ Rowbatham, D.S., Mapstone, N.P., Trejdosiewicz, L.K., et al. 1995. *Mycobacterium paratuberculosis* DNA not detected in Crohn's disease tissue by fluorescent polymerase chain reaction. *Gut* 37:660-667.
- ³⁷ Wu, S.W, Pao, C.C., Chan, J., Yen, R.S.B., 1991. Lack of mycobacterial DNA in Crohn's disease tissue. *Lancet* 337:174-175.
- ³⁸ Anonymous. Facts about the epidemiology of inflammatory bowel diseases. 1999. Crohn's and Colitis Foundation of America. Online, available at: <http://www.cffa.org/medcentral/library/basic/news0129.htm>
- ³⁹ Anonymous. Australian Crohn's and Colitis Association. 1999. Online, available at <http://www.accaq.org.au/whatis/causes.htm>
- ⁴⁰ Anonymous. National Association of Thorn's and Colitis. 1999. Online, available at <http://www.nacc.org.uk/ibd.htm>
- ⁴¹ Collins, M.T., 1998. *Mycobacterium paratuberculosis*: a potential food-borne pathogen?. *J Dairy Sci.* 80:3445-3448.
- ⁴² Streeter, R.N., Hoffis, G.F., Bech-Nielsen, S., et al. 1995. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. *Am J Vet Res.* 1995. 56:1322-1324.
- ⁴³ Taylor, T.K., Wilks, C.R., McQueen, D.S., 1981. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. *Vet Rec* 109:532-533.
- ⁴⁴ Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. *J. Clin. Microbiol.* 30:166-171.
- ⁴⁵ Millar, D. S., Ford, S. J., Sanderson, J.D., et al. 1996. IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurized cows' milk in England and Wales. *Appl. Environ. Microbio.* 62:3446-3452.
- ⁴⁶ Grant, I.R., Ball, H.J., Neill, S.D., Rowe, M.T., 1996. Inactivation of *Mycobacterium paratuberculosis* in cows milk at pasteurization temperatures. *Appl Envir Micro* 62:631-636.
- ⁴⁷ Stabel, J.R., Steadham, E.M., Bolin, C.A., 1997. Heat inactivation of *Mycobacterium paratuberculosis* in raw milk: are current pasteurization conditions effective? *Appl Envir Micro* 63:4975-4977.
- ⁴⁸ Sung, N., Collins, M.R., 1998. Thermal Tolerance of *Mycobacterium paratuberculosis*. *Appl Envir Micro* 64:999-1005.
- ⁴⁹ Stabel, J.R., 1998. Johne's Disease: a hidden threat. *J Dairy Sci* 81:283-288.
- ⁵⁰ Steele, M.L., McNab, W. B., Poppe, C., Mansel, W. Griffiths et al. 1997. Survey of Ontario bulk tank raw milk for food-borne pathogens. *J Food Prot.* 60:1341-1346.
- ⁵¹ Jayarao, G. 1999. A study on the prevalence of pathogens in bulk tank milk. Page 148-149 in Proc. National Mastitis Council., Natl. Mast. Coun. Madison, WI.
- ⁵² Rohrbach, B.W., Draughon, F.A., Davidson, P.M., Oliver, S.P., 1992. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in bulk tank milk: risk factors and risk of human exposure. 55:93-97.
- ⁵³ Padhye, N.V., Doyle, M.P. 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. *Appl Envir Micro.* 57:2693-2698.