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REVIEW

Emerging Pathogenic Bacteria: *Mycobacterium avium* subsp. *paratuberculosis* in Foods

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Abstract

Mycobacterium avium paratuberculosis (MAP), the cause of Johne's disease in animals, may be a causative agent of Crohn's disease (CD) in humans, but the evidence supporting this claim is controversial. Milk, meat, and water could be potential sources of MAP transmission to humans. Thus, if the link between MAP and Crohn's disease is substantiated, the fact that MAP has been detected in retail foods could be a public health concern. The purpose of the present study was to review the link between MAP and CD, the prevalence of MAP in foods, heat inactivation, control of MAP during food processing, and detection methods for MAP. Although MAP positive rates in retail milk in nine countries ranged from 0 to 2.9% by the culture method and from 4.5 to 15.5% by PCR, high temperature short time pasteurization can effectively control MAP. The effectiveness of pasteurization to inactivate MAP depends on the initial concentration of the MAP in raw milk. Development of highly sensitive and specific rapid detection methods for MAP may enhance investigation into the relationship between MAP and CD, the prevention of the spread of MAP, and problem-solving related to food safety. Collaboration and efforts by government agencies, the dairy industry, farmers, veterinarians, and scientists will be required to reduce and prevent MAP in food.

Key words: *Mycobacterium avium* subsp. *paratuberculosis*, dairy products, food safety, Crohn's disease

Introduction

The mycobacteria are weakly Gram positive, slender, rod-shaped, acid-fast, aerobic, and slow-growing bacteria, which include some significant pathogens and many non-pathogens. The property of acid-fastness, due to waxy materials in the cell walls, is particularly important for recognizing mycobacteria (Holt *et al*., 1994).

The *Mycobacterium avium* complex is an important subgroup of mycobacteria that can survive outside an animal host. As a member of the *M. avium* complex, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) grows extremely slowly, taking 16 weeks to produce visible colonies on media (Collins, 1997), needs mycobactin J for *in vitro* growth (Merkal and McCullough, 1982), and is the causative microorganism for the granulomatous ileitis in ruminants called Johne's disease (JD). Johne's disease results in chronic, infectious enteritis, causing diarrhea, weight loss and death (Collins, 1996; Grant, 2005).

It has been suggested that a relationship between MAP infection and Crohn's disease (CD) exists because of the similar pathology of CD in humans and JD in animals (Chiodini, 1989; Shanahan and O'Mahony, 2005; Thompson 1994). However, there are many arguments both for and against MAP being the aetiological agent of CD (Griffiths, 2006, 2009; Mendoza *et al*., 2009; Sartor, 2005; Shanahan and O'Mahony, 2005).

Milk, meat, and water could be a potential vehicle of transmission of MAP from animals suffering from JD (Ayele *et al*., 2001; Collins, 2003a; Eltholth *et al*., 2009). Also, if MAP is present in raw milk and can potentially survive the pasteurization process, then the possibility also exists for MAP to be present in milk products, such as butter, cream, cheese, yoghurt, cheese, ice cream and infant formula (Grant *et al*., 2001). Several researchers have reported the isolation of MAP from retail pasteurized milk (Grant *et al*., 2002; Ikonomopoulos *et al*., 2005; Millar *et al*., 1996).

MAP has been considered as an emerging food pathogen (Collins, 1997; Greenstein and Collins, 2004; Griffiths, 2009; Kousta *et al*., 2010; Skovgaard, 2007). The

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aim of this paper is to review the information related to Crohn's disease, prevalence, inactivation, control, prevention and detection of MAP, as an emerging pathogen.

Crohn's Disease and *Mycobacterium avium* subsp. *paratuberculosis*

Crohn's disease was firstly described in 1913 by Dalzeil, who proposed MAP as the aetiological agent of the disease. It is a chronic, remitting and relapsing disorder, similar to inflammatory bowel disease, and most commonly affects the small and large intestine (Feller *et al*., 2007; Mendoza *et al*., 2009). Chiodini *et al*. (1984) have isolated 3 strains of MAP from CD patients, Linda, Dominic and Ben, and these were genetically identical to strains of MAP isolated from cattle that were able to cause JD in infant goats (van Kruiningen *et al*., 1986). There have been many reports of MAP being cultured from or its unique DNA fragment being present in tissues from CD patients (Green *et al*., 1989; Thompson 1994; Vary *et al*., 1990).

Crohn's disease is a debilitating and expensive disease that is growing in incidence in both developing and developed countries. Incidence rates of CD in Europe range from 0.7 to 9.8 cases per 100,000 persons and in Asia range from 0.5 to 4.2 per 100,000; with the lowest recorded rates of new cases occurring in South Africa (0.3-2.6 per 100,000) and Latin America (0-0.03 per 100,000) (Panes *et al*., 2007). Other studies have reported the prevalence of CD to range from 26 to 198.5 in North America, between 8.3 and 214.0 in Europe, and in Asia between 3.6 and 50.6 (Loftus, 2004). Yang *et al*. (2008) stated that the incidence and prevalence of CD in Korea was still low compared with those in Western countries but are rapidly increasing. They determined adjusted prevalence rate of CD per 100,000 inhabitants in 2005 was 11.24.

The relation between MAP and CD has been debated since MAP was detected from CD patients, but the association remains controversial. Several reviews have summarized arguments for and against MAP being the causative agent in CD (Griffiths, 2009; Nacy and Buckley, 2008; Sartor, 2005). The main arguments proposed as supporting a link between MAP and CD are i) the clinical and pathological similarity between CD in humans and JD in animals (Chiodini, 1989); ii) the detection of MAP in tissues, blood, and breast milk from CD patients (Chiodini *et al*., 1984; Naser *et al*., 2000, 2004); iii) the high prevalence of MAP DNA in biopsies from CD patients (Feller *et al*., 2007); and iv) the efficacy of antimycobacterial drugs in the treatment of CD (Shafran *et al*., 2002). In addition, Paustian *et al*. (2008) reported that both cattle and humans are susceptible to infection by MAP isolates with similar genotypes.

In contrast, studies indicating that MAP does not play a causative role in the aetiology of CD have been reported. There are differences in the clinical and pathological responses in CD and JD (Griffiths, 2009), MAP was detected at the same level in patients with ulcerative colitis, CD and controls (Naser *et al*., 2004), and several studies have failed to find MAP in CD patients (Baksh *et al*., 2004; Bernstein *et al*., 2003; Fujita *et al*., 2002). Jones *et al*. (2006) also suggested that the prevalence of CD in dairy farmers was not different from the general population, and there was no association between CD and the occurrence of bovine paratuberculosis. Other studies have shown that the effects of antimycobacterial drugs are transient and that CD is primarily an autoimmune disease that may be triggered by many factors (Griffiths, 2009).

Although the causal role of MAP in the aetiology of CD can neither be confirmed nor excluded with certainty, MAP may play a role in the context of secondary infection, which may exacerbate the disease, or represent non-pathogenic colonization (Feller *et al*., 2007).

Transmission of *Mycobacterium avium* subsp. *paratuberculosis*

MAP can replicate only when it is in animals. It cannot multiply outside the animal in nature. If soil or water is contaminated with *M. paratuberculosis*, the bacterium can survive for more than a year because of its resistance to heat, cold and drying (Collins, 2003b).

During the course of JD, most infected animals do not develop clinical disease but may excrete the bacterium. MAP spreads through the blood and lymph vessels of infected animals to multiple internal organs and is excreted in their faeces and milk (Ayele *et al*., 2001). Infection of newborn animals occurs by oral ingestion of the pathogen, or calves may acquire infection *in utero*. The main infection route is fecal-oral by exposure to manure from infected adult cattle or their environment to young cattle (Ayele *et al*., 2001; Sweeney, 1996). MAP infection can be diagnosed by examination of blood or serum, stool, milk, biopsies of bowel tissue, and tissue from bowel secretions.

Nielson and Toft (2009) estimated the true prevalence among cattle to be approximately 20% in several countries in Europe. Seroprevalence in dairy cattle in Canada ranged from 1.3 to 7.0% at animal level and 9.8 to 40.0%

at herd level (Tiwari *et al*., 2006). In the USA, the average herd prevalence among dairy cattle was 21.6% in 1996 (USDA, 1997).

The first isolation and identification of MAP in Korea was reported in 1984 from feces of cattle infected with JD and exhibiting clinical and pathological symptoms (Jeon *et al*. 1984). Park *et al*. (2006) reported that the true prevalence of bovine paratuberculosis in Korea was estimated to be 7.1% from tests performed using two different ELISA: an 'in house' modified absorbed ELISA and a commercial ELISA.

Mycobacterium avium subsp. *paratuberculosis* in Foods

Dairy foods

People may be exposed to MAP by the consumption of milk and meat from infected cattle (Ayele *et al*., 2001; Collins, 2003a; Grant, 2005). Cheng *et al*. (2005) suggested that the increase in production and consumption of cow's milk in China has resulted in an increased incidence of CD.

The prevalence of MAP in raw milk detected by the culture method was 3.3-4.8% for the UK (Grant *et al*., 2002, 2005b; Millar *et al*., 1996), and 11.6% in the USA (Sweeney *et al*., 1992). Hwang *et al*. (2009) evaluated the relation between milk traits and paratuberculosis in a dairy herd in Korea, and reported that MAP-herd prevalence for bulk milk was 5.2% and MAP- prevalence in individual dairy cows was 5.7%.

The prevalence of MAP in retail milk and dairy products is summarized in Table 1. The presence of MAP in retail milk was firstly investigated by Millar *et al*. (1996), and MAP positive pasteurized milks by the culture method were first reported in the UK by Grant *et al*. (2002). MAP positive rates ranged between 0 and 2.9% by the culture method, between 4.5 and 15.5% by PCR.

Nauta and van der Giessen (1998) assessed the point estimate of the exposure level to be about 0.5 CFU/L pasteurized milk, which was primarily due to milk from clinically affected animals entering the supply. Even in countries that have not reported MAP in retail milk, including Korea, based on the JD prevalence rates, there is a strong possibility that MAP could be present in retail milk.

In studies in Greece and the Czech Republic using a culture-based detection method, MAP positive rates in retail infant powdered milk and cheese were higher than those of retail milk (Hruska *et al*., 2005; Ikonomopoulos *et al*., 2005). Although no viable or few MAP were cultured, the fact that MAP DNA was found in the retail cheese indicated that cheese could be a vehicle for human exposure to MAP.

Although MAP is widely present in the food chain and the DNA of MAP can be recovered from the intestine of CD patients, the results do not compellingly implicate MAP as a casual agent in CD, neither do they definitively

Table 1. Prevalence of MAP in retail dairy products

Dairy products	Country	Culture method			PCR and related methods			Reference
		No. of samples	No. of positive	(%)	No. of samples	No. of positive	(%)	
Milk	UK and Wales				322	22	6.8	Millar *et al*. (1996)
	Canada				710	110	15.5	Gao *et al*. (2002)
	UK	567	10	1.8	567	67	11.8	Grant *et al*. (2002)
	Ireland	77	0	0.0				O'Doherty *et al*.(2002)
		9[a]	0	0.0				O'Doherty *et al*.(2002)
		357	0	0.0	357	35	9.8	O'Reilly *et al*. (2004)
	USA	702	20	2.8				Ellingson *et al*. (2005)
	Czech Republic	244	4	1.6				Ayele *et al*. (2005)
	Argentina	70	2	2.9				Paolicchi *et al*. (2005)
	Venezuela	83	0	0.0				Mendez *et al*.(2006)
	Italy	22	0	0.0	22	1	4.5	Lillini *et al*. (2007)
Cheese	Greece	42	2	4.8	42	21	50.0	Ikonomopoulos *et al*. (2005)
	Czech Republic	42	1	2.4	42	5	11.9	Ikonomopoulos *et al*. (2005)
	USA	98	0	0.0	98	23	23.5	Clark Jr. *et al*. (2006)
	Swiss	143	0	0.0	143	6	4.2	Stephan *et al*. (2007)
Powdered infant milk	Europe				51	25	49.0	Hruska *et al*.(2005)

[a]Goat's milk

exclude the possibility (Sartor, 2005). Therefore, by applying the "precautionary principle" the intake of MAP cells should be minimized in people at highest risk, e.g. in newborns, children and genetically susceptible persons, namely patients suffering from CD and their direct relatives. The national certification of paratuberculosis-free herds should be strongly supported to decrease the risk for children and other people at higher risk (Hruska *et al*., 2005).

Meat

Collins (2003a) suggested that MAP contamination of beef production could occur both pre- (via the blood system) and post- (via fecal contamination) slaughter as with other microbial contaminants. Ground beef has the highest risk of being a vehicle for MAP exposure of humans.

MAP was isolated from ileocecal lymph nodes and feces from 65/189 (34.4%) dairy and 9/350 (2.6%) beef cows, associated with muscle used in ground beef product. It was also isolated from the liver of 15/189 (7.9%) dairy and 1/350 (0.3%) beef cows, and from superficial cervical and popliteal lymph nodes of 6/189 (3.2%) dairy and 1/350 (0.3%) beef (Rossiter and Henning, 2001). It was concluded that the occurrence of MAP in superficial cervical and popliteal lymph nodes in the total market cow population was very low.

In contrast, Jaravata *et al*. (2007) reported that no MAP was detected from 200 retail ground beef samples by multiplex real-time PCR assay and conventional culture assay. The prevalence on beef carcasses of MAP DNA in Canada was 6 to 54% for beef carcasses using the IS900 PCR assay, and 4 to 20% when the F57 sequence was detected (Meadus *et al*., 2008). The authors concluded that MAP DNA on carcasses was probably derived from small numbers of MAP from the environment resulting from contaminated animal hides.

Mutharia *et al*. (2010) investigated the effects of cooking on the survival of MAP in beef and hamburger patties. They concluded that MAP was likely to be inactivated when meat was cooked to a well-done condition. Whittington *et al*. (2010) reported that the possibility of survival of MAP in red meat cooked to recommended temperatures was low. This study also showed that MAP was less heat tolerant in lamb skeletal muscle fluid than in milk, and the total thermal exposure of MAP during baking of lamb roasts in domestic ovens resulted in a reduction in count of MAP of more than 20 log cycles in most cases.

Control of *Mycobacterium avium* subsp. *paratuberculosis*

Thermal inactivation of MAP in milk

The heat tolerance of MAP in milk has been a focus of attention due to the fact that MAP has been detected in pasteurized milk. Chiodini and Hermon-Taylor (1993) reported that heat treatment at 63°C for 30 min resulted in a 1 log cycle reduction in counts of bovine MAP isolates, and conditions simulating High temperature short time (HTST) pasteurization, 72°C for 15 s, resulted in slightly greater inactivation; with more than 95% of the organisms destroyed. Grant *et al*. (1996) confirmed that MAP in milk was more heat resistant than *M. bovis* and MAP could survive HTST pasteurization when 10^3 to 10^4 MAP cfu/mL were present in milk samples before heat treatment. The D-values of MAP were determined as 11.76, 21.8, 47.8, and 228.8 s at 71, 68, 65, and 62°C, respectively, and were considerably higher than those for *Listeria*, *Salmonella*, *Coxiella* spp. and *M. bovis* (Sung and Collins, 1998). Grant *et al*. (1999) examined the effect of heating temperature and time on the inactivation of MAP, and showed that a longer holding time was more likely to achieve complete inactivation of MAP in milk than a higher pasteurization temperature. These results have been questioned by Cerf and Griffiths (2000) on the basis of their thermodynamic feasibility and by Lund *et al*. (2002), who identified limitations with the equipment used for the study. Grant *et al*. (1999) were also able to detect MAP after heat treatment at 80 and 90°C for 15 s. The frequency of MAP detected decreased with increases in HTST pasteurization temperature, and homogenization increased the lethality of the subsequent heat treatment. The MAP cells would have been present as predominantly declumped cells in raw milk, which may explain the greater inactivation achieved by the combination of pasteurization and homogenization (Grant *et al*., 2005a). McDonald *et al*. (2005) conducted an efficacy of pasteurization on heavily artificially contaminated MAP in milk and detected a few viable MAP in milk pasteurized at 72°C for 15 s, 75°C for 25 s, and 78°C for 15 s.

The heat resistance of MAP may be due to seven highly expressed stress-response proteins, including GroES heat shock protein, alpha antigen, alpha antigen 85 complex B (Ag85B) and fibronectin-binding protein (Sung *et al*., 2004).

There are several other studies that show HTST pasteurization to be adequate to ensure the absence of viable MAP in fluid milk (Klijn *et al*., 2001; Pearce *et al*., 2001; Rademaker *et al*., 2007; Stabel and Lambertz, 2004).

Klijn *et al*. (2001) concluded an inactivation of MAP of 4 to >8-log cycles was achieved during industrial pasteurization. Pearce *et al*. (2001) tested the survival of a bovine and human strain of MAP following heat treatment in a pilot-scale pasteurizer operating under validated turbulent flow, and reported that no MAP strains survived heating at 72°C for 15 s. They proposed that an additional safeguard is the widespread commercial practice of pasteurizing 1.5 to 2°C above the recommended minimum temperature of 72°C. Stabel and Lambertz (2004) studied inactivation of MAP in milk using ultrahigh temperature milk that was inoculated with 10^8 and 10^5 cells/mL of three strains of MAP. They pasteurized the milk in a slug-flow pasteurizer unit and a laboratory scale pasteurizer unit, and concluded that treatment of milk regardless of bacterial strain or pasteurizer unit resulted in an average 5.0- and 7.7- $\log_{10}$ kill for the low and high concentration of inocula, respectively, and mentioned that the U. S. minimum standards for batch and high-temperature short-time pasteurization of grade A milk significantly reduced the survival of MAP. Rademaker *et al*. (2007) also concluded that HTST pasteurization conditions of 15 s at or above 72°C resulted in a >7 log reduction of MAP. Lynch *et al*. (2007) reported that MAP was not detected in milks pasteurized at 72.5°C for 27 s regardless of whether the milk was homogenized or not.

To rationalize the disparate results obtained from the studies on the heat stability of MAP, Hope *et al*. (1996) stated that survival of MAP in experimentally inoculated batches of milk in small-scale commercial units could not be directly extrapolated to commercial pasteurization of naturally infected milk in dairy factories because of differences in the thermosusceptibility of laboratory cultured mycobacteria and the features of the pasterization unit. Klijn *et al*. (2001) assumed that the experimental conditions of MAP heat inactivation studies of different research groups varied significantly and led to considerable differences in results; a theory supported by Lund *et al*. (2002). The results obtained by Mendez *et al.* (2006) also suggest that laboratory contamination may play a significant role when studying mycobacteria and led Cerf *et al*. (2007) to conclude that higher frequencies of MAP in pasteurized milk were due to improper pasteurization and cross-contamination in the analytical laboratory. Consequently, the effectiveness of pasteurization in inactivating MAP depended on the initial concentration of the MAP in raw milk (Eltholth *et al*., 2009), but all studies in which validated turbulent flow was achieved in the heat-exchanger have demonstrated that HTST pasteurization can effectively control MAP.

Inactivation of MAP by lactic acid bacteria

Lactobacillus GG might be effective in improving gut barrier function and clinical response in pediatric patients with mildly to moderately active Crohn's disease (Gupta *et al*., 2000). With regards to inactivation of MAP by lactic acid bacteria, the *in vitro* inhibition of MAP by probiotic strains and cheese lactic acid bacteria isolates was investigated, and it was shown that MAP growth was inhibited (delayed) when supplemented with supernatants from a number of *Lactobacillus paracasei* isolates. In addition, MAP could not be detected by culture method up to 50 d when co-inoculated with probiotic strains in sterile milk for 48 h (Donaghy *et al*., 2005). It was suggested that the in vitro inhibitory effect of some lactobacilli on MAP growth may be due to factors other than acid production. Probiotic mechanisms of action relative to therapy for inflammatory bowel disease have been reported. However, the efficacy of probiotics in treatment or maintenance of remission of CD needs to be verified (Shanahan, 2000). For example, a meta-analysis performed by Rahimi *et al*. (2008) failed to demonstrate the efficacy of probiotics in maintaining remission and preventing clinical and endoscopic recurrence in CD.

Inactivation of MAP during cheese ripening

The behavior of MAP during cheese ripening was investigated by Sung and Collins (2000), who reported that a lower pH was associated with faster inactivation of MAP, but NaCl had little or no effect on MAP inactivation rates during ripening of Hispanic-style soft white cheese (Queso Fresco). However, they were able to demonstrate that heat treatment of the raw milk used for cheese manufacture, coupled with a 60 d curing period would inactivate about 10^3 MAP cfu/g. Spahr and Schafroth (2001) also concluded that the most important factors responsible for the death of MAP in model cheese were the temperature applied during cheese manufacture and the low pH at the early stage of cheese ripening. In hard (Swiss Emmentaler) and semi-hard (Swiss Tisliter) cheese between 10^3-10^4 MAP CFU/g would be inactivated after 90-120 d of ripening. Similarly, Donaghy *et al*. (2004) observed a slow gradual decrease in the count of MAP in Cheddar cheese over the ripening period. In all cases where high levels (>10^4 CFU/g) of MAP were present in 1-day old cheeses, the organism was recovered after the 27-wk ripening period. At low levels of contamination (10^1 to 10^2 CFU/g), only one of the three strains of MAP

used was recovered from the 27-wk-old cheese.

Non-thermal pasteurization of MAP

Rowan *et al*. (2001) investigated the use of Pulsed Electric Fields (PEF) to inactivate MAP. A PEF treatment at 50°C with 2,500 pulses at 39 kV/cm field strength reduced the level of viable MAP cells by 5.9 log CFU/mL in cow's milk, The inactivation was due to damage of the cell membrane. They indicated that the application of high-intensity PEF kills MAP in a test liquid and in milk when carried out at moderately elevated temperatures.

Grant *et al*. (2005b) reported that centrifugation of preheated milk (60°C) at 7,000 g for 10 s and microfiltration with pore size 1.2 μm was able to remove 95-99.9% of cells from MAP spiked milk. They concluded that physical removal, centrifugation and microfiltration may have potential application within the dairy industry as a pretreatment of raw milk to remove MAP. However, this study was performed using a laboratory microcentrifuge and syringe filter.

Lopez-Pedemonte *et al*. (2006) examined the possibility of reducing MAP in milk by means of high hydrostatic pressure treatment and confirmed that an average MAP reduction of 10^4 CFU/mL was obtained after treatment with 500 MPa. Donaghy *et al*. (2007) also reported that pressure above 500 MPa resulted in significant reductions in viable MAP cells in spiked milk samples. Nevertheless, even when combined with pasteurization, MAP could survive some pressure treatments. Recovery of MAP treated with high pressure was affected by the recovery medium used; with better survival rates found with 7H9 and 7H10 medium than those on HEYM (Donaghy *et al*., 2007; Lopez-Pedemonte *et al*., 2006).

UV treatment of milk would appear to have a limited ability to reduce numbers of MAP. A reduction of 0.5-1.0 log of MAP in milk was achieved by a dose of 1000 mJ/mL (Altic *et al*., 2007). Donaghy *et al*. (2009) also concluded that the use of UV radiation alone did not represent an alternative to current pasteurization process for control of MAP in milk.

Detection methods for MAP

When MAP exists as a cell wall deficient form, spheroplast, isolation of MAP from human subjects is very difficult (Chiondini *et al*., 1986). The diagnostic methods for MAP, which can be applied to food include bacterial culture, PCR, and immunological techniques (Collins *et al*., 2006).

Bacterial culture is the 'gold standard' for detecting MAP infection, but culture methods may take 16 wk or more, and may not detect viable but non-culturable cells (Collins, 1997; van der Giessen *et al*., 1992). These methods also have limited sensitivity (30-50% in JD infected animals) (Whitlock *et al.*, 2000). Media used for the primary isolation of MAP can be classified as egg-based media such as Herrold's egg yolk medium and Löwenstein-Jensen medium; serum-based media including Dubos medium; and synthetic media such as Middlebrook and Watson-Reid medium (Allen, 1998; Middlebrook and Cohn, 1958; Nielson *et al*., 2004; Saxegaard, 1985; Whipple *et al*., 1991; Whittington *et al*., 1999). The requirements and media for isolation of MAP strains by culture are directly related to the MAP strain and not to the host animal species. The use of inappropriate media affects the detection rate and therefore leads to false-negative results (De Juan *et al*., 2006). The most widely-used agar-based media, '7H' media, were developed by Middlebrook and Cohn (1958), and are comprised of a series with changing formulae. Middlebrook 7H10 supplemented with oleic acid, albumin, dextrose, and catalase (OADC) is the most useful of the agar-based media, and Middlebrook 7H9 medium, which requires the addition of an albumin-dextrose-catalase (ADC) supplement before use is the most useful of the liquid media (Allen, 1998). Selective media for MAP by the addition of antimicrobials are used to reduce contamination by fungi and bacteria, an example of which is PANTA (polymyxin B, amphoterin B, nalidixic acid, trimethoprim, azocillin), vancomycin, nistatin, and carbenicillin (Ellingson *et al*., 2005; Pearce, 2001; Spahr and Schafroth, 2001; Sung and Collins, 2000).

Molecular methods such as PCR, theoretically reducing the detection time to 1 to 3 d, have been able to confirm the presence of MAP DNA. These assays have limited sensitivity and specificity, and they cannot distinguish between live and dead MAP cells. Green *et al*. (1989) designated IS900, containing a single copy of a mycobacterial insertion, as a highly specific marker for the precise identification of *Mycobacterium paratuberculosis*. Vary *et al*. (1990) achieved highly sensitive and rapid detection of MAP by using PCR with IS900, which took only hours to perform compared with 6 to 12 wk for the culture method. The restriction enzyme analysis of IS*1311*, an insertion sequence common to MAP, can be used to distinguish between and within species (Whittington *et al*., 1998). Recently, DNA sequences for MAP identification, F57 (Strommenger *et al*., 2001), and ISMap02 (Stabel and Bannantine, 2005) were developed as additional tools for

PCR.

Immunological assays such as enzyme-linked immunosorbent assay (ELISA), agar gel immunodiffusion assay (AGID), and complement-fixation test (CF), which rely on the presence of MAP antibodies in samples, are commonly used to diagnose paratuberculosis in cattle (Colgrove, 1989; Sockett *et al*., 1992). ELISA is fast, less expensive, sensitive and specific test for serum antibodies to MAP (Colgrove, 1989; Park *et al*., 2006), however, it can be used to diagnose JD.

Bacteriophages can be used in a variety of ways to detect MAP. With the understanding of a phage's host range and with decades of historical experience in the application of phage typing schemes, it was evident that phage could successfully function as bacterial-specific indicators. Consequently, phage-mediated detection assays are more efficient, sensitive, and faster than the venerable phage plaque assays (Griffiths, 2010; Ripp, 2010).

Stanley *et al*. (2007) were the first to report that MAP in milk could be detected using the *FASTPlaque*TB (Biotec Laboratories Ltd., Ipswich, UK) assay based on phage amplification technology and PCR-based identification method. Because the *FASTPlaque*TB assay is not specific for MAP, a PCR-based method was introduced to amplify MAP-specific sequences from the DNA of the mycobacterial cell detected by the phage. They were able to detect 1×10^2 CFU/mL of MAP cells in just 24 h as phage plaques. Foddai *et al*. (2009) reported that *FASTPlaque*TB phage amplification assay, incorporating a D29 mycobacteriophage with an optimized burst time of 90 min, was able to detect 1 to 10 CFU/mL of MAP in spiked milk or broth within 48 hours. Luciferase-encoding mycobacteriophage has also been used as a reporter, Sasahara *et al*. (2004) showed that phAE85 was most effective to detect MAP within 24-48 h, and its detection limit was 1000 CFU/mL. Recombinant lytic phage TM-4 were used, but the sensitivity was low (Riska *et al*., 1997).

Conclusion

MAP is the causative agent of Johne's disease in animals, and there is a possibility that MAP plays a role in Crohn's disease in humans; although this remains speculative. However, the fact that MAP can be detected in food, albeit rarely, raises the issue of the safety of such food. MAP prevalence in the food chain should be surveyed more rigorously because the fact that MAP can exist in retail food has been confirmed. The level of MAP in milk has not been reported in Korea, which might be a reflection of the prevalence of JD in herds. The seroprevalence of MAP in Korean dairy cattle is 6.1% with herd prevalence being 41.7% (Lee and Jung, 2009) so the possibility that MAP could be detected from milk in Korea may exist. In the case of retail milk, even though HTST pasteurization has been demonstrated as effective for the control of MAP, the organism has been isolated from pasteurized product. MAP is a very fastidious pathogen, therefore development of rapid and accurate detection methods for MAP with regard to their specificity and sensitivity is inevitably necessary to prevent the problems and possibilities which are predicted and presumed in public health and food safety as well as to investigate the relation between MAP and CD. Collaboration and effort by government agencies, dairy industry, farmers, veterinarians and scientists will be required for the reduction and prevention of MAP in food.

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