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Assessment of Sources of Exposure for *Mycobacterium avium* subsp. *paratuberculosis* in Food and Water

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Abstract *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is believed to be the causative agent of paratuberculosis (Johne's disease) in ruminants. MAP infection has also been proposed as the cause of Crohn's disease (CD) in humans. An increasing number of recent studies suggest some association between MAP and CD, however a cause-effect relationship has yet to be proved or disproved. Infected cattle appear to be the most important source of human exposure to MAP, and the associated suspected vehicles of transmission are milk, dairy products and beef.

Other possible routes of human exposure to MAP are via contaminated water supplies, vegetables and fruits.

A low level survival of MAP has been demonstrated in some surveys of commercially pasteurized milk and retail cheese in several countries outside Canada. Viable MAP has been identified in 1.6-2.9% of pasteurized retail milk and 3.6% of retail cheese samples in several countries. In addition, viable MAP has been found in the intestine and associated lymph tissues of diseased animals as well as in organs other than the gut. The primary purpose of this article is to review the recent scientific evidence on the potential human exposure to MAP via foods and water.

Keywords *Mycobacterium avium* subsp. *paratuberculosis*, food, water

1. Introduction

Paratuberculosis in ruminants, also known as Johne's disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It is a slowly developing infectious disease characterized by chronic degenerative granulomatous enteritis affecting the distal part of the small

intestine, colon and associated lymphoid tissue [1]. Clinical signs of the disease include persistent diarrhea in some species (cattle), reduction of milk yield, weight loss and progressive emaciation. Paratuberculosis is a progressive disease; affected animals become increasingly emaciated and usually die as the result of dehydration and severe cachexia. It has been suggested that young

animals, less than 6 months old, are the most susceptible to MAP infection [2]. Clinical signs of the disease are usually not detected until the animals are 2 years old. Some of the infected animals never develop clinical signs, but they continue to intermittently shed the organism and serve as a source of MAP to infect their off-spring and other animals [3].

In addition to cattle, other ruminants such as bison, deer and elk have been increasingly farmed in Canada and cases of MAP infection in these ruminants have been recognized [4]; [5]; [6]; [7]; [8]; [9]. Natural infection with MAP and outbreaks of the disease have also been reported in wild ruminants, including key deer, bighorn sheep, Rocky Mountain goat, and saiga antelope [10]; [11]; [12]). Nonruminant wildlife such as primates, foxes, weasels, stoats, crows, rooks, jackdaws, rat, wood mice, hares, rabbits and badgers are also known to be susceptible to MAP infection [13]. In view of the widespread susceptibility of many animal species, MAP has been proposed as a zoonotic pathogen that could cause infection or disease in humans.

Crohn's disease is an inflammatory bowel disease (IBD) that affects primarily the small intestine and colon, but also causes lesions in other parts of the gastrointestinal tract in humans. The major symptoms are weight loss, abdominal pain, diarrhea, fever, and fatigue. Crohn's disease is chronic and debilitating, often requiring multiple surgical resections of affected intestine. There is no known drug cure, and treatment and disease management are supportive. The disease often begins between the ages of 16 and 25 years, and lasts a lifetime. The primary goal of treatment is to control the disease by increasing the length and frequency of disease-free remissions. While an infectious origin for CD has long been suspected, it is clear that genetic and immunologic factors may also play important roles in CD. An increasing number of studies and recent meta-analysis [14] suggest an association between MAP and CD, however a cause-effect relationship has yet to be proved or disproved. This article makes no conclusion regarding the role that MAP might play in the etiology of CD, but it examines the potential for human exposure to this organism from food sources in Canada. Although scientific evidence remains inconclusive, limiting human exposure to MAP is viewed by many as an appropriate precautionary measure.

The true prevalence of MAP in the Canadian food supply is not known. Suspected vehicles of transmission of MAP to humans are milk, dairy products (for example: cheese, cream, butter and yogurt), beef, and water. However, it is reasonable to assume that any product contaminated with faecal material of bovine, other animal or possibly human origin could be at risk for MAP contamination. Due to the recent focus on milk as a source of MAP, there is more data on MAP in milk than in other foods. Therefore, milk and the effectiveness of pasteurization will be the main focus of this document. Other dairy products, beef, vegetables and fruit products, fish and shellfish, water and the environment will be discussed in proportion to the available information. In terms of food safety, it is important to determine the efficacy of food processing technologies, such as heat treatment, for controlling MAP- contaminated foods. This review will address recent scientific evidence, and provide a preliminary assessment of human exposure to MAP via food and water, summarizing the potential distribution of MAP in the Canadian food supply.

2. Diagnostic methods

Mycobacterium avium subspecies *paratuberculosis* is an acid-fast, intracellular, extremely slow-growing bacillus. The organisms are very small (0.5 x 1.5 μm), and naturally occur as clumps of up to several hundred bacterial cells. Due to a high cell wall lipid content, mycobacteria resist decoloration with acid alcohol during the acid-fast staining procedure (Ziehl-Neelsen or Kinyoun's stain), retain the initial dye (carbol fuchsin) and appear red (acid-fast). Cell wall deficient (CWD) forms of MAP are believed to be important in CD [15] and they can not be detected by acid-fast staining. MAP is believed to be an obligate pathogenic parasite of animals with the ability to grow and multiply only within host cells, most often macrophages [16]. For *in vivo* growth, MAP obtains iron from host iron-binding proteins, particularly transferrin. When grown *in vitro*, the organism requires exogenous mycobactin (an iron-chelating agent produced by all other mycobacteria) for growth [1].

Currently, there are several tests used to diagnose MAP infection, which can be divided into two groups. The first group is directed at detection of the organism (MAP) and includes microscopic examination of faecal and tissue smears, standard bacterial culture, radiometric liquid media and DNA probe or polymerase chain reaction (PCR). These tests typically have high specificity, and their sensitivity increases as the disease progresses. The second group includes indirect tests and consist of assays that detect cell-mediated or humoral immune responses, such as the gamma interferon (IFN-γ) assay and enzyme-linked immunosorbent assay (ELISA). Test specificity is generally quite high (97-99%), however the sensitivity is often poor at less than 50% in faecal culture-positive animals [17]; [18]; [19] or 20% in low incidence populations. Determination of the sensitivity and specificity of a diagnostic test for this disease is difficult because of the long and variable period of subclinical infection, and imperfect "gold standards" used to define

the infection status of an animal. Overall, the current gold standard for confirming that an animal is infected is a culture of the mesenteric lymph nodes and ileum.

ELISA has been the most widely used serological test for the diagnosis of MAP infection. The assay is rapid, cost-effective and suitable for screening a large number of animals. Early infection with MAP is generally difficult to detect due to the lack of antibody production in early stages of infection, rather than the inability of the test to detect serum antibodies. However, detection improves as the disease progresses. The test reportedly has a low sensitivity in young, recently infected animals, and therefore cannot be used to detect the disease on a newly exposed farm [20]; [21]; [22]. A United States Department of Agriculture (USDA) ELISA with improved specificity, evaluated by researchers at the University of Wisconsin, has been recommended as a preliminary screening test to determine whether MAP infection exists in a herd [23]; [24]. In MAP-positive herds, ELISA should be followed by PCR assay or bacteriological culture of faecal samples to confirm the infection in ELISA-positive animals, and also to identify infected animals that are not producing serum antibodies (and are not detectable by the ELISA). Therefore, when confidence in the absence of MAP infection is required, two different tests should be performed, in other words, performing multiple diagnostic tests in parallel can improve the sensitivity of MAP testing.

Assays that detect IFN-γ, an effector of cell-mediated immune response to MAP infection, are considered by some authors to be superior to other immunological assays for the detection of early infection with MAP [25]. However, low test sensitivity and specificity have been reported [26].

Hulten *et al*. (2000a) [27] reported a novel in-situ hybridization method for the detection of cell wall deficient MAP (spheroplasts) in tissue samples. The authors injected beef samples with MAP spheroplasts, as well as a number of intact or spheroplastic control microorganisms to ensure specificity and sensitivity of the method (*M. smegmatis, M. tuberculosis, Helicobacter pylori* and *Escherichia coli*). Since only those samples injected with the cell wall deficient (CWD) or spheroplastic forms of MAP hybridized with the probe, and not with acid-fast MAP, this method has the potential for detecting subclinical CWD MAP infections. The authors [28] have since used this technique in tissues of CD patients.

Bacteriological culture is the most definitive and widely used diagnostic method for the detection of MAP. However, a major drawback of this method is that it can take between 8 to 16 weeks (and sometime up to 1 year) for the organism to grow. Due to the length of incubation, contamination by fungi and other bacteria frequently occurs. Therefore, a decontamination step is necessary to selectively kill non-mycobacterial organisms. The difficulty with this process, and a source of controversy in mycobacterial methodology, consists of obtaining adequate decontamination without inactivating MAP or inhibiting its growth. A decontamination step has been shown to have an adverse effect on the detection of MAP and therefore the sensitivity of the bacteriological culture [29]. Culture of the agent from clinical or environmental samples, including food, consists of the following phases: 1) concentration using centrifugation, filtration, sedimentation, or immunomagnetic separation; 2) decontamination and preincubation; and 3) culture using conventional bacteriological media, radiometric culture media or non-radiometric automated culture systems. Hexadecylpyridinium chloride (HPC) and benzalkonium chloride are the most commonly used decontaminants, together with various antibiotics such as vancomycin, amphotericin, and nalidixic acid. In addition, a processing method based on the use of a zwitterionic detergent (C_{18}-carboxypropylbetaine (CB-18)), has been reported [30]. The lower detection threshold of this method was associated with its decreased impact on the viability of mycobacteria, and also with the ability of the detergent to disperse the organisms that usually clump in processed sediment. A publication by Dundee *et al*. (2001) [31] discussed the comparative evaluation of four decontamination protocols for the isolation of MAP from milk and reported large discrepancies in the effectiveness of commonly used methods. The four methods were: 1) treatment with 0.75% hexadecylpyridium chloride (HPC) for 5 h; 2-3) Cornell methods using brain heart infusion broth containing 0.75 and 0.9% HPC, respectively; and 4) CB-18 method. Both Cornell methods resulted in poor recovery of MAP from milk (<1% of milk samples spiked with 10^6 cfu/ml). The CB-18 method showed some promise with a detection rate of up to 17%. However, its minimum detection limits were 10^5-10^6 cfu/40 ml milk. The 0.75% HPC method was the only sensitive means of detecting MAP in milk, able to detect 10-100 cfu/40 ml. This poor recovery of MAP, following a decontamination process, may result in an underestimation of the true number of organisms present in milk.

The existence of different genotype variants of MAP strains has been well documented [32]; [33]. The strains appear to have a certain degree of host specificity as well as different growth requirements [33]; [34]. Therefore, success in isolating MAP by bacteriological culture also depends on the culture media used. A commonly isolated strain from cattle (C) grows well on HEYM (Herrold's egg yolk medium) and is enhanced by the presence of sodium pyruvate, while Lowenstein-Jensen (LJ), Middlebrook 7H10 and 7H11 without pyruvate

supplement are recommended for promoting the growth of sheep (S) strains [35]. Donaghy *et al*. (2003) [36] evaluated three commonly used culture media: HEYM supplemented with vancomycin, amphotericin B and nalidixic acid (HEYM/VAN); BACTEC 12B with polymixin, amphotericin B, nalidixic acid, trimethoprim and azlocillin (BACTEC/PANTA PLUS); and Middlebrook 7H10/PANTA for the recovery of MAP from cheese. The authors reported that 7H10/PANTA and to lesser extent HEYM/VAN, were very effective in recovery of MAP and inhibiting the growth of starter culture and contaminating microflora. Middlebrook 7H10/PANTA was also very effective in recovering MAP from cheese without applying a preliminary decontamination step (0.75% HPC for 5h), known to adversely affect the isolation of MAP by culture. Finally, Donaghy *et al*. (2003) [36] reported on a culture method for the recovery of MAP from cheddar cheeses. Basic 7H10-PANTA solid media was most efficient at recovery of MAP during cheddar manufacture and ripening, as opposed to Herrold Egg Yolk Medium and BACTEC 12B liquid medium. The Becton Dickinson mycobacterial growth system (radiometric BACTEC) is commonly used for culture and detection of MAP. The main advantages of the system are the ability to grow MAP from wide variety of animal species and to detect the organism faster than the standard bacteriological culture. A few preliminary studies reported the non-radiometric mycobacterial growth indicator tube (MGIT) culture system (modified by addition of mycobactin and egg yolk emulsion) as successful in growing MAP from animal and human specimens [37]; [38]. The MGIT culture system has several advantages over the radiometric BACTEC system, such as the fact that it does not require the use of radioactive materials and there is no need for an expensive automated reader. Both use liquid broth and can therefore be classed as short-term culture systems. The MGIT system also contains L-asparagine, pyroxidine, trace elements, biotin and glycerol, and was proposed to be more suitable than the BACTEC system for recovery of MAP. Similar observations were reported by Naser *et al*. [39]and [40] and Thomas *et al*. (2005) [41] who found MGIT to be more sensitive than BACTEC for the detection of MAP in both humans and animals, while Grant *et al*. [29]; [42] reported that the radiometric BACTEC and MGIT have similar recovery rates of MAP in milk. Following the decontamination procedure, both systems had a detection threshold of 100-1000 cells/ml of milk. Considering that low numbers of MAP are generally present in milk [43]; [44]), false-negative culture results can be expected.

As an alternative to culture-based systems, two rapid systems for enumeration of viable MAP in milk have been recently reported. D'Haese *et al*. [45], [46] reported a rapid method for enumeration of viable MAP in milk based on solid-phase cytometry (SPC). Following an extensive decontamination protocol, MAP is retained on a filter and fluorescently labeled with a living-organism specific stain (ChemChrome V6). The recovery rate for MAP in spiked milk was 73% (2 cells/ml), however, milk was spiked with cell-wall competent forms of MAP while cell-wall-deficient forms of MAP may be the contaminant of concern in fluid milk. The potential for cross-reaction of antibodies with other bacteria commonly found in milk could be a drawback of this assay. Stanley *et al*. (2002) [47] proposed the use of phage amplification to detect viable MAP in milk in 24-48 h. However, limitations include decontamination, which would kill the phage and have possible inhibitory effects on the assay.

Molecular methods: Detection of the insertion element IS*900* (which occurs as 14-18 copies in the chromosome of MAP) using PCR is a rapid, specific and relatively sensitive test for the detection of MAP in milk and other clinical and environmental specimens. It has been routinely used to confirm MAP isolated from bacteriological culture. Unfortunately, due to the inhibitors present in milk and faecal specimens, and difficulties associated with the disruption of MAP cell wall to release DNA, the assay lacks desired sensitivity, and its detection threshold has been reported to be 1000 or more MAP organisms per ml of milk [43]. Some of the difficulties with MAP detection have been overcome by using pre-PCR processing steps such as immunomagnetic separation (IMS) or IMS coupled with a bead-beating procedure to selectively separate and concentrate MAP cells in specimens (and release DNA from captured MAP cells), with a reported detection threshold of 20 cfu/ml to <10 cfu/ml of milk [48]; [49]. Studies using a real-time PCR assay in milk and water successfully detected <100 MAP cells/ml of sample [50]; [51]. Buergelt and Williams (2004) [52] reported that nested PCR assay applied on milk samples was able to detect most of the clinically and subclinically MAP-infected cows, indicating its potential as a sensitive MAP screening tool. Although PCR is a rapid and highly specific assay, it cannot distinguish between viable and dead cells. Some studies have reported that certain environmental mycobacteria contain a homologue to the IS*900* locus, which can lead to the production of false-positive results by this assay [53]; [54]. However, appropriately designed PCR primers [55], or restriction endonuclease analysis of IS*900* PCR products [53], can readily distinguish between the "true" and "false" IS*900* products.

More recently, an article by Beumer *et al*. (2010) [56] reporting detection of MAP by PCR in 81% of drinking water and biofilm samples has triggered further controversy about the value of detection of MAP PCR using only the IS*900* sequence from bulk DNA extracts. However, Chiodini and Chamberlin (2011) [57] have

challenged this potential for MAP to be a widespread contaminant of the environment, and report only very low incidence of MAP in drinking water. Both groups [56], [57] agree that MAP should be positive for IS*900* and 251F, but instances of strains which are IS*900* negative but 251F positive and vice-versa [57] further confuse the issue of MAP identification. Many groups routinely use more than one locus for MAP identification.

Other MAP specific genetic elements such as F57, Hsp X and ISMav2 have also been recently investigated as alternatives to IS900 for MAP detection by PCR [58]; [59]; [60]. Alexander *et al.* (2009) [61] reported details on the insertion and deletion events that have defined MAP from a wide range of clinical samples and concluded that in true MAP species LSP^{A}8 was always absent. Clearly, definitive specifications for the use of molecular methods for MAP detection are still in a state of flux, and need to be resolved. The issue of how much credence one should give to studies reporting a MAP positive result only on the detection of IS*900* from environmental samples is questionable.

Co-culture with free living protozoa: The co-culture of MAP with certain free-living protozoa can improve their detection [62]; [63]. While this can be demonstrated routinely in the laboratory, the extent to which it occurs in the environment or in food is unknown. In an analogy to legionellae, incorporation of MAP into free-living protozoa could amplify the potential for contact and exposure of humans. Although free-living protozoa are ubiquitous and found in dairy operations and meat processing plants, their potential synergistic relationship with MAP in these environments is unclear at present.

3. MAP prevalence

Milk and other dairy products

Milk

Milk may be contaminated with MAP by two routes: organisms directly shed into the milk via the udder, or secondly, by contaminated faecal material. Even a small amount of faecal matter could potentially introduce high populations of MAP into raw milk. It has long been known that MAP can be cultured from the milk of clinically infected cows with JD [64]. However, subclinically infected animals can also shed the organism in milk [44]. The authors [44] reported that 11.6% of asymptomatic carriers shed an average of 4 x 10^{-2} cfu/ml to 16x/10^{-2}cfu/ ml of milk. In this study, MAP was cultured from the milk of 19% of healthy cows that were heavy faecal shedders of MAP and from the milk of 11% and 3% of healthy cows that were intermediate or light shedders of the organism, respectively. Clinically infected cows can shed from <100 cfu/ml to as high as 1000 cfu/ml of milk [43]; [44]; [65]. Of 81 supramammary lymph node samples collected from asymptomatic cows, 22 (27%) were culture-positive for MAP [44]. In a study reported by Streeter *et al*. (1995) [3], the organism was cultured from the colostrum of 22.2% and milk of 8.3% of faecal culture positive clinically normal animals. MAP is likely to be present in the faeces of an infected cow in much higher numbers than those reported in milk. It has been estimated that the number of viable bacilli present in a clinical case of paratuberculosis in cattle is between 10^{5} and 10^{6} cfu/g, and may even exceed 10^{8} cfu/g of faeces ([66]; [67].

Based on more recent serological surveys of dairy cattle, animal-level prevalence rates in Canadian provinces range between 2.4 and 9.1% [68]. As mentioned above, both symptomatic and asymptomatic animals sporadically shed large numbers of organisms in faeces. Therefore, since JD is present in the Canadian dairy cattle, it seems inevitable that MAP will find its way into bulk tank raw milk.

It is difficult to estimate the level of contamination of bulk tank milk without knowing the level of milk contamination on the farm; for example, depending on the number of MAP positive cows in the herd and when the milk is pooled, what is the on-farm raw milk prevalence? Without these numbers, and with the low level of recovery of live MAP from milk, it is difficult to reliably comment on the degree of contamination in raw or retail milk. A study to determine the prevalence of milk shedders (ELISA and culture) in MAP positive herds should be combined with raw milk culture for MAP to best estimate how much of the organism is actually present in bulk milk.

Pasteurized milk

Several investigators have tested the survival of MAP in pasteurized milk using a variety of lab-scale pasteurization systems and, commercial size pasteurization facilities [65]; [69]; [70]; [71]; [72]. Viable MAP was found in retail milk by research groups in Czech Republic, UK, USA and India [73]; [74]; [75]; [76].

Because of the difficulty in culturing MAP, survival studies following pasteurization have been problematic. Table 1 summarizes pasteurization studies for inactivation of MAP from milk. Grant *et al*. (1999) [77] suggested that at concentrations above 100 cfu/ml, MAP can survive the minimum HTST pasteurization (72˚C for 15 seconds), possibly through the clumping of cells in naturally-contaminated raw milk. This study also suggested that increasing the holding time of HTST from 15 to 25 seconds could inactivate high levels of MAP (10^{6} CFU/ml). Increased temperatures did not have this same effect. Since 1993, several studies have demonstrated the

ability of MAP to survive batch or HTST pasteurization [78]. These studies have been criticized due to the aforementioned variability in laboratory scale pasteurization methods, and because spiking raw milk may not represent a natural contamination process, since the laboratory strains may be more or less resistant to heat treatment. It should be noted that most of the studies used milk spiked with laboratory-grown MAP, instead of naturally-contaminated milk. In summary, factors suspected to affect survival include clumping of cells, presence/absence of cell-wall, phase of growth, and the number of bacilli present in milk before pasteurization [16]

Type of sample	Type of pasteurization	Equipment	Reduction / Survival of MAP	Reference
Spiked milk (2 bovine strains, 2 human strains)	Standard* HTST**	Laboratory stirred batch / double boiler	1 log or less	[70]
Spiked milk 100 cfu/ml (3 heat resistant bovine strains)	HTST	Laboratory pasteurising units	Not effective***	[77]
Spiked milk (ranged from $7x10^3$ to $16x10^3$ cfu/ml) (1 type strain, 1 human strain, 3 bovine isolates)	63°C, 15 seconds 66°C, 15 seconds 69°C, 15 seconds and 72°C, 15 seconds (HTST)	Laboratory unit – turbulent flow	Effective, but pasteurized milk likely to harbour low levels of viable MAP	[71]
Spiked milk (2 possible mixtures; 4 field isolates, or 5 field isolates per mix)	Combinations: 72, 75, 78°C and time intervals of 15, 20, and 25 seconds	Industrial pasteurizer designed for research purposes	> 6 log reduction May still have some viable cells.	[79]
Naturally infected milk	HTST and 72°C, 15 and 25 seconds	Commercial scale unit	6.9% of 144 samples positive for viable MAP post pasteurization	[69]
Spiked milk (5.0-7.7 log) (3 field strains)	Standard 65.5°, 16 seconds HTST 71.7°C, 20 seconds and 74.4°C, 15 seconds	Slug-flow and lab scale pasteurizer units	5-7.7 log kill May still have some viable cells.	[80]
Spiked milk (10^3-10^6 cfu/ml)	Ranging: 67°C, 15 seconds up to 135°C, 5 seconds	Industrial pasteurizer designed for research purposes	5-7.0 log kill May still have some viable cells.	[72]
* Standard pasteurization is defined as 63°C for 30 min ** HTST is defined as 72°C for 15s *** This study showed that increasing the time for pasteurization from 15-25s increased the effectiveness of pasteurizations				

Table 1. Summary of pasteurization studies for inactivation of MAP from milk

Although all research groups attempted to reproduce as closely as possible the industrial pasteurization process, the methodology and equipment used varied greatly, which makes it very difficult to compare the results obtained. Pearce *et al*. (2001) [71] made an effort to mimic commercial pasteurization by reproducing conditions that can provide for a turbulent flow of the fluid milk travelling up the holding tube, and tested kinetic data at several points along the process. In turbulent flow conditions, the layers of milk intermix, which makes the velocity of all milk particles travelling in the pipe roughly the same. This is in contrast to laminar flow conditions that are sometimes observed in smaller, lab-scale pasteurizers due to their smaller pipe diameter and lower product velocity. Laminar flow conditions are generally considered as the worst-case scenario because they affect the holding time, as the velocity of milk particles in the pipe is greatest at the center which can often lead to particles in the center travelling up to twice as fast as the average particle. Proper system design is of paramount importance to ensure that the legal holding time for pasteurization is achieved under laminar flow conditions. MAP levels in spiked milk ranged from 0.7×10^3 to 16×10^3 cfu/ml, with the raw whole milk being heated for 15 seconds at 72, 69, 66 and 63°C. There were no significant differences in the heat resistance of strains used (human and cattle isolates). No strains survived at 72°C for 15 seconds, and only one strain had survivors at 69°C for 15 seconds. The mean extrapolated decimal reduction time (D-value, time to kill 1 $\log_{10}$ concentration of bacteria) at 72°C or D_{72} for the five strains of MAP examined was <2.03 seconds, representing a 7 $\log_{10}$ kill at the 95% confidence interval. Similar results were reported by [79], and [80]. The study by Stabel and Lambertz (2004) [80] demonstrated a 5.0 - 7.7 $\log_{10}$ kill of MAP at 71.7°C for 15 seconds using two different pasteurizers, three different bovine field strains and high (10^8) and low (10^5) inoculation levels. These results suggested that properly pasteurized milk is likely to harbour a low number of viable MAP.

More recently, Hammer at al. [72] showed that low levels of MAP can be detected after heating skim milk, whole milk and cream. The samples were spiked with 10^3 to 10^5 cfu/ml at time-temperature combinations ranging from 15 seconds at 67°C up to 5 seconds at 135°C. A 5.0-7.0 $\log_{10}$ reduction of viable MAP cells was reported. According to the statistical analysis, survival was dependent on the heating temperature and the holding time, but not on the initial bacterial count. It was suggested that MAP survives best in whole milk followed by cream and skim milk. Neither additional upstream nor downstream homogenization led to a significant inactivation during heat treatment. The same applied to double pasteurization. The factors possibly influencing the heat resistance include effects of cell clumping, mechanisms of heat inactivation and physiological state of the cells.

Grant *et al*. (2002a) [69] conducted pasteurization trials using a commercial-scale unit (turbulent flow) to examine the survival of MAP in naturally-infected milk. Ten (6.7%) of 144 samples from the pasteurization trials were positive for viable MAP. Samples held for 15 and 25 seconds at 72°C were both positive, indicating the naturally contaminating MAP in raw milk may be able to survive the pasteurization process of a commercial scale pasteurizer. The researchers did allow 24-72 h between heat treatment and diagnostics, which may have allowed for the recovery of sub-lethally injured cells. Furthermore, a 1999-2000 study [74], found viable MAP in 1.6% (4/244) of raw milk and 1.8% (10/567) of commercially pasteurized milk samples in the UK; the 10 positive pasteurized milk samples came from 8 (3.3%) of the 241 different dairies that participated in the survey. Seven of the culture-positive milk samples had been heat-treated at 72 to 74° C for 15 seconds; the remainder had been treated at 72 to 75°C for an extended holding time of 25 seconds. In addition, MAP DNA was detected by immunomagnetic separation PCR in 7.8 and 11.8% of the raw and pasteurized milk samples, respectively. The reason for higher MAP detection in pasteurized than raw milk is not known. However, it can be speculated that the homogenizer or turbulent flow through the pasteurizer dispersed clumps of MAP, resulting in more colonies and more accessible MAP DNA from the same number of cells. The possibility of contamination of pasteurized milk is unlikely, since dairies whose pasteurized milk samples contained viable MAP tested culture negative for coliforms. This suggests that proper pasteurization time and temperatures were reached, and that post-pasteurization contamination did not occur. Table 2 lists a summary of the presence of MAP in tested milk and dairy products.

Location	Prevalence detected by PCR (%) (detected by culture)	Matrix	Reference
Canada (Ontario)	15 % (0 %)	Commercially pasteurised milk	[85]
USA (California,Minnesota, Wisconsin)	2.8 (2.8 %)	Commercially pasteurised milk	[75]
Ireland	9.8	Commercially pasteurised milk	[81]
	12.9 (0.3%)	Raw milk	
England & Wales	7	Commercially pasteurised milk	[82]
UK (England)	11.8 (1.8%)	Commercially pasteurised milk	[74]
	7.8 (1.6%)	Raw milk	
Switzerland	19.7	Raw bulk-tank milk	[83]
Czech Republic	(1.6%)	Commercially pasteurised milk	[73]
India	(58%)	Commercially pasteurised milk	[76]
Argentina	(< 3)	Commercially pasteurised milk	[84]
Czech Republic and Greece	30.9 (3.6%)	Feta, hard, semi-hard and soft cheeses made from bovine, sheep and goat milk	[87]
USA (Minnesota,Wisconsin)	5 (0%)	Retail cheese made from pasteurized milk	[88]
Switzerland	4.2 (0%)	Raw milk cheese	[89]
7 European Union countries	49 (0%)	Powdered infant formula	[92]

Table 2. International data on the prevalence of MAP in retail dairy products

In 2004, the Food Safety Authority of Ireland (FSAI) reported on a survey of Irish bulk raw and commercially pasteurized milk [81]. MAP DNA was detected in 12.9% (50/389) of raw and 9.8% (35/357) of pasteurized milk samples using immunomagnetic separation (IMS)-PCR. Culturable MAP was found in only one raw milk sample (0.3%). The authors concluded that since no viable MAP was isolated from commercially pasteurized milk, their pasteurization procedures (at least 71.1°C for 15 seconds or any equivalent combination) are considered effective.

However, comparison of MAP detection rates in raw milk by PCR and culture suggests insufficient sensitivity of the culture method and a high risk of false-negative culture results. Similar results were reported by Millar *et al*. (1996) [82] who conducted an extensive study of retail pasteurized milk in England and Wales and found that 7% of retail milk samples tested positive for MAP by PCR, while in Switzerland, 19.7% of raw bulk-tank milk samples contained MAP DNA [83].

In a US study [75], viable MAP was found in 2.8% (20/702) of retail milk samples by two culture methods and PCR. The samples were obtained from supermarkets in California, Minnesota and Wisconsin. Similar results were reported from the Czech Republic [73] using a culture method. Viable MAP was present in 1.6% (4/244) of commercially pasteurized (71.7° for 15 seconds) retail milk samples collected from the country's supermarkets and stores. The authors also detected viable MAP from 2% (2/100) of locally pasteurized (71.7°C for 15 seconds) milk samples obtained from herds known to be infected with MAP, and 0% (0/100) from a herd characterized as JD free. In addition, researchers in Argentina isolated viable MAP from 2.9% (2/70) of commercially pasteurized milk samples; one from pasteurized and the other from ultra-pasteurized (138° C for 30 seconds) milk [84]. Both culture positive samples were also positive with IS*900*-PCR. Viable MAP was reported in 72% (13/18) of commercially available pasteurized milk samples tested in India [76]. The authors also found viable MAP in 56% (5/9) of commercially available pasteurized milk products.

Researchers at the University of Guelph reported on an investigation of the presence of MAP in pasteurized milk obtained at retail outlets and dairy plants in southwest Ontario [85]. From 710 milk samples tested, 110 (15%) were positive by IS*900* nested PCR. Each sample was tested three times and the results were as follows, expressed by number of positive reactions out of three tested: 73 x 1/3; 32 x 2/3; 5 x3/3. No viable MAP was isolated from the broth and agar cultures of 44 PCR positive and 200 PCR negative retail milk samples tested. The authors stated several possible reasons for not detecting MAP, such as that culture methods used were not sensitive enough, sample contamination or the presence of low number of viable organisms which were undetected by culture. Also, a survey of time/temperature combinations used for fluid milk pasteurization across Canada showed that all temperatures used were above 72°C (72.9-83.5°C) [85]. However, 41% (13/32) of the facilities used a holding time of less than 25 seconds, with 9% (3/32) below 20 seconds. With the exception of one facility that used a time/temperature combination of 72.9°C and 19 seconds, all Canadian facilities with holding times less than 25 seconds used high temperatures ($\geq$73°C).

In conclusion, based on a review of papers published in English between 1980 and 2010, the effectiveness of pasteurization with respect to the destruction of MAP is still unclear. The combined data suggest that current pasteurization practices have the potential to eliminate the concentration of MAP likely to be found in commercial milk supplies. However, the presence of viable MAP in retail samples of pasteurized milk in multiple studies demonstrates that current practices may allow for the survival or reintroduction of MAP at some low frequency. The exception is the study from India [76] which reported a high level of MAP survival in pasteurized milk. It should be taken into consideration that India is a developing country with a less effective public health control over the milk industry, compared to developed countries. Cerf *et al*. (2007) [86] assessed the probability of detecting MAP in 50-ml samples of pasteurized milk at less than 1% using a quantitative model and Monte Carlo simulation. Therefore, we conclude that the presence of viable MAP in pasteurized milk occurs infrequently, and that consumer exposure to MAP through pasteurized milk in Canada may occur at low levels.

Cheese and other dairy products

To-date, surveys of retail cheese products for the presence of MAP have been reported from Greece, Czech Republic, the USA, and Switzerland ([87]; [88]; [89]). To our knowledge, investigations on the presence of MAP in Canadian retail cheeses have not been conducted. Three regulatory options for producing microbiologically safe cheese products are available in Canada: cheese can be prepared either from i) pasteurized, ii) thermised or iii) raw milk; if produced from thermised or raw milk, cheese by law must be held at 2° C or higher for a period of 60 days or more from the date of the beginning of the manufacturing process. However, these conditions may not be sufficient to inactivate MAP if present in the milk.

Sung and Collins (2000) [90] studied the effect of pH, salt (pH 6.0, 2% [wt/vol] NaCl) and heat on the viability of MAP in soft white Hispanic-style cheese. Results indicated that salt had little to no effect on MAP

inactivation rates; a decreasing D-value was associated with decreasing pH, and heat-treated MAP cells were inactivated faster than non-heat-treated cells. Overall, the 60-day curing period resulted in a 2-log reduction in the number of heat-treated MAP cells per gram in cheese (initial concentration of MAP was 10^6 cfu/ml of milk), suggesting that the heat treatment and mandatory 60-day curing period are likely important elements in reducing the organism in this product. Due to the popularity of raw milk cheese in Canada, this issue may warrant additional research and discussion.

Donaghy *et al*. (2004) [91] prepared cheddar cheeses from pasteurized milk artificially-contaminated with high (10^4-10^5cfu/ml) and low (10^1-10^2 cfu/ml) inocula of three strains of MAP, a reference strain, (NCTC 8578) and two strains (806PSS and 796PSS) previously isolated from pasteurized milk. The D-values were variable depending on the strain of MAP. The D-values for strains 806PSS, 796PSS and NCTC 8578 were 107, 96 and 90 days, respectively. Strain 806PSS, was the only one culturable from 27-week-old cheese spiked at the low inoculum level.

Ikonomopoulos *et al*. (2005) [87] investigated the presence of MAP in retail cheeses (feta, hard, semihard and soft) manufactured from bovine, sheep and goat milk in Greece and Czech Republic. Viable MAP was isolated from 3 of 84 (3.6%) cheese samples, while PCR detected MAP DNA in 26 of 84 (30.9%) samples. The highest proportion of MAP-positive samples was reported for two brands of feta cheese (10 and 14.3%), prepared with a mixture of sheep and goat milk. The results indicate that retail cheeses may be an important means of human exposure to MAP. A similar study was conducted in Wisconsin and Minnesota [88], reporting MAP DNA in 5% of the retail cheese samples manufactured from pasteurized milk. No viable MAP was isolated in this study, which may suggest either an effective pasteurization process or lack of sensitivity of the culture method. The decontamination procedure was much harsher and the incubation time was shorter compared to the study by Ikonomopoulos *et al*. (2005) [87]. The researchers also raised a question about the ability of heat-inactivated MAP to elicit an immune response and hence play a role in the etiology of CD. More recently, Swiss researchers [89] reported on the prevalence of MAP in Swiss retail raw milk cheeses. Although the study included only 143 cheese samples, 6 (4.2%) of them contained MAP DNA (F57 sequence). No viable MAP was isolated in the cheese samples tested in this study.

MAP DNA was found in powdered infant milk products from 10 producers operating in 7 European Union countries [92]. Of the 51 samples tested, 25 (49%) were IS*900* PCR positive, but no viable MAP was found. To our knowledge, there are no reported studies on the presence of MAP in dairy products such as yogurt, butter, and cream. However, considering that the organism has been found in pasteurized milk in some countries, there is a possibility that it can be found in other dairy products.

Exposure estimate for MAP in pasteurized milk

Exposure estimates are determined by the prevalence of MAP infection in cattle, the rate of shedding among infected animals, the level of contamination of milk and dilution effects, the efficacy of pasteurization, the organism survival in milk and the milk consumption rate.

Nauta and Van der Giessen (1998) [93] employed a modelling approach to estimate human exposure to MAP via pasteurized milk. Looking at a farm with a high prevalence of JD, it was estimated that the concentration of MAP was 5.4 x 10^{-3} cfu/ml of pasteurized milk. Theoretically, the major contribution to contamination came from clinically affected animals, with the contribution from subclinically-infected cows appearing to be minimal. The authors reasoned that removing clinically affected cattle from the production chain would reduce the point estimate of exposure by about 99%, from 5.4 x 10^{-3} cfu/ml to 0.06 x 10^{-3} cfu/ml. However, the approach used in this example was based on limited data and a rough estimation. Sweeney *et al*. (1992) [44] reported 4 x 10^{-2} cfu/ml to 16x/10^{-2}cfu/ ml of milk from asymptomatic cows infected with MAP, while estimations suggest that due to faecal contamination of milk, exposures may be as high as 10^4 cfu/ml [65]. The authors of the modelling study referred to the fact that there are no adequate published numbers for the concentration of MAP in raw milk, and that the pasteurization experiments used vary greatly in their methodology, making comparison difficult. Only when such data are available, will modelling approaches be useful.

Data on Canadian milk consumption rates are very limited. Statistics Canada reported that the average milk consumption rate among Canadians in 2004 was 85.5 litres per capita per year, which is comparable to the rates reported in New Zealand (96.7 litres/capita), the European Union (78.4 kg/capita) and the United Kingdom (112.4 kg/capita) ([94]; [95]).

Meat and meat products

The high prevalence of MAP in cattle delivered to slaughter for human consumption would result in great expense if all MAP positive cattle were diverted. There is reportedly a high load of MAP observed in the gastrointestinal tract of infected animals. Thus, given the lack of evidence that there is risk to humans, cattle that test fecal culture positive for MAP are not diverted. As a

result, contamination of raw meat by MAP-containing feces could occur and this can be considered as potential source of human exposure to the organism.

A recent Canadian study reported infrequent contamination of beef carcasses with MAP [96]. Cattle in later stages of JD may have heavily contaminated lymph tissues that are commonly included in trimmings used for ground (minced) beef production ([97]; [98]). Since ground beef (hamburgers) are not always eaten well-cooked, the survival of MAP following cooking could be a possibility. The organism's resistance to standard hamburger cooking processes has been recently reported [99]. The authors concluded that small numbers of MAP cells may survive meat cooked to a medium-rare condition (63°C). However, it was found that there is a low probability of survival of MAP when meat is cooked to a well-done condition (75°C). Studies on MAP inactivation in beef are uncommon. To our knowledge, two studies calculated D and z values for MAP in meat ([100]; [101]). The z-value is the temperature elevation required to reduce the D-value by 1 $\log_{10}$ unit. Using lamb skeletal muscle homogenate fluid, the authors calculated D_{60} values from 8 to 11 min, D_{65} from 26 to 35 seconds and D_{70} from 1.5 to 1.8 seconds. Values for z, for the S and C strains were 4.21°C and 4.51°C, respectively. A reduction of at least 5 logs in viable counts of MAP required heating at 70°C for 15-20 seconds or at 75°C for less than 5 seconds (C strain), and 10-15 seconds at 70°C or less than 5 seconds at 75°C (S strain). In addition, Saucier and Plamondon (2011) [101] reported that in ground beef, D_{70} values for both MAP strains were 12 ± 1 and 13.1 ± 0.3. The z-values ranged from 5.6 ± 0.1°C to 5.7 ± 0.1°C. Other authors [102] reported that cooking wiener-type sausages for 2 min at 70°C resulted in a 12D reduction in *M. avium*.

Although MAP infection is often restricted to the intestine and mesenteric associated lymph nodes, in advanced disease, the organism can be widely distributed throughout the body of infected animals [103]. MAP in cattle has been isolated from the liver, kidney, spleen, lung, heart, short ribs, ribs-prepared muscle, tenderloin, shin-shank, reproductive organs, semen, milk, and lymph nodes associated with muscle and organs other than the gut ([97]; [98]; [104]; [105]). A study conducted by Rossiter and Henning (2001) [97] examined the presence of MAP in thin dairy and beef market cows at three slaughter plants in the US. Culture of faeces and ileocecal lymph nodes classified 34.4% of dairy cows and 2.6% of beef cows as MAP infected, while liver and other lymph nodes were infected in 7.9% of dairy and 0.3% of beef cows. Therefore, the results indicate that in cattle which are systematically infected, MAP may be found in lymph nodes which may be incorporated into ground beef products. MAP DNA was detected in 19.8% of slaughtered healthy dairy cows in Switzerland [106]. In total, 8.9% (9 of 101) of the cows were positive for MAP DNA in fecal samples, 4.9% (5 of 101) in jejunal LNs, 0.9% (1 of 101) in ileum tissue, 2.9% (3 of 101) in diaphragmatic muscles, and 3.6% (3 of 84) in milk. Similarly, viable MAP was reported in the diaphragm muscle in 13% (6 of 47) of both clinically and subclinically infected cattle [107]. The infected animals also had heavy bacterial loads in mesenteric LNs, ileocecal valve, ileum and jejunum. Brady *et al*. (2008) [108] found that MAP was widely distributed in the tissues of 17 of 21 cows which were examined, including three clinically normal animals. MAP was also found in the mammary tissues of 7 of the cows, including two clinically healthy animals.

To our knowledge, there have been only two reported surveys on the presence of MAP in retail ground beef [109], [110]. The first study included 200 samples of ground beef obtained from retail stores in California, USA. All samples tested negative for the presence of MAP using multiplex real-time PCR and conventional culture. In addition, a government lab in British Columbia, Canada conducted testing of several hundred ground beef samples from Canadian retail stores, and all samples tested negative [110]. Table 3 summarizes the prevalence of MAP in tested beef and beef product.

In conclusion, current studies demonstrate that there is a possibility that MAP can be present in meat and meat products in low concentrations. Since meat is not always eaten well cooked, the organism could be present when consumed. However, the results suggest that conventional cooking methods should be sufficient to inactivate low concentrations of MAP.

Matrix	Location	Prevalence detected by PCR (detected by culture)	Matrix (detailed)	Reference
Dairy Cattle	Switzerland	19.8%	Variable by tissue tested including fecal (8.9 %), jejunal LN (4.9 %), ileum tissue (0.9 %), diaphragm muscle (2.9 %)	[106]
	USA	34.4%	Feces and ileocecal lymph node	[97]
	USA	7.9%	Liver and other lymph nodes	
	Spain	13 % *	Diaphragm muscle	[107]
	Canada (Manitoba)	4.5%	Blood	[68]
Beef Cattle	USA	2.5%	Feces and ileocecal lymph node	[97]
	USA	0.3%	Liver and other lymph nodes	
	Spain	13 % *	Diaphragm muscle	[107]
	Canada (Manitoba)	1.7 %	Blood	[68]
Groun beef	USA (California)	0 % (0%)	Retail ground beef	[109]
	Canada	0 %	Retail ground beef	[110]
* In this study, cows were classified by the owners as having clinical signs consistent with paratuberculosis if they showed or had shown persistent diarrhea, weight loss, and / or low milk production, and / or had tested positive in a paratuberculosis enzyme-linked immunosorbent assay (ELISA) and / or a fecal PCR.				

Table 3: The prevalence of MAP in tested beef and beef products

Water, produce and environment

A possible route of transmission of MAP to cattle, from cattle and other ruminants to humans, and from humans to humans, is through water.

Application of contaminated field run-off are likely to be important, if water is a vehicle for the presence of MAP in source waters that are treated for human consumption.

The survival of MAP in the environment for prolonged periods of time ([111]; [112]; [113]), and its presence in the faeces of cattle (cattle with severe JD can shed 5 x 10^{12} cfu per day) [103], suggests that surface and ground water may be a potential reservoir of MAP infection. To-date, studies have shown a variable presence of the organism in potable water [114], [56] as well as untreated water entering water treatment facilities [111]. Other studies have demonstrated the ability of MAP to adapt within protozoa and survive traditional water treatment practices [115], [116]. Some studies have shown that the organism remained viable for at least 163 days in river water and at least 270 days in pond water [1]. Pickup *et al*. (2005) [117] also showed that a MAP bovine strain remained detectable in model water lake systems for at least 632 days by culture and at least 841 days by RT-PCR.

MAP and other mycobacteria of the *M. avium* complex which are widely distributed in the environment have been cultured from potable water sources, and shown to cause infections (mycobacterioses) in immunocompromised hosts [118,] [119]. Other environmental mycobacteria have also been found in water distribution systems [120]. The only known case of MAP detection in potable water [114] found that the *Mycobacterium* (originally called *M. avium*) isolated from the municipal water supply of a major city in the US, was in fact MAP. More recently, MAP was detected in a high percentage of samples (81 and 88%) in one survey in the mid west United States, but a lower percentage in a subsequent national survey [56]. The potential for temporal and spatial variation in the presence of MAP in drinking water raises interesting questions concerning the potential for exposure, and clearly MAP occurrence in drinking water needs more investigation. *M. avium* detected in drinking water are often not typed to determine if they could be MAP. The Public Health Laboratory Service in the UK failed to detect viable MAP in samples of untreated and treated drinking water [16]. However, a survey of untreated surface water, entering 9 water treatment centers across Northern Ireland, showed that of the 192 one litre water samples tested, 9 were IMS-PCR-positive and 8 were culture positive for MAP [111]. A recent study [121], detected viable MAP in treated water which is used for the production of potable water. These findings indicate that MAP can survive sufficiently in the environment for water receiving agricultural runoff to be a possible route of human exposure. Since there is evidence to suggest that MAP can survive chlorine disinfection [122], the possibility of human exposure to

this organism through water consumption exists. It may also have implications for those who come into contact with the contaminated water (agricultural runoff) through recreational activities.

Recently, the UK Food Standards Agency conducted a case-control study and found no association between consumption of water and dairy products potentially contaminated with MAP, and subsequent development of CD [123], [124]. However, in the same study, consumption of pasteurized milk and fruits was associated with a reduced risk of the disease, while meat intake was associated with an increased risk of developing CD.

A number of environmental, opportunistic mycobacteria, have been shown to be relatively resistant to chlorine or chloramine concentrations used in municipal water treatment [125]. Most of the *M. avium* strains tested were highly resistant to chlorine, chloramine, chlorine dioxide and ozone [125]. It was also noted that cells of the more slowly growing strains were more resistant to chlorine than rapidly growing cells, and that water-grown cells were 10-fold more resistant than medium-grown cells. The contact time values for the effect of chlorine on MAP have been estimated to be up to 580-2300 times greater than those for *E. coli* [126]. To date, the efficacy of water treatment facilities in removing or inactivating MAP present in water destined for human consumption has not been thoroughly investigated. Only one study has been identified on the effect of chlorine on MAP [122]. MAP was not completely inactivated by chlorine at levels as high as 2.0 μg/ml for a contact time of 30 min, when the initial inoculum levels were approximately 10^6 cfu/ml. This translates to 2 ppm or 2 mg/L; notwithstanding the initial disinfectant (chloramine, chlorine, ozone, etc.), the manager of a water treatment system in Ontario must maintain a free chlorine residual of 0.2 ppm (Ontario Drinking Water Guidelines; provinces mandate specific guidelines in Canada). If MAP is able to withstand treatment, there is a potential for the organism to remain viable in water distribution system biofilm.

Several research reports have also focused on environmental mycobacteria surviving within amoebae. The protozoan host can provide protection for MAP bacilli under adverse environmental conditions and thus prolong its survival in lake and river water [115], [116]. *M. avium* was demonstrated to grow saprozoically (in other words, living in decaying organic matter; especially denoting certain protozoa) on the products secreted by the amoeba *Acanthamoeba polyphaga*, and survive within the outer walls of the double-walled cysts of *A. polyphaga*; it was unclear whether multiplication occurred in the cysts [115]. *M. avium*, *M. fortuitum* and *M. marinum* were shown to be very successful at growing within the amoeba host [116]. Bull *et al*. (2002) [127] reported that MAP can survive for up to one year within *A. polyphaga*; whether it was able to multiply was not clear. They also investigated gene expression of MAP within *A. polyphaga* using microarray analysis and identified expression specific to intracellular localization of MAP, which may prove significant in MAP pathogenesis. A recent publication on the replication and long-term persistence of bovine and human strains of MAP within *A. polyphaga*, showed that MAP was able to survive and replicate within *A. polyphaga* over periods of up to 24 weeks [63]. They also found that human MAP isolates, obtained directly from infected human intestinal tissues, survived in cultures of amoebae for almost 4 years. At the end of the study period, the organisms were still intact and thus, no upper limit on their persistence was established.

In conclusion, MAP can survive for a prolonged period of time in water systems and has also been shown to survive chlorination. The efficacy of water treatment and water resources used for domestic consumption will need to be reevaluated, as more evidence on the survival of MAP in water becomes available. Therefore, more research is needed to determine the survival of the organism during the water treatment process and its presence in water destined for domestic supply. To our knowledge, neither data regarding the presence of MAP in Canadian water, nor published studies looking at MAP in Canadian municipal water, currently exist.

Other potential sources of human exposure to MAP are aerosols, contaminated environment, vegetable and fruit products. Mycobacteria are found in aerosols, indoor swimming pools and hot tubs [128], [129]. Exposure to MAP via aerosols has been implicated in a study that investigated the presence of MAP in the water of the river Taff in South Wales, and its association with CD clusters in the city of Cardiff [117]. The river runs off of hill pastures grazed by livestock in which Johne's disease is endemic. Of the 96 daily samples tested, 31 (32.3%) were positive by PCR, and 12 of these 31 (66%) grew bovine MAP strains after 8 to 11 months of incubation. Previous epidemiological research in the city of Cardiff demonstrated a significant increase of CD in most of the districts that bordered the river Taff [117]. The researchers [117] hypothesized that after rains wash MAP into ground waters and rivers, contaminated river runs through the city where aerosols from surface water expose the residents to inhalation of MAP, a risk well characterized for other environmental mycobacteria [128]. Pulmonary involvement in CD has been reported [130], and inhalation has been recently suggested as a potential route of MAP infection for cattle [131]. However, given the tissue tropism of MAP, the principal clinical manifestation that eventually emerges is chronic enteritis [132].

Persistence of MAP in commonly used dairy manure treatment systems (thermophilic composting at 55°C, low-temperature composting at 25°C, and liquid lagoon storage at room temperature) has been studied recently [133]. Composts are often used for residential gardening and organic farming markets (for vegetable production) and are periodically applied to agricultural land as a fertilizer, and therefore, can serve as a potential source of human exposure to MAP. The study showed that after initial inoculation of 10^6 cfu/g, MAP was viable on day 0 in all treatments and later remained culturable only in the liquid storage treatment through day 56. MAP DNA was detectable by PCR through day 56 in all treatments and up to 175 days in liquid storage treatments. Overall, results show that MAP may persist for more than 2 months at unculturable levels (detectable by PCR) regardless of whether the manure is composted at 55°C, 25°C, or is liquid stored under anaerobic conditions. Pavlik *et al*. (2002) [134] reported that MAP can survive for one year in manure, can be found in larvae and subsequently in adult flies that eat contaminated manure and can be shed by invertebrates living in manure or soils contaminated with MAP. Lately, research on the long-term persistence of MAP in the farm dam water and sediment suggests that the aquatic environment is a greater risk than pasture and soil with respect to long-term persistence of the organism [112]. MAP survived for up to 48 weeks in dam water and/or sediment in the shade, and 36 weeks in the semi-exposed location. Survival in soil and faecal material in the terrestrial environment in the shaded location was only 12 weeks. Similarly, dry, warm soil (30°C), was found to be the most significant factor in reducing the numbers of MAP in soil [135]. Wet and dry cyclic conditions, common in Canada, resulted in intermediate recovery of MAP; UV exposure (with the exception of increasing soil temperature) had no noticeable effect on MAP survival. The current evidence suggests that at the present prevalence in Canadian dairy herds, persistent environmental contamination must also be considered when assessing sources of MAP throughout the country.

A sewage sludge pool was implicated as a source of epidemic *Mycobacterium xenopi* pulmonary infections in Pecs, Hungary [136]. All patients and asymptomatic individuals testing positive for *M. xenopi* lived in close proximity to a local sewage sludge pool or were exposed to the sludge via manure distribution for fertilizer in local parks and home use. For those in close proximity to the sludge pool, exposure to dust during the summer when the pool dries completely, was suggested; this is consistent with the ability of *M. xenopi* to grow at temperatures around 42°C [137]. *M. xenopi* was isolated from sewage inflow as well as several locations and depths within the sludge pool, which dries every summer. Although sewage treatment was not discussed in the paper, it was noted that the heating of sewage, before use as a fertilizer should be implemented as a health precaution. Treatment of abstracted surface water intended for human consumption in the UK often employs counter-current dissolved air flotation filtration (COCODAFF) to remove suspended solids [138]. The removed product is a brown sludge which collects in slurry pits where it dries out. As in the Pecs, Hungary example above, this material was found to be strongly MAP-positive. Since it is usually trucked back onto farmland, this establishes a cycle of environmental contamination. The use of agricultural and human waste as fertilizers should be investigated, as they may prove to be a potential source of MAP, especially if not properly treated.

Vegetables and fruits may become contaminated with MAP and therefore serve as another potential source of human exposure to MAP when MAP-infected slurry is applied to gardens or agricultural land as fertilizer. Pavlik *et al*. (2002) [134] recovered MAP from the stems, leaves and fruits of vegetables (tomato, radish, lettuce) grown on soil artificially-contaminated with MAP-containing manure. Presently, there are no other reported studies looking at the contamination of vegetables and fruits with MAP. However, considering the organism's long-term persistence in the environment, survival during manure treatments, and the common use of manure as a fertilizer, further research is needed to clarify the risk of human exposure to MAP via contamination of fruit and vegetable products.

Fish and shellfish are potential sources of exposure to MAP, since MAP is present in contaminated water. However, to our knowledge, there have been no published studies regarding the presence of MAP in fish and shellfish.

4. Conclusion

It is likely that MAP may be present in raw milk and other dairy products in Canada, as well as at low levels in raw ground beef and beef products. However, to-date, no viable MAP has been reported in any dairy or beef products in Canada. Given the evidence that on occasion, the organism may be capable of surviving commercial milk pasteurization and that common cooking practices may be insufficient to control MAP in beef, sheep, goat and wild ruminants, the potential exposure of Canadians to low levels of MAP via these foods, exists. Other vehicles such as vegetables and fruits, water, and the environment associated with farms housing MAP positive livestock, may also be involved in the transmission of the organism. There is a need for increased research on the prevalence of MAP in Canadian environments, food and water supplies. Therefore, we

conclude that at present, the public health significance of MAP in Canada is unknown.

5. Further research

In light of the control of MAP in the food supply (on-farm, processing, consumer levels), the following research needs have been identified:
Each identified research/data gap was ranked as low, medium or high in terms of importance.

"High Ranking" (H)
"Medium Ranking" (M)
"Low Ranking" (L)

1) **Prevalence of viable MAP in the food supply:**
i) Prevalence of MAP in meat
• Prevalence of viable MAP in ground beef & select cuts (H)
• Prevalence of MAP on carcasses (H)
• Prevalence of viable MAP from farm to retail in all animals/animal products (Canadian national survey) (L)

ii) Prevalence of MAP in dairy
• Prevalence of MAP in powdered dairy products (for example, powdered infant formula) (H)
• Prevalence of MAP in dairy products in Canada (national survey) (H)

iii) Prevalence of MAP in water, fruits & vegetables
• Prevalence of MAP in produce (H)
• Prevalence of MAP in irrigation water (H)
• Prevalence of MAP in water used for washing produce (H)
• Prevalence of viable MAP in drinking water in Canada (national survey) (H)

2) **Prevalence of MAP in the environment:**
• Prevalence and survival of MAP in environmental samples (H)
• Prevalence of viable MAP in Canadian rivers (L)

3) **Pathways in beef:**
• Extent to which contamination contributes to MAP load in (ground) beef (H)
• Contamination of ground beef with MAP via lymph nodes (H)
• Blood-borne organisms in beef from cattle with Johne's disease (M)

4) **Improved methodology:**
• Improvement in methods for detection of viable MAP in foods, water, including cell-wall deficient (CWD) forms (H)

5) **Molecular characterization and virulence:**
• Molecular characterization and comparison of MAP strains from human (clinical), animal, food and environmental sources in Canada (Are all MAP strains equal or are some more pathogenic?) (M)

6) **Prevalence of Crohn's disease:**
• Prevalence of Crohn's disease in people exposed to herds with/without JD (longitudinal study) (M)

7) **Effectiveness and validation of processing interventions:**
• What time/temperature combinations kill MAP in relevant food matrices? (H)
• Survival of MAP in beef products following cooking to recommended temperatures (H)
• Effect of changes in pasteurization parameters (temperature/time) on viable MAP in milk and dairy products (H)

• Responses of MAP to sublethal stress (M)
• Effective pasteurization temperatures in eradicating viable MAP when studied at bulk/commercial level (M)
• Effect of various processing conditions/ in-plant interventions (for example: lactic acid; steam/water pasteurization, UHP, carcass irradiation) on the survival of MAP (M)
• Specific associations of MAP with protozoa (M)
• Alkaline phosphatase as an indicator of the presence of MAP in pasteurized milk (L)
• MAP survival in raw milk cheese (L)
• Effect of chemical treatments normally used on fresh cut fruits/vegetables (ppm chlorine) on survival of MAP (L)
• Effective treatment of water for viable MAP (L)

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