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Review

Mycobacterium avium subsp. *paratuberculosis* in Dairy Products, Meat, and Drinking Water†

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ABSTRACT

Mycobacterium avium subsp. *paratuberculosis* (*Map*) is the cause of Johne's disease, a chronic infection of the gut, in ruminant animals that provide milk and/or meat for human consumption. *Map* also may be involved in Crohn's disease and type 1 diabetes in humans. Although the role of *Map* in human diseases has not been established, minimizing the exposure of humans to the organism is considered desirable as a precautionary measure. Infected animals can shed *Map* in feces and milk, and the organism can become disseminated in tissues remote from the gut and its associated lymph nodes. The presence of at least some *Map* in raw milk and meat and in natural waters is likely, but the numbers of *Map* in those foods and waters should be reduced through cooking or purification. The available information relating to *Map* in milk and dairy products, meats, and drinking water is reviewed here for assessment of the risks of exposure to *Map* from consumption of such foods and water.

Johne's disease, a chronic granulomatous enteritis of cattle and other ruminants, is caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). The infection usually is acquired by young animals through ingestion of *Map (34)*. Infection of the fetus also can occur. Whether the course of the disease is the same when animals are infected before rather than after birth is uncertain *(220)*. Clinical symptoms of weight loss and diarrhea usually develop in cattle when animals are 2 years or older *(213),* but symptoms can appear in younger sheep and deer *(25, 119, 133)*. However, even older infected animals may not have poor body condition and/or loose feces, whereas animals not infected with *Map* may show such clinical signs *(125)*.

Map is present in domesticated ruminants in most regions of the world. Determination of the *Map* infection status of individual animals or herds can be difficult. Consequently, estimates of the prevalence of *Map* in animals and herds also is difficult. However, the available information indicates that there is wide regional variation in *Map* prevalence. For cattle, estimated animal prevalences have ranged from <2 to >20%, and estimated herd prevalences have ranged from <10 to >50% *(1, 96, 103, 108, 132, 141, 201)*. In some regions, the prevalence of *Map* in beef cattle is apparently about half the prevalence in dairy cattle *(74, 144)*. Data on *Map* infection of sheep and goats are limited, but prevalences of up to 20% in European herds have been suggested *(141)*.

Map ingested by calves invades the ileum and is found in lymph nodes associated with the gut soon after infection *(226)*. Invading *Map* cells are taken up by macrophages but survive and proliferate in those cells *(90)*. Granulomata that may contain only a few *Map* cells develop at the sites of infection. However, the infection tends to spread to involve much of the small intestine and parts of the large intestine in some species, and lesions of the gut wall become heavily colonized by *Map (33)*. The organism can be present in the white cell fraction of blood from infected animals *(13)* and can be widely disseminated in the abdominal organs and in lymph nodes remote from the gut *(20)*. Disseminated infections have been thought to occur only when the disease was far advanced. However, recent study results suggest that episodic disseminated infections can occur at early stages of the disease and in young animals *(41, 226)*. Cattle that are infected with *Map* shed the organism in feces sporadically from about the age of 1 year *(218)*.

When the ruminant gut is colonized by *Map,* the tissues develop a characteristic histological and gross appearance, and eventually the absorption of nutrients is compromised *(33)*. In human patients with Crohn's disease (a chronic inflammatory condition of the gut), the appearance of the gut wall is similar to that seen in animals with Johne's disease *(29)*. Consequently, it has long been postulated that *Map* may cause or be otherwise involved in Crohn's disease *(168),* but the available evidence of this involvement has been inconclusive. *Map* has been found in gut tissues from patients with Crohn's disease but has not been found consistently *(61)*. When detected, *Map* has been present in only small numbers, whereas large numbers of the organism are frequently found in the gut tissues of ruminants and

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other animals infected with *Map (101)*. In some studies, *Map* was detected in blood from patients with Crohn's or other forms of inflammatory bowel disease and from healthy control individuals *(10, 104)*. In other studies, *Map* was detected in blood from only patients with inflammatory bowel disease *(160, 170)* or was not detected in blood from either patients with Crohn's disease or control individuals *(148)*. Epidemiological and other indirect evidence for a possible link between *Map* and Crohn's disease is also inconclusive *(102)*. Thus, the involvement of *Map* in Crohn's disease, as either the primary or a contributory cause of the condition, has not been established *(89, 122, 164)*. However, the pathogenicity of *Map* for ruminant and nonruminant animals other than humans is incontrovertible *(4)*. *Map* infection of an immunocompromised patient also has been reported *(159),* and Dow *(50)* hypothesized that *Map* is a cause of type 1 diabetes. Therefore, minimizing the extent to which humans are exposed to *Map* is widely considered desirable as a precautionary measure *(75)*.

Transmission of *Map* to humans most likely would occur via (i) consumption of milk from herds that include infected animals and products prepared from such milk, (ii) consumption of meat and organ tissues from infected animals or animals contaminated by feces shed by infected animals, and (iii) drinking or bathing in waters contaminated with *Map* from feces of infected animals. In this review, current information on *Map* in food and drinking water was assessed to determine the risks of human exposure to *Map* from each of those potential proximate sources.

A report by the National Advisory Committee on Microbiological Criteria for Foods *(139)* was prepared in 2007, updated in 2009, and recently published, after the preparation of the present review. The present review necessarily refers to many of the publications presented in the Advisory Committee's report, but it also includes material that was outside the Committee's terms of reference, was unavailable, or could be given only limited consideration at the time of final preparation of the Committee's report. However, methods for *Map* isolation and procedures for detecting *Map* in milk, which are only summarized in the present review, are presented and considered in detail in the Committee's report.

ISOLATION AND DETECTION OF *MAP*

Isolation of *Map*. Strains of *Map* have been classified as S or ovine strains, which are found mainly in sheep, and C or bovine strains, which predominate in cattle and species other than sheep *(37)*. On the basis of molecular analyses, the S and C groups have been classified as type I and type II strains, respectively *(27)*; an additional group of type III or intermediate strains also has been identified *(86)*. However, the host specificities ascribed to S and C strains have been questioned *(62)*. Most mycobacteria grow slowly, and *Map* is the most slowly growing of the mycobacteria that can be cultivated in microbiological media *(151)*. Generation times at 37°C apparently can range widely from more than one to several days *(111, 225)*. Consequently, liquid or solid media inoculated with samples for recovery or detection of *Map* are incubated at 37°C for at least 6 weeks; incubation for 12 to 16 weeks is most common. Most strains require the siderophore mycobactin J for acquisition of iron when growing in microbiological media *(121)*. Therefore, media for recovery of *Map* from foods are supplemented with mycobactin J.

Map can be grown on or in various mycobactin-supplemented media formulated for the cultivation of mycobacteria in general, such as Herrold's egg yolk, Lowenstein-Jensen, or Middlebrook media. The media usually are further supplemented with additional materials to provide substrates that enhance *Map* growth or act to counter growth inhibition by fatty acids or oxidizing compounds *(40, 192, 225)*. However, no single medium can support the growth of all strains of the organism *(166, 200)*. The use of multiple media is apparently required if recovery of *Map* is not to be biased by the choice and use of a single medium.

Because of the necessary use of complex media and the long times required for cultivation, *Map* is likely to be overgrown by other organisms naturally present in samples unless such organisms are inhibited or destroyed. Control of competing organisms is achieved by supplementing media with antibiotics *(45)* or subjecting samples to decontamination treatments to which *Map* are relatively resistant *(165, 199)*. Often, both methods of control are used *(123, 215)*. Although *Map* is resistant to somewhat harsh decontamination treatments, the treatments may inactivate some and perhaps large portions of the *Map* present in a sample *(52, 178),* thus either reducing or precluding recovery of *Map (83, 157)*.

The resistance of *Map* to decontaminating reagents is due at least in part to the thick cell envelope rich in mycolic acids with chain lengths of ≥C60, which is common to mycobacteria *(22, 228)*. Because of this waxy cell envelope, *Map* and other mycobacteria are not readily decolorized with acid alcohol after staining *(44)*. The Ziehl-Neelsen method, in which staining is achieved with a hot solution of carbolfuchsin followed by a decolorizing treatment, is widely used to establish the presence of acid-fast bacilli in samples, cultures, or colonies suspected of containing *Map (227)*.

The waxy cell envelope also makes *Map* and other mycobacteria highly hydrophobic *(221)*. Consequently, *Map* cells in aqueous suspensions have a strong tendency to aggregate *(17)* and to associate with fats in foods and tissue homogenates *(66)*.

Molecular methods. Because of the difficulties and long times associated with cultivation of *Map,* foods have often been tested by indirect methods for the presence of the organism. Enzyme-linked immunosorbent assays (ELISAs) for detection of *Map* antibodies in blood have been adapted for detection of *Map* antibodies in milk *(209),* but a positive ELISA result does not establish that *Map* as such is present in milk *(175)*. The time required to isolate *Map* from enrichment cultures in which acid-fast organisms have grown greatly extends the time required for detection of *Map*. Consequently, the presence of *Map* in such cultures is

usually confirmed by a PCR test for a genetic sequence unique to *Map*. Because the biochemical activities of *Map* are generally nonspecific and weak, the identities of isolates suspected of being *Map* are commonly confirmed by a PCR test *(98)*.

Most PCR assays developed for *Map* have targeted the repetitive insertion sequence IS*900,* which was thought to be specific to *Map (36, 85)*. However, mycobacteria other than *Map* have been found to carry IS*900*-like elements with nucleotide sequences that are up to 94% identical to the nucleotide sequence of *Map* IS*900 (56, 134, 138)*. Some PCR systems that target IS*900* also can give false-positive results with DNA from mycobacteria other than *Map* and with DNA from other types of organisms *(131, 196)*.

In response to the uncertainty about the specificity of PCR systems that target IS*900* for identification of *Map,* several other target sequences for use in *Map* identification systems have been proposed: IS*Map02,* IS*Mav2, hspX,* locus 255, and F57 *(8, 23, 180, 188, 197)*. The F57 sequence appears to have been the most widely used of these targets. Both single-round and nested PCR systems that target the F57 sequence have been reported to be highly specific for *Map (73, 131, 198)*.

Some researchers have suggested that the sensitivity of tests targeting sequences such as F57, which occur as single copies in the *Map* genome, may be lower than that of tests that target the multicopy IS*900* sequence *(54, 173)*. However, other researchers found that the number of copies in the genome of the targeted sequence had little effect on the sensitivity of the PCR system *(131)*. The achievable detection limits for all target sequences might then be between 10 and 100 genomes per PCR for single-round PCR assays and one genome per PCR for nested PCR assays *(208)*. Because of the very high sensitivity of nested PCR tests, cross-contamination during manipulation of test materials must be scrupulously avoided to prevent false-positive results.

Because extraneous *Map* DNA and DNA in moribund cells will be detected in addition to DNA in viable *Map* cells, detection of *Map* DNA alone does not prove that viable *Map* cells are present in a sample. Detection of an appropriate mRNA by a reverse transcriptase PCR assay and detection of DNA from *Map* cells lysed by mycophages have been applied to confirm the presence of viable *Map* cells with a PCR method. The half-life of bacterial mRNA is generally short, so detection of mRNA can be viewed as an indication that gene transcription in viable cells was occurring when samples were processed *(93, 130)*. The assumption that detection of mRNA indicates the presence of viable cells has been questioned *(189),* but the technique has been used to detect viable pathogens in beef *(124)* and *Map* in enrichment cultures *(137)*.

Mycobacterium tuberculosis in clinical samples can be rapidly detected by treating sample preparations with a broad spectrum mycophage to infect viable cells *(147)*. Phage particles that have not infected cells are inactivated, and the preparation is mixed with a suspension of a quickly growing mycobacterium and plated to obtain a mycobacterial lawn. Infected cells from the sample lyse to release phages, which give rise to plaques in the mycobacterial lawn. The identities of the organisms that initiated plaque formation can be determined by PCR testing of agar plugs excised from plaques. This method has been applied for detection of viable *Map* in raw, naturally contaminated milk *(183)* and in inoculated milk subjected to pasteurization treatments *(63)*. In a study in which milk was tested by culture, a quantitative PCR assay, and the phage method, 29 and 22% of the samples of raw cow's milk were positive for *Map* by PCR and the phage method, respectively, but $<1\%$ of the samples were positive by culture *(19)*. Thus, the phage method seems be an effective means of rapidly determining the presence of viable *Map* in foods.

Confirmed detection of *Map*. The examination of foods for viable *Map* would be greatly facilitated if it were established that viable *Map* can be reliably detected by rapid PCR-based methods, but both the PCR and phage methods have been only sparingly used for that purpose, probably because of their relative novelty. Thus, for findings reported in the literature to date, the detection of *Map* can be considered as confirmed by isolation of slowly growing, mycobactin-dependant acid-fast bacilli that are positive for IS*900* and/or F57. When detection does not involve isolation of organisms and identification of all the distinguishing characteristics in isolates, the possibility of false-positive *Map* results must be considered.

MILK AND DAIRY PRODUCTS

***Map* in raw milk.** *Map* can infect lymph nodes associated with the mammary gland and be shed in colostrum and milk from asymptomatic animals and those with clinical signs *(67, 187, 193)*. Shedding of *Map* in milk is apparently intermittent *(138)* as is shedding of the organism in feces *(214)*. During the milking of both infected and uninfected animals in herds that have some infected animals, milk may sometimes be contaminated with fecal material that could contain *Map (145)*. The presence of *Map* in raw milk from infected herds would then be expected.

Data on *Map* in raw cow's milk are available for milk from *Map*-infected animals, milk from individual animals not identified as being infected by *Map,* and milk from bulk containers at farms or milk processing plants (Table 1). In three studies of milk from *Map*-positive animals, *Map* was recovered from $\geq 12\%$ of the tested samples *(67, 68, 193)*. *Map* was recovered from similarly large fractions of samples from individual animals in India that were not known to be infected with *Map (169)* and from bulk containers of milk in Austria and Mexico *(60, 127)*. In other studies, *Map* was recovered from $\leq 2\%$ of samples from individual animals or bulk containers *(80, 143, 146, 172, 174, 195)*. The recovery of *Map* from large proportions of milk samples from infected animals would be expected, and *Map* is apparently prevalent among cattle in India and Mexico. However, the recovery of *Map* from a large proportion of samples from Austrian bulk milk is difficult to explain because the prevalence of *Map* in Austrian dairy cattle is apparently no greater than that in dairy cattle from

TABLE 1. *Surveys of raw cow's milk for the presence of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*)*

Source of samples[a]	Country	No. of samples[b]	Type of test[c]	Positive samples		Detection limit (CFU/ml)	Reference
				No.	%		
Map-positive animals	United States	77	Culture	9	12	ND[d]	*193*
	Denmark	11	Culture	5	45	<100	*68*
			PCR	2	12	>100	
	Canada	134	Culture	46	34	ND	*67*
			PCR	38	28	2	
			N-PCR	72	54	ND	
Animals	Poland	87	Culture	2	<2	ND	*195*
			PCR	18	21	ND	
	Brazil	222	PCR	8	4	ND	*26*
	India	16[e]	Culture	7	44	ND	*169*
			PCR	1	6	ND	
Bulk milk containers	United Kingdom	244	Culture	4	2	0.08	*80*
			IMS-PCR	19	8	0.02	
	Ireland	310	Culture	0		ND	*143*
	Ireland	389	Culture	1	<1	ND	*146*
			IMS-PCR	50	13	ND	
	Switzerland	100	PCR[f]	3	3	<100	*18*
	Austria	243	Culture	38	16	ND	*127*
			IMS-PCR[g]	47	19	<20	
	Iran	110	N-PCR	12	11	ND	*87*
	Denmark	143	PCR	19	13	5	*94*
	Czech Republic	251	Culture	5	2	ND	*172*
			PCR	85	34	ND	
	Brazil	16	PCR	0		ND	*26*
	Cyprus	220	Culture	0		ND	*174*
			PCR	63[h]	29	5	
	Mexico	14	Culture	10	71	ND	*60*
			PCR	14	100	ND	

[a] Samples were from single animals or bulk containers of milk at farms or milk processing plants.
[b] Each sample was subjected to all tests listed for the survey.
[c] Tests were culture of decontaminated samples on solid or in liquid media (culture), or detection of *Map* DNA by a PCR or nested PCR (N-PCR) procedure, without or with collection of *Map* by immunomagnetic separation (IMS). PCR tests were for IS*900* unless indicated otherwise.
[d] ND, no detection limit was identified.
[e] Six samples possibly were from more than one animal.
[f] PCR tests were for F57.
[g] PCR tests were part of a commercial *Map* detection system with no specified PCR target.
[h] Number of samples positive for IS*900*; 14 of the samples were positive for F57 as well.

other European countries where bulk milk was tested for *Map (51)*.

In studies in which *Map* was recovered from ≥16% of cow's milk samples, the proportion of tested samples that were *Map* positive by PCR assay, with or without immunomagnetic separation of *Map* from samples, was similar to the proportion of samples that were *Map* positive by culture (Table 1). Similar results from culture and PCR assay are expected when the numbers of *Map* cells in samples are high. In two studies, fewer samples were *Map* positive by PCR assay than by culture *(68, 169),* which suggests that the PCR procedures in those studies were problematic. In all studies in which the proportion of culture-positive samples was low or none were found and samples were also tested by PCR assay, the proportion of PCR-positive samples was much higher or substantial. No study produced negative *Map* results for all samples tested by some PCR procedure.

In studies of *Map* in sheep's and/or goat's milk, *Map* was recovered in only one study, and then from only one sample (Table 2). *Map* was detected in few samples of such milks that were tested by immunomagnetic separation PCR assay. In contrast, *Map* was detected in large proportions of samples of sheep's and/or goat's milk from Switzerland and Italy that were tested by nested PCR procedures, and three samples of bulk goat's milk from Mexico were positive by PCR *(60)*. The general failure to recover *Map* from sheep's and goat's milk might be due in part to those animals being infected by S (or type I) strains of the organism, which grow very slowly and so are more difficult to recover. The large proportion of *Map*-positive samples of sheep's and goat's milk in Switzerland and Italy also might reflect a high incidences of *Map*-infected animals in these countries and the high sensitivity of nested PCR tests. However, the reported findings suggest that, in at least some regions,

TABLE 2. *Surveys of raw goat's and sheep's milk for the presence of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*)*

Milk type	Source of samples[a]	Country	No. of samples[b]	Type of test[c]	Positive samples		Detection limit (CFU/ml)	Reference
					No.	%		
Goat	Animals	Norway	340	Culture	0		10	*43*
				IMS-PCR	5	>1	1	
		India	20[d]	Culture	1	5	ND[e]	*171*
	Bulk containers	United Kingdom	90	Culture	0		ND	*82*
				IMS-PCR	1	>1	ND	
		Cyprus	13	Culture	0		ND	*19*
				PCR	0		ND	
		Mexico	3	Culture	0		ND	*60*
				PCR	3	100	ND	
Sheep	Animals	Switzerland	344	N-PCR	79	23	ND	*135*
		Australia	76	Culture	0		ND	*110*
	Bulk containers	United Kingdom	14	Culture	0		ND	*82*
				IMS-PCR	0		ND	
		Cyprus	5	Culture	0		ND	*19*
				PCR	1	20	ND	
		Switzerland	63	N-PCR	15	24	ND	*135*
Sheep and goat	Animals	Italy	145[f]	N-PCR	28	19	ND	*140*
	Bulk containers	Cyprus	19	Culture	0		ND	*19*
				PCR	1	5	ND	

[a] Samples were from single animals or from bulk containers of milk at farms.
[b] Each sample was subjected to both tests where two tests are listed for a survey.
[c] Tests were culture of decontaminated or not decontaminated samples on solid or in liquid media or detection of *Map* DNA by a PCR or nested PCR (N-PCR) procedure, without or with collection of *Map* by immunomagnetic separation (IMS). All PCR tests were for IS*900*.
[d] Two samples from each of 10 animals.
[e] ND, no detection limit was identified.
[f] Five samples from each of 29 animals.

sheep's and goat's milk is generally less contaminated with *Map* than is cow's milk.

The findings of a recent study suggest that other species of mycobacteria, which may also be present in milk, could give rise to erroneous results for samples of milk tested for *Map*. Isolates of the quickly growing *Mycobacterium porcinum* were obtained from 8% of bulk milk samples from 631 dairy cow herds in northern Italy *(196)*. The isolates carried an IS*900*-like sequence that gave a positive result with the commonly used IS*900* primer pair p90/91 *(129)*. When *M. porcinum* was spotted onto slants of Herrold's egg yolk agar that had been spread with *Map* cultures, growth of *Map* was totally inhibited. Clearly, raw milk can be contaminated with *Map*; but the presence of mycobacteria other than *Map* in milk samples can produce false-positive PCR results or false-negative results for recovery of *Map,* and these possibilities must be considered when assessing reports of the presence of *Map* in milk. Further investigation of how tests for *Map* in milk might be affected by the presence of other mycobacteria is obviously required.

***Map* in pasteurized milk.** The findings for raw milks indicate that generally very few samples of bulk raw milk are likely to be *Map* positive by cultivation. That must suggest that *Map* would likely be recovered rarely if at all from pasteurized milk. While that was so in three studies of pasteurized cows' milk on retail sale, in four other such studies the fractions of samples from which *Map* were recovered were similar to or much greater than the fractions of bulk raw milk samples from which *Map* were generally recovered (Table 3). When samples of pasteurized milk were tested by both culture and PCR, *Map* were detected by PCR in substantial fractions of samples even when none were recovered from any sample by culture.

PCR procedures will detect DNA from inactivated as well from viable cells. Pasteurization could then be expected to have no effect on the prevalence of *Map* DNA in milk. Consequently the prevalence of *Map* DNA in bulk raw milk and pasteurized milk prepared from it should be much the same. The results reported for surveys of *Map* in pasteurized milk offered for retail sale seem agreeable with that expectation. However, the cultivation of *Map* from some samples of pasteurized milk suggests that the organism can survive some commercial pasteurizing treatments. In two studies the fractions of samples of pasteurized milk from which *Map* were recovered were not much less than the fractions of such samples among samples of bulk raw milk from countries other than Austria (Tables 1 and 3). In two other studies, in the United Kingdom and India, *Map* were recovered from large fractions of pasteurized milk samples *(128, 169)*. The findings of the Indian study might be explained by ineffective pasteurizing of heavily contaminated bulk raw milk. Such an explanation for the findings of the United Kingdom study seems untenable, given the low levels of *Map* contamination of United Kingdom bulk raw

TABLE 3. *Surveys of pasteurized cow's milk from retail containers from which samples were tested for the presence of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*)*

Country	No. of samples collected	Type of test[a]	No. of samples tested	Positive samples		Detection limit (CFU/ml)	Reference
				No.	%		
United Kingdom	312	Culture	54	15	28[b]	ND[c]	*128*
		PCR	312	22	7	<300	
United Kingdom	567	Culture	567	10	<2	0.08	*80*
		PCR	567	67	12	0.02	
Ireland	77	Culture	77	0		ND	*143*
Canada	710	Culture	244	0		ND	*65*
		N-PCR	710	110	15	ND	
Ireland	357	Culture	357	0		ND	*146*
		PCR	357	35	10	ND	
United States	720	Culture	720	20	3	>1	*53*
India	27	Culture	27	18	67	ND	*169*
		PCR	27	9	33	ND	

[a] Tests were culture of decontaminated or not decontaminated samples on solid or in liquid media or detection of *Map* DNA by a PCR or nested PCR (N-PCR) procedure. All PCR tests were for IS*900*.
[b] Eighteen PCR-positive and 36 PCR-negative samples were tested; 50% of these positive and 17% of these negative samples were culture positive for *Map*.
[c] ND, no detection limit was identified.

milk *(80)*. However, the various findings of viable *Map* in pasteurized milk would seem to indicate that at least some commercial pasteurization treatments are ineffective for inactivating *Map* in milk.

***Map* in dairy products other than milk.** In developed countries, most cheese is made from pasteurized milk. However, many types of cheese continue to be made from raw milk or milk that has been subjected to heating treatments less severe than those used for pasteurization *(113)*. *Map* obviously may be present in raw milk and can survive subpasteurization heating treatments *(181)*; it also may be present in pasteurized milk. The microbiological safety of cheese is assured by salting of the curd and the growth of inoculated or naturally occurring lactic acid bacteria, with consequent production of organic acids and decline of the cheese pH during maturation under controlled conditions *(11)*.

The *D*-value is the time required for a 1-log reduction in the population of a specified bacterium under specified inactivation conditions. Four studies of the inactivation of *Map* inoculated into cheeses have been reported. The *D*-values for the various strains of *Map* used in three of the studies under ripening conditions that differed for each cheese ranged from 28 to 107 days (Table 4). The highest *D*-values were obtained for the cheese with the lowest pH. That might not be expected, because *D*-values for two of the strains in acetate buffer decreased with decreasing pH *(191)*. The *D*-value for the one of those strains that was inoculated into soft cheese was 37 days when the culture was heated to 62°C for 4 min before it was inoculated into the cheese. The *D*-value was 60 days when the culture was not heated. The authors therefore suggested that when cheeses were made from heated but not pasteurized milk and ripened for 60 days or more, as is the usual and often mandated practice, the populations of *Map* in the cheese would be reduced by about 3 log units. The findings of other workers also indicate that ≥2-log reductions in *Map* numbers would occur during ripening of cheese *(178)*. However, Cirone et al. *(31)* observed only about 1-log reductions in *Map* during maturation of cow's and goat's milk cheeses for 60 days. Donaghy et al. *(46)* found that at least some strains of *Map* tended to concentrate in the curd when it was formed. Because of that concentration and the slow decline in *Map* numbers observed in the study, these authors suggested that mild cheddar cheese, which is ripened for 112 days or less, might pose a relatively high risk of consumer exposure to *Map*.

Seven surveys of *Map* in cheeses or other milk products have been reported (Table 5). Hruska et al. *(97)* detected *Map* DNA from presumably inactivated *Map* in about half of 51 samples of dried milk foods for infants from seven European Union (EU) countries. A similarly high prevalence of *Map* DNA was found for Greek cheese (feta) made from mixtures of sheep's and goat's milk *(99)*. The prevalence of *Map* DNA in Cyprus cheeses made from cow's, sheep's, and goat's milk was 25% *(19)*. However, the prevalence of *Map* DNA in cheeses or curd made from

TABLE 4. *Reported inactivation of* Mycobacterium avium *subsp.* paratuberculosis *during ripening of inoculated cheeses*

Cheese type	Ripening		pH	*D*-value(s)	Reference
	Time (days)	Temp (°C)			
Soft	28	4	6.2	60	*191*
Hard	120	12, 22[a]	5.7	28	*178*
Semihard	120	15	5.7	46	*178*
Cheddar	189	10	5.2	107, 96, 90[b]	*46*

[a] Cheese was ripened at 12°C for 10 days, 22°C for 60 days, and then 12°C for 50 days.
[b] *D*-values are for three strains of *Map*.

TABLE 5. *Reports of the detection of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*) in dairy products other than milk by PCR or culture*

Product	Country	No. of samples	Type of test	Positive samples		Detection limit (CFU/g)	Reference
				No.	%		
Infant food formula	EU	51	PCR-IS*900*	25	49	ND[a]	*97*
			PCR-F57	18	35	ND	
Curd	United States	98	PCR-IS*900*	5[b]	5	ND	*32*
			Culture[c]	1[d]	2	ND	
Cheese	Greece	42	PCR-IS*900*	21	50	ND	*99*
			Culture[c]	2	5	ND	
	Czech Republic	42	PCR-IS*900*	5	12	ND	*99*
			Culture[c]	1[d]	2	ND	
	Cyprus	28	PCR-IS*900*	7	25	ND	*19*
			Culture	0	0	ND	
	Scotland, United Kingdom	28	Culture	7	25	ND	*224*
	Switzerland	143	PCR-F57	6	4	ND	*185*
			IMS-culture[e]	1[d]	1	40	
Various[f]	India	9	Culture	5	56	ND	*169*
			PCR-IS*900*	7	78	ND	

[a] ND, no detection limit was identified.
[b] Samples also were positive for *hspX*.
[c] Samples were decontaminated and inoculated onto agar slants.
[d] *Map* was not isolated.
[e] *Map* cells were collected by immunomagnetic separation (IMS) and inoculated into liquid media.
[f] Included ice cream and flavored milk.

cow's milk in the Czech Republic, the United States, and Switzerland was ≤12% *(32, 99, 185)*. Treatment and processing procedures for the milk used for the production of the cheeses and curds examined in the Czech Republic and the United States were not reported, but the milk used for the U.S. products probably were pasteurized. The Swiss cheeses were all prepared from raw milk.

Map DNA or acid-fast cells were detected in material obtained from inoculated slants of one or two *Map*-selective media in three of four studies with cheeses and in one study with curd. However, no *Map* isolates were obtained in any of the studies. Consequently, the Swiss and U.S. researchers concluded that the presence of viable *Map* in the cheeses they studied had not been established *(32, 185)*. Whether viable *Map* was detected in the products examined by other researchers also must be questioned. The low prevalence of *Map* DNA in most of cheeses and curds examined seems to be compatible with the generally low prevalence reported for *Map* in bulk milk. The high prevalence of *Map* in Greek and Cyprus cheeses might be due to high prevalence of *Map* in livestock providing milk for localized cheese production *(99)*. However, the findings for Greek and Cyprus cheeses obviously contrast with the low prevalences of *Map* found for sheep's and goat's milk in other countries (Table 2). The high reported prevalence of viable *Map* in Scottish cheeses made from pasteurized cow's milk as well as from raw cow's and sheep's milk *(224)* seems contrary to the various findings for United Kingdom raw and pasteurized milk. Similarly, the very high prevalence reported for *Map* DNA in infant foods manufactured in the EU *(97)* seems to be at variance with the generally low prevalences found for *Map* in EU bulk milks, even when the concentrating effects of drying processes are taken into account. The finding of *Map* in most of a few samples of Indian milk products is, however, agreeable with the apparently common presence of the organism in Indian pasteurized milk *(169)*.

Effects of pasteurizing treatments on *Map* in milk. The likelihood of *Map* being at least occasionally present in raw milk prompted investigations of the efficacies of pasteurizing treatments for inactivating *Map* in milk. In most such studies, the effects of various heating treatments on several strains of *Map* inoculated alone into cow's milk were investigated. The levels of inoculation ranged from <2 to >8 log CFU/ml, and in most of the studies *Map* strains were inoculated at two or more levels. Feces from infected animals were used as the inoculum in one study *(156)*. In another, pasteurization of naturally contaminated milk was investigated *(81)*.

Because heating to pasteurizing temperatures should inactivate at least some *Map* cells, detection of *Map* DNA in pasteurized milk is obviously not useful for determining the effects of a treatment. Therefore, in several, mostly earlier studies *Map* was recovered from pasteurized milk by spreading undiluted and diluted samples of milk on selective agars. Detection levels with such methods are necessarily ≥1 CFU/ml. In most later studies, 50- or 100-ml samples of milk were centrifuged to pellet cells. In some studies, the resuspended pellet was decontaminated with cetylpyridinium chloride, and the preparation was centrifuged again to obtain a pellet. In other studies, a final pellet was obtained from both the pellet and the cream layer formed during centrifugation of a sample. Whatever procedure was adopted, the final pellets were resuspended in small volumes

TABLE 6. *Laboratory studies of pasteurization inactivation of* Mycobacterium avium *subsp*. paratuberculosis *(*Map*) inoculated into milk*

Equipment	Treatment		Level of detection (CFU/ml)	Maximum inoculum (log CFU/ml)	No. of samples	*Map*-positive samples		Minimum reduction (log CFU)	Reference
	Temp (°C)	Time				No.	%		
Test tubes	63	30 min	2	5	36	36	100	<1	*30*
	72	15 s			36	36	100	<1	
Test tubes	63.5	30 min	2	7	66	63	95	2	*76*
	71.7	15 s			66	60	91	2	
Test tubes	65–76	30 min	5	8	16	5	31	2	*182*
Laboratory pasteurizer	65–75	15 s		6	12	0		>6	
Capillary tubes	63	30 min	4	5	30	0		>5	*105*
	72	15 s			30	0		>5	
Laboratory pasteurizer	72	15 s	0.04	3	117	7	6	<1	*77*
Laboratory	≤90	15 s	2	6	54	18	33	6	*79*
pasteurizer	72	25 s			9	0		>6	
Test tubes	63	30 min	3	7	7	0		7	*65*
	72	15 s			11	2	18	5	
Laboratory	63	30 min	0.04[a]	8	36	4	11	4	*181*
pasteurizers	66	16 s			36	34	94	1	
	72–74	15 s			108	8	7	4	

[a] Level determined experimentally was <10 CFU/ml.

of diluent, and portions of each suspension were spread on selective agars in up to five tubes and/or inoculated into liquid media. With such procedures, *Map* could in most instances be detected, at least in principle, at levels between about 1 CFU/10 ml and 1 CFU/100 ml of milk. However, actual levels of detection usually were somewhat higher. Thus, in a study in which the level of detection was in principle less than 1 CFU/10 ml, the level determined experimentally was more than 1 CFU/ml *(181)*.

Three early laboratory studies were carried out with inoculated milk in test tubes. In those studies, the milk was subjected to heating treatments similar to those used for pasteurizing in holding tanks (holder pasteurizing) (e.g., 63°C for 30 min) or for high-temperature short-time (HTST) pasteurizing (e.g., 72°C for 15 s) (Table 6). The findings of those studies indicated that the reductions in *Map* numbers achieved with either type of treatment could be ≤2 log units. Consequently, in two of those studies *Map* was recovered from most or all samples of pasteurized milk *(30, 76)*. In the third study, holder pasteurization in test tubes at temperatures up to 76°C was ineffective. However, HTST pasteurization in a laboratory pasteurizer, with treatments as mild as 65°C for 15 s, resulted in >6-log reductions *(182)*. Subsequent studies with milk heated in test tubes, capillary tubes, or laboratory pasteurizers all indicated that both holder and HTST pasteurizing could be effective for inactivating *Map* in milk (Table 6), with minimum reductions for holder pasteurization of 4 to 7 log units *(65, 181)*. Reductions with HTST pasteurization were up to 6 log units *(77–79)*.

In all studies with pilot scale or industrial pasteurizing equipment, the level of detection of *Map* was ≤1 CFU/10 ml of milk (Table 7). In three studies with such equipment, heating at ≥72°C for ≥6 s reduced the populations of *Map* inoculated at ≤5 log CFU/ml to undetectable levels of <1 CFU/40 ml *(117, 152, 156)*. In other studies, *Map* was detected in some samples of milk subject to comparable treatments. In most instances, the surviving *Map* cells were too few to enumerate. In a study with naturally contaminated raw milk in which the number of *Map* cells was very low, *Map* was recovered from some samples of pasteurized milk *(81)*. In one study, increasing the treatment temperature from 72 to 82°C had little effect on *Map* survival, and increasing the treatment time from 15 to 60 s had no effect *(84)*. In another study, surviving *Map* cells were detected in samples regardless of the level of inoculation, the treatment temperature, or the time *(88)*.

The disparate results obtained in various studies are mirrored by the *D*-values that have been reported for *Map* in milk heated to inactivation temperatures (Table 8). The *D*-values obtained in some studies suggest that *Map D*-values at 72°C could be about 10 s. If this were the case, then usual HTST pasteurization would be relatively ineffective for inactivating *Map (105, 190)*. In other studies, *D*-values at 72°C have been estimated at between 1 and 2 s, in which case HTST pasteurization could be expected to reduce *Map* numbers by >7 log units *(152, 156)*. The range of *D*-values reported for *Map* probably reflects both differences in the procedures used for heating samples and differences between *Map* strains in their susceptibility to inactivation by heat. Despite the differences in *D*-values, all estimates of *z*-values (the change in temperature required for a 10-fold change in *D*-value) for *Map* were similar.

Various explanations for the survival of *Map* in milk subjected to pasteurization treatments have been proposed. Hydrophobic cells such as those of *Map* tend to accumulate at liquid surfaces and on the sides of tubes. Thus, in studies in which milk was heated in open tubes, *Map* at the milk surface or in liquid films on the sides of tubes above the level of the water in the bath used for heating the tubes can

TABLE 7. *Studies of inactivation of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*) in milk pasteurized with pilot scale or industrial equipment*

Equipment	Treatment		Level of detection (CFU/ml)	Maximum inoculum (log CFU/ml)	No. of samples	*Map*-positive samples		Minimum reduction (log CFU)	Reference
	Temp (°C)	Time (s)				No.	%		
Pilot scale	63, 66	15	0.025	4	12	12	100	<1	*152*
	69				6	1	17	>4	
	72				6	0		>6	
Industrial	73	15	0.04	NI[a]	72	4	6	ND[b]	*81*
		25			72	6	8		
Pilot scale	68–79	18	0.02	5	282	77	27	<0	*88*
	71–77	18		4	98	50	51	<2	
	72–90	18–60		5	146	146	100	<3	
Pilot scale	65–85	15–60	0.1	5	816	27	3	<2	*84*
Industrial	72–78	15–25	0.004	3	20	3	15	3	*123*
Pilot scale	60–70	6–15	0.023	5	≃108	>90	>83	0	
	72–90				≃24	0		>4	*156*
Pilot scale	72.5	27	0.02	5	16	0		>4	*117*

[a] No inoculum; naturally contaminated milk was used for the study.
[b] ND, not determined.

escape heating to the intended temperature *(116)*. Such inadequate heat treatment of milk in some parts of the equipment also may occur with laboratory pasteurizers. In those processors, milk is heated while stationary in a treatment chamber, but parts of the inlet and outlet tubes remain above the level of the water used for heating *(64)*.

Repeated sampling of open tubes would be highly likely to give erroneous results because of milk splashing onto tube sides above the level of the heating water and carryover on pipette surfaces of organisms that had accumulated at the milk surface *(47, 116)*. Inadequate heating also might occur in pasteurizers in which milk is treated during continuous flow along a length of tubing (the holding tube). This holding tube issue may be particularly problematic when the flow is laminar rather than turbulent *(92)* because with laminar flow the fluid particles that move fastest travel at up to twice the average rate of flow *(163)*. There also will be a range of fluid particle residence times when turbulent flow is maintained within the holding tube *(202)*. However, in most reported studies with pilot scale or industrial pasteurizers, the operating conditions were arranged to ensure both turbulent flow of the milk and treatment of the fastest moving particles for the specified time. Such conditions are mandated for industrial HTST pasteurization of milk *(205)*.

Some researchers have suggested that apparent or actual heat resistance of *Map* might result from the clumping of cells; each clump of cells would be detected as a single CFU. In principle, even if all but one of the cells in a clump were inactivated, the clump could still give rise to a colony. Thus, the presence of large clumps in a suspension could result in tailing of the *Map* inactivation curve *(106)*. Tailing as a result of clumping was found or suggested in several studies *(76, 79, 84, 88)*. Some cells within a clump also might be protected from heat inactivation. Slowing of the heating of cells within clumps could be trivial *(39)*. However, bacteria are more resistant to dry than to wet heat, and *Map* within a clump might then be protected from heat inactivation if the clump provided a nonaqueous environment for some cells. Cells entrained within globules of milk fat also might be protected from heat inactivation *(118, 167)*. The role of cell clumps in the apparent or actual protection of *Map* from heat inactivation has been investigated in several studies. In some studies, treatments of suspensions (e.g., homogenization) to reduce clumping enhanced heat sensitivity *(81, 84, 162)*. In other studies, such treatments were without effect *(105, 156, 190)*. The presence of *Map* within macrophages that were inoculated into milk also had no effect on the ability of *Map* to survive pasteurization *(88, 179)*.

Lund et al. *(116)* reviewed the information available in 2001 that related to the possible survival of pasteurization treatments by *Map* in milk. These authors concluded that findings of similar small numbers of *Map* surviving pasteurization regardless of the initial numbers present in the milk could be artifactual. In addition, they suggested that the presence of *Map* in pasteurized milk could be due to inadequate holding during pasteurization, leaks in valves or

TABLE 8. *Ranges of the decimal reduction times (*D*-values) at pasteurization temperatures that have been determined for* Mycobacterium avium *subsp.* paratuberculosis *in milk*

Temp (°C)	*D*-value (s)[a]	Reference(s)
60	846–6,492	*105*
62	112–309	*190*
63	12–174	*63, 105, 152*
65	20–71	*117, 190*
66	6–7	*152*
68	9–24	*63, 190*
69	4	*152*
71	10–13	*190*
72	1–4	*63, 152, 156*

[a] Temperature changes required for a 10-fold change in the *D*-value (the *z*-value) have been reported as 6.6 to 8.8°C *(152, 156, 190)*.

TABLE 9. *Reports of* Mycobacterium avium *subsp.* paratuberculosis *(*Map*) recovered from or detected in swab samples of the surfaces of cattle carcasses*

Carcass type	Stage of processing	No. of carcasses	Type of test[a]	*Map*-positive samples		Reference
				No.	%	
Culled cattle	After skinning	150	N-PCR	65	43	*126*
	After dressing	150	N-PCR	56	37	
Fed cattle	After skinning	100	N-PCR	32	32	
	After dressing	100	N-PCR	23	23	
Culled cattle	Before skinning	343	PCR	273	80	*210*
	After skinning	338	PCR	117	35	
			Culture	172	51	
	After dressing	302	PCR	30	10	
			Culture	3	1	
Fed cattle	Before skinning	243	PCR	3	1	
	After skinning	245	PCR	3	1	
			Culture	0		
	After dressing	210	PCR	4	2	
			Culture	0		

[a] PCR and nested PCR (N-PCR) procedures targeted IS*900*.

heat exchangers of the pasteurizing equipment, or recontamination of pasteurized milk by *Map* that survived in the dairy plant environment.

Subsequently, Cerf et al. *(28)* reviewed the information on *Map* and milk pasteurization available in 2007. These researchers constructed a model for identification of level of *Map* contamination in raw milk that would be required for *Map* to be present in pasteurized milk at the sample prevalence reported by Ayele et al. *(6),* Ellingson et al. *(53),* and Grant et al. *(80).* Cerf et al. concluded that the numbers of *Map* in raw milk, the prevalence of the organism in livestock, and/or contamination of raw milk with animal feces would have to be improbably high for the reported prevalence in milk samples to be reached. They then suggested that the findings of *Map* in pasteurized milk were due to leaks in pasteurizing equipment, inadequate heating of milk because of fouling of pasteurizer heat exchangers, or contamination of samples during laboratory analysis. The results of various recent studies largely support the contention that current HTST pasteurization procedures for milk are adequate for inactivating *Map* when these procedures are properly used *(117, 123, 156).*

MEAT

***Map* on dressed carcasses.** Animals that carry *Map* and are farmed to provide meat for human consumption include cattle, buffalo, sheep, goats, and deer. The carcasses of these animals are skinned during the carcass dressing process. During dressing, all carcasses that are skinned will inevitably be contaminated with fecal organisms that are transferred from the hide to the meat *(70).* Some carcasses also may be contaminated with feces spilled from the intestine. These fecal organisms include both bacteria shed by the animal and bacteria acquired from other animals, directly or via the pen and transport facilities *(158, 176).* Animals reared specifically for their meat are generally slaughtered while young *(3, 177)* when shedding of *Map* is likely to be limited. In contrast, animals culled from dairy or breeding herds are mostly more than 3 years old *(107, 142),* and some animals culled from *Map*-infected herds may be shedding *Map* in high numbers *(115).* Thus, *Map* should be more prevalent on dressed carcasses from culled animals than on those from younger animals.

The results from two studies in which swab samples from beef carcass at North American meat processing plants were examined for *Map* are summarized in Table 9. In one study, swab samples collected at three plants from the carcasses of older culled cattle and young fed beef cattle after skinning and after completion of the dressing process were tested by PCR procedures for the presence of the IS*900* and F57 sequences *(126).* The IS*900* sequence was detected by nested PCR in large percentages of samples from skinned but not eviscerated carcasses of both older and younger animals. The prevalence of the F57 sequence was much lower. The percentage of samples from dressed and decontaminated carcasses that were positive for IS*900* or F57 were generally smaller. However, at one plant the skinned carcasses of culled cattle provided fewer IS*900*-positive samples than did the dressed carcasses. The higher prevalence of *Map* DNA on the dressed than on the skinned carcasses could have been due to the redistribution of *Map* on carcass surfaces during carcass dressing operations.

When the samples were tested by real-time PCR assay, only 2 of the 500 samples were positive for IS*900,* and none were positive for F57. The researchers concluded that the amount of *Map* DNA present on the carcasses was small and therefore that the number of *Map* cells on the carcasses, viable or otherwise, was also small. Those findings suggested that viable *Map* would be recoverable from carcass surfaces only infrequently.

In the second study, most swab samples from the hides of carcasses of culled cattle were positive for IS*900 (210).* The percentage of IS*900*-positive samples from skinned carcasses was smaller, and the percentage from dressed

carcasses was still smaller. Half or more of the IS*900*-positive samples from skinned or dressed carcasses also were positive for the IS*MAP02* sequence. The skinned and dressed carcasses also were tested for viable *Map* cells. *Map* was recovered from half the skinned but few of the dressed carcasses. Few samples from hides or carcasses from fed cattle, i.e., beef cattle finished in feedlots, were positive for IS*900*. IS*MAP02* was detected in <1% of samples from skinned carcasses and was not detected in samples from dressed carcasses. *Map* was not recovered from the carcasses of fed cattle.

The obvious difference between the two studies in the findings for *Map* DNA on the carcasses of fed cattle might have arisen because of differences between the plants involved in the studies. In the first study, samples were collected at plants that processed culled and fed cattle, whereas in the second study the plants from which samples were collected were reported to process one or the other but not both types of animal. Fed cattle at the plants involved in the first study might have been contaminated by *Map* from plant pens that had held culled cattle. Whatever the reasons for the discrepancies between the findings of the two studies, both sets of findings suggest that decontaminating treatments that are routinely applied to beef carcasses may remove *Map* from carcass surfaces. The findings of the second study indicate that much of the *Map* DNA on dressed carcasses that is detected by PCR could be derived from organisms that were inactivated by pasteurizing and other decontaminating treatments routinely applied to carcasses at most North American beef packing plants *(71)*. Thus, both studies indicate that few viable *Map* are likely to be present on the surfaces of beef carcasses at the end of dressing processes at North American packing plants. As yet there has been no report of beef or other carcasses produced in countries outside North America being examined for *Map*. However, carcass decontaminating treatments are not permitted elsewhere *(16)*. It is then possible that viable *Map* may be more prevalent, and present in higher numbers on dressed carcasses produced in countries outside North America.

***Map* in muscle and organ tissues.** In addition to the possibility of *Map* being present on carcass surfaces, *Map* may be present within muscle and organ tissues used for human food. *Map* has been recovered from or detected in the liver, kidney, spleen, and other organs of fetuses from *Map*-infected cattle *(24, 194)*. Calves infected as fetuses may develop symptoms of Johne's disease only when they are 2 years or older *(120)* as is usual with animals infected after birth. *Map* was recovered from the liver of a calf shortly after experimental infection, but in other experimentally infected animals *Map* was not subsequently recovered from tissues other than gut and its associated lymph nodes *(226)*. However, the organism survives and proliferates within macrophages and can certainly be disseminated to organs and lymph nodes not associated with the gut when the disease is far advanced *(207)*.

The presence of *Map* in tissues other than those of the intestine and its associated lymph nodes was investigated in four studies *(5, 6, 20, 150)* of the prevalence of disseminated *Map* infections among older animals (Table 10). Most of the animals used in these studies were culled from dairy herds known to be infected with *Map*. In all these studies, *Map* was detected by recovery of colonies from tissue homogenates. The findings of the four studies appear to be generally in agreement; *Map* was found particularly in the liver and in at least some samples from most other tissues that were examined. The disseminated *Map* infection of an animal could not be predicted from the presence or lack of clinical signs or from results of testing of blood by ELISA or testing of feces for the presence of viable *Map (5, 20, 41, 150)*.

When all the animals in a closed herd of dairy cattle that had become infected with *Map* were slaughtered, *Map* was recovered from the gut tissues more often than from the feces of these animals *(91)*. The animals with *Map*-positive gut tissues included 7 (16%) of 44 animals that were ≤18 months old and 2 (6%) of 36 fetuses. *Map* was recovered from other than gut tissues from 13 (10%) of all the animals and 1 (3%) of the fetuses.

Map was detected by PCR in the blood of 11% of 262 Indian cattle that were 3 years or older but in only 1 (1%) of 78 sheep *(13)*. In contrast, *Map* was not detected in blood from five cattle with advanced Johne's disease *(137)*. However, Coelho et al. *(35)* found that 19% of 300 pooled samples of blood from groups of five Portuguese sheep were positive for *Map* by PCR.

Few studies have been conducted to examine muscle tissues for the presence of *Map*. Antognoli et al. *(5)* sampled tissues from two muscles of each of 40 culled cows but recovered no *Map* from those samples, despite finding the organism in samples of liver from 17 of these animals. However, Alonso-Hearn et al. *(2)* recovered *Map* from diaphragm muscle obtained before the removal of viscera from 6 of 47 culled cattle and from the blood of 1 of those animals.

Mutharia et al. *(137)* tested for *Map* in raw, frozen, and cooked muscle tissues from five culled dairy cows with advanced Johne's disease. Freezing appeared to have little effect upon the recovery of viable *Map*. With a level of detection of 40 CFU/g, *Map* was recovered from 1 of 50 samples of raw muscle tissue. The organism was detected by PCR in enrichment cultures that were positive for growth in 6 of the 50 samples of raw muscle tissues, 1 of 15 samples cooked to 61°C, and 1 of 40 samples cooked to ≥70°C. *Map* was recovered from samples of chopped mesenteric lymph nodes from the infected animals. These samples included some that had been cooked within hamburger patties. These studies with lymph node tissues that contained *Map* at $>10^3$ CFU/g indicated that some *Map* cells may survive usual cooking to temperatures generally regarded as adequate for assuring the microbiological safety of meat *(204)*.

The *D*-values at 70°C and *z*-values for *Map* in meat substrates determined in two studies were >10 s and <6°C *(155)* and <2 s and about 4°C *(219)*, respectively. Despite those differences, the findings from both studies indicate that *Map* would be unlikely to survive in meat cooked to a

TABLE 10. *Reports of* Mycobacterium avium *subsp*. paratuberculosis *(*Map*) recovered from or detected in tissues other than gut or associated lymph nodes from commercial animals that provide meat for human consumption*

Animal type	No. of animals	Type of test[a]	No. (%) of animals with *Map*-positive tissues						Reference
			Blood	Abdominal organs[b]	Thoracic organs[c]	Reproductive organs[d]	Lymph nodes[e]	Muscle	
Culled cows	132	Culture	NT[f]	32 (24)	NT	NT	39 (30)	NT	*150*
Culled bulls	4	Culture	NT	1 (25)	NT	4 (100)	1 (25)	NT	*6*
Culled cows	40	Culture	NT	17 (43)	4 (10)	6 (15)	8 (20)	0	*5*[g]
Culled cows	21	Culture	NT	8 (38)	14 (67)	8 (38)	16 (76)	NT	*20*
Culled cattle[h]	262	N-PCR	30 (11)	NT	NT	NT	NT	NT	*13*
Culled cattle	47	Culture	1 (2)	NT	NT	NT	NT	6 (13)	*2*
Map-positive cows	5	Culture[i]	0	3 (60)	NT	1 (20)	1 (20)	1 (20)	*137*
		PCR[j]	0	4 (80)	NT	0	0	4 (80)	
Cow herd									*91*
Fetus	36	Culture	NT	1 (3)	0	0	0	NT	
≤1.5 yr	44	Culture	NT	0	0	1 (2)	0	NT	
1.6–3.0 yr	30	Culture	NT	2 (7)	2 (7)	1 (3)	1 (3)	NT	
3.1–8.0 yr	45	Culture	NT	1 (2)	0	2 (2)	1 (2)	NT	
>8.0 yr	12	Culture	NT	0	1 (8)	0	0	NT	
Fed cattle	14	Culture	NT	5 (36)	NT	NT	0	NT	*21*
Sheep	78	N-PCR	1 (1)	NT	NT	NT	NT	NT	*13*
Sheep	1,500[k]	PCR	≥56 (≥4)[l]	NT	NT	NT	NT	NT	*35*

[a] All PCR and nested PCR (N-PCR) procedures targeted IS*900*.
[b] Liver, kidney, and/or spleen and associated lymph nodes.
[c] Heart and/or lungs and associated lymph nodes.
[d] Mammary glands and testes and associated lymph nodes.
[e] Lymph nodes of the head, neck, shoulder, thigh, etc.
[f] NT, tissue was not tested.
[g] These findings are also reported elsewhere *(41)*.
[h] Cattle were 3 years or older.
[i] IS*900* was detected in growth medium inoculated with sampled tissues.
[j] *Map* was recovered from IS*900*-positive growth medium that had been inoculated with muscle tissue samples.
[k] Samples of blood from five animals were combined for testing.
[l] Fifty-six of 300 composite samples were positive for IS*900*.

well-done condition. The recovery of *Map* from some well-done hamburger patties in the study conducted by Mutharia et al. *(137)* might have resulted from uneven heating of the meat, which can occur during grilling of meat patties *(149)*.

Samples of meat from commercial beef strip loins from young animals and mesenteric lymph nodes from young animals also were tested for *Map (137)*. Enrichment cultures that were not positive for growth were tested by PCR, and 4 of 12 of these cultures were positive for IS*900*. These findings suggested that small numbers of *Map* cells can be present in lymphatic and muscle tissue of young animals. That hypothesis is supported by the finding of *Map* in liver, spleen, kidney, and intestinal tissue of cattle younger than 2 years old *(21)*. In contrast, Wells et al. *(210)* detected *Map* by PCR for IS*900* in ileocecal lymph node samples from only 1 of 232 fed cattle but in 34% of such samples from 330 culled cows. The results for fed cattle may be due in part to the lower prevalence of *Map* in beef cattle than in dairy cattle, which would make up a substantial portion of the culled animals. However, these finding are not wholly reconcilable with those of various reports that indicate that infection with *Map* is commonly acquired by calves or fetuses or with those of surveys that indicate that the prevalence of *Map* in U.S. beef cattle is about 8% *(95)*.

Map can obviously be present in the intestinal tissues of both young and old animals and in the feces of animals both without and with symptoms of overt disease. Gut tissues that are used for human food, such as beef tripe, sheep stomach, and natural casings, might then be contaminated with large numbers of *Map* cells from feces spilled on the gut collection table or conveyors *(69)* or in lymphatic tissue remaining on casings after processing *(109, 222)*. The presence of *Map* on or in stomach tissues used for food and in natural casings does not seem to have been investigated. Although it is unlikely that *Map* would survive the harsh bleaching and/or prolonged cooking or salt preservation of those foods *(7, 9, 223),* some investigation of *Map* contamination of those tissues is warranted.

Organs of meat animals that are used for food include liver, kidney, and heart; spleen in chopped meat preparations; and udders, testicles, and lungs in some ethnic dishes. All can evidently be contaminated with *Map* in older animals and probably occasionally in younger animals. The *Map* populations in the tissues or associated lymph nodes of

some of these organs may be $\geq 10^2$ CFU/g *(137)*. However, the available information on the *Map* populations in organs and lymphatic tissues is very limited, and no information is available on *Map* in meat products that can contain such tissues.

The few data available on *Map* in muscle tissues indicate that the organism may sometimes be present in small numbers in muscle tissue from young and older animals. This age and tissue distribution obviously requires further investigation, as does the survival of *Map* in cooked, processed, and fermented meats *(55)*. The *Map* populations in meat tissues are likely to be much reduced, if they are not entirely eliminated, by cooking meats to a well-done condition. However, muscle and some organ tissues such as liver are commonly undercooked because many consumers prefer underdone meat *(114),* and some meat may be eaten raw *(48)*. There are no reports of the effects of preservative treatments or fermentation process on the survival of *Map* in meat.

***Map* in retail meat.** Culling of animals infected with *Map* is an essential element of Johne's disease control programs *(49),* and culling of dairy cattle identified as shedding large numbers of *Map* cells is apparently a common practice *(115)*. Because *Map* is not recognized as a human pathogen by regulatory authorities, there are no restrictions on the slaughter of *Map*-infected animals to provide meat for human consumption, although emaciated animals may be excluded. Thus, meat from *Map*-infected animals can enter the human food supply. Meat from animals culled because of this disease probably is relatively common in ground beef. However, only one survey of *Map* in meat presented for retail sale has been reported. In that survey, 200 retail packs of ground beef obtained from three supermarkets were tested for *Map* using a real-time PCR assay with a level of detection of 10 CFU/g *(100)*. No *Map* DNA was detected, although the product was likely to include large and variable portions of meat from culled animals. This finding agrees with the hypothesis that *Map* populations in muscle tissues from older and younger animals are likely to be small.

WATER

In various surveys of water and soil for nontuberculous mycobacteria that are opportunistic pathogens for humans, *M. avium* subsp. *avium* and other organisms of the *M. avium* complex have been recovered from natural waters, potable water systems, hot water systems, and forest, acidic, and potting soils *(57)*. Procedures that would allow the recovery of *Map* were not used in these surveys. Unlike other *M. avium* complex organisms, *Map* probably cannot grow outside a host except when cultivated in the laboratory, but both members of the *M. avium* complex and *Map* can survive ingestion by and may grow within protozoa *(136, 186)*. Protozoa can be found in meat processing plants *(206)* and probably in other food processing plants and in water and soil. *Map* also may be able to grow in biofilms in potable water and hot water systems *(112, 161)*. *M. avium* subsp. *avium* can readily form biofilms in drinking and hot water distribution systems *(58, 59),* and possibly *Map* may sometimes be a component of biofilms in which *M. avium* subsp. *avium* predominates.

Map shed in feces can survive for prolonged periods in soil, water, and sediment, although survival is affected by soil and water composition and is reduced by exposure to sunlight *(42, 216, 217)*. In a study of *Map* in biofilms on water trough materials immersed in trough water that had been inoculated with *Map,* the organisms persisted on the trough materials for the 1-year duration of the study *(38)*. Estimation of *Map* populations from quantitative PCR data indicated that the *Map* population in tank water declined rapidly, the number of cells on some materials declined slowly, but *Map* persisted at constant high populations on concrete and galvanized steel. *Map* has been recovered from and detected in river, lake, and reservoir water and in sediments in the United Kingdom *(153, 211)*. *Map* also has been recovered from water entering a treatment plant and from solids extracted from the water during treatment *(154)*. The survival of *Map* in water treated for drinking and in biofilms in potable water systems is expected because, like other *M. avium* complex organisms, *Map* is relatively resistant to inactivation by chlorine *(184, 212)*. *Map* was detected by quantitative PCR in approximately 90% of both water and biofilm samples from 31 cold water faucets in southwestern Ohio *(12)*. However, *Map* and other mycobacteria tend to be associated with particles in water because of their hydrophobic tendencies and so are removed with particles during water purification *(15, 203)*. This particle attraction and removal could explain, at least in part, findings that seem to disagree with those for the Ohio faucets. An organism initially identified as *M. avium* but subsequently identified as *Map* was isolated from only 1 of 68 potable water systems in the Los Angeles area *(72, 130)*. *Map* also was detected by nested PCR in only 1 of 54 samples of sediment from domestic water tanks in the United Kingdom despite that fact that *Map* was detected in 40 of 70 samples of water entering the treatment plant that supplied water to the tanks *(154)*.

Other data on the presence of *Map* in drinking water appear to be lacking, and the matter obviously requires further investigation. The available data suggest that people may be exposed to *Map* from natural waters, by direct contact or in aerosols formed at water surfaces, or by dust from contaminated soils *(14)*. However, exposure to *Map* in water treated for drinking should be limited as long as the water is not contaminated after treatment.

CONCLUSIONS

False-positive and false-negative results from tests for *Map* in foods often cannot be reliably excluded, and any estimation of *Map* populations in food samples is highly uncertain *(139)*. Based on these considerations and the relatively few and somewhat contradictory findings that have been published, any conclusion about the exposure of consumers to *Map* in foods or drinking water must be tentative. Consumers may certainly be exposed to *Map* from

raw milk and dairy products made from raw milk, inadequately pasteurized milk and milk pasteurized with defective equipment, organ tissues and to a lesser extent muscle tissues from at least older food animals, and perhaps drinking water. However, the *Map* populations to which consumers would commonly be exposed by consumption of water or any of those foods would be small.

The numbers of viable *Map* cells in foods or drinking waters that are likely to be encountered by consumers probably will be reliably determined only when better methods have been developed for enumerating *Map* in complex menstrua, which may often be contaminated with other mycobacteria, including some that are closely related to *Map*. Whether the apparently small populations of *Map* in foods and drinking water will pose health risks to some consumers can be established only when the current uncertainties about the involvement of *Map* in Crohn's disease and/or diabetes and the effects of *Map* infection on apparently healthy people have been resolved.

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