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Detection of Viable *Mycobacterium avium* subsp. *paratuberculosis* in Retail Pasteurized Whole Milk by Two Culture Methods and PCR

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ABSTRACT

Cattle with Johne's disease can shed live *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in their milk, and MAP can survive under simulated commercial pasteurization conditions. In several studies conducted in the United Kingdom and Canada, MAP DNA has been detected in retail pasteurized milk samples; however, in one study in the United Kingdom viable MAP was identified in commercially pasteurized milk. A double-blind study involving two laboratories was undertaken to evaluate retail pasteurized whole milk in the United States. Marshfield Clinic Laboratories used solid culture medium (Herrold's egg yolk agar slants with mycobactin J and amphotericin B, nalidixic acid, and vancomycin), and TREK Diagnostic Systems, Research and Development used liquid culture medium (ESP culture system). Cultures at both laboratories were confirmed by PCR. A total of 702 pints of retail whole milk were purchased in three of the top five milk-producing states (233 from California, 234 from Minnesota, and 235 from Wisconsin) over a 12-month period and were tested for the presence of viable MAP. The criteria used for identifying samples as positive for viable MAP were similar to those followed by most laboratories (positive culture with PCR confirmation). The combined data from the two laboratories revealed the presence of viable MAP in 2.8% of the retail whole milk pints tested. Although the number of samples containing viable MAP was similar among states ($P > 0.05$), there was a seasonal effect on the presence of viable MAP in retail milk ($P = 0.05$). More MAP-positive samples were identified during the third quarter of the year (July through September). Of the 22 brands of retail milk tested, 12 (55%) yielded at least one sample positive for viable MAP.

Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic granulomatous infection of the intestinal tract of wild and domestic ruminants. The symptoms of this disease include diarrhea, reduced milk production, emaciation, and ultimately death in infected animals, and infections result in significant economic losses for individual farms and the dairy industry as a whole. Estimated losses in the United States alone are as high as $250 million annually *(21).* In the National Animal Health Monitoring System '96 study, on approximately 22% of U.S. dairy farms at least 10% of the herd was infected with MAP *(19).*

The traditional ''gold standard'' testing method for Johne's disease (MAP infection) is fecal culture. This culture method is time-consuming, requiring a long incubation period of 8 to more than 16 weeks for bacterial recovery. Because of these difficulties, several PCR tests have been developed to detect MAP *(7, 12).* Although these assays offer the benefits of sensitivity and speed, they cannot distinguish between viable and nonviable MAP cells.

Clinical similarities have been observed between Johne's disease in cows and Crohn's disease in humans, and both viable MAP and MAP genetic material have been found in some patients diagnosed with Crohn's disease *(2, 3, 5, 30).* However, no cause and effect relationship between MAP and Crohn's disease has been defined and no documentation proving zoonotic transmission from a cow to a human has been recorded *(33).* If there is a causal relationship between MAP and Crohn's disease, investigations into possible vectors through which MAP is spread should provide useful information.

Cattle infected with MAP can shed live organisms in their milk *(23, 25, 27, 28).* Some researchers have demonstrated that MAP is not able to survive commercial pasteurization *(23, 24)* or have been critical of the differing pasteurization methodologies used *(15),* whereas other researchers have found that this organism can survive pasteurization under conditions simulating those used in commercial facilities *(4, 9, 10, 16, 26).* In early studies of commercial milk in the United Kingdom and Canada, MAP was detected by PCR assay, although no viable organisms were cultured *(8, 17).* In a recent study in the United Kingdom, viable MAP was found in 1.8% of commercially pasteurized milk samples *(11).*

To our knowledge, retail milk in the United States has not been tested for viable MAP. The present study was designed to evaluate the pasteurized whole milk supply in the United States by testing retail milk purchased in three of

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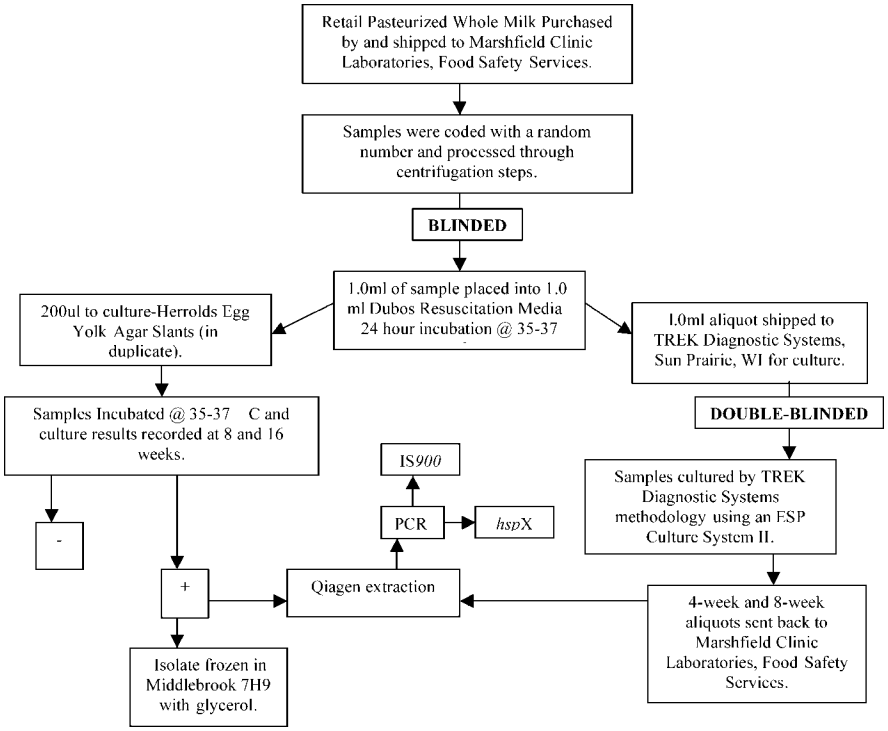

FIGURE 1. *Flow chart of sample routing and coding and where each procedure was performed.*

the top five milk-producing states (California, Minnesota, and Wisconsin) for the presence of viable MAP.

MATERIALS AND METHODS

Sample collection. During a 12-month period from May 2002 through April 2003, 702 pints of retail pasteurized whole milk were tested by two culture methods (solid and liquid media) for viable MAP, and the presence of MAP genetic material was confirmed by PCR assay. Representatives from Marshfield Clinic Laboratories, Food Safety Services purchased pints of whole milk at retail stores each month and shipped them on ice in coolers to Marshfield Clinic Laboratories (Marshfield, Wis.). Ten pints of milk were obtained from both the northern and southern regions of California, Minnesota, and Wisconsin (20 samples per state per month). In the first month of the study, representatives from Marshfield attempted to purchase as many different name brands of milk as possible in each state. Representatives obtained 10 different brands from California, 8 from Minnesota, and 6 from Wisconsin. In subsequent months, replicates of the initial brands were purchased at the same retail stores for the remainder of the study. Upon receipt of the samples at Marshfield, the temperature and condition of each sample was determined. Samples that were damaged or expired or were at temperatures above 10°C were discarded to ensure sample integrity. Samples were stored at 4°C for no longer than 48 h before processing.

Double-blind process. Before processing at Marshfield, all samples were entered into a database containing information such as the brand name, expiration date, and state where the milk was purchased. Each milk sample was then assigned a random number from Research Randomizer (an online random number generator, www.randomizer.org). After the centrifugation and concentration steps and the 24-h resuscitation step, aliquots of each sample were shipped to TREK Diagnostic Systems, Research and Development (Sun Prairie, Wis.). Upon arrival at TREK, all sample aliquots were recoded with a new random number, tested with the ESP Culture System II, and then returned to Marshfield for PCR confirmation. Figure 1 illustrates the routing of samples, when they were blind coded, and what tests were performed at each location. The identity of each sample and the corresponding results were revealed upon completion of all culture and PCR assays.

Sample centrifugation and concentration. The outside of each milk container was decontaminated with 70% ethanol. Using aseptic technique, two 40-ml aliquots of each retail milk sample were placed into 50-ml conical tubes. One milk sample in each processing batch (a processing batch was defined as all the samples from 1 month from one state: approximately 20 pints) was spiked with 500 μl ($>10^6$ organisms) of log-phase MAP (ATCC 19698). This positive control sample was processed in the same manner as all other samples in that batch to ensure that viable MAP could be recovered from positive samples. The 50-ml conical tubes containing milk samples were centrifuged at 14,000 × *g* for 30 min at 4°C. The supernatant was discarded, and the resulting pellet was resuspended in 1 ml of phosphate-buffered saline (PBS), transferred to a 2-ml microcentrifuge tube, and centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in PBS to a final volume of 1 ml. Two aliquots from each pint of retail milk were then combined into a single 2-ml microcentrifuge tube and centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in PBS to a final volume of 1.5 ml. One milliliter of this resuspended sample was placed into 1 ml of Dubos medium containing polymyxin B sulfate, amphotericin B, carbenicillin, and trimethoprim *(13)* and incubated for 24 h at 35 to 37°C for further culture work.

Marshfield Clinic Laboratories culture method. After the 24-h resuscitation in Dubos medium, 200 μl of each sample was inoculated in duplicate onto Herrold's egg yolk agar slants with mycobactin J and amphotericin B, nalidixic acid, and vancomycin (HEYA; Becton Dickinson, Sparks, Md.). One milliliter of each sample was sent to TREK to be tested using their ESP II instrument. The HEYA slants were incubated horizontally with the caps loosened at 35 to 37°C until the surface of the slant was dry (approximately 1 week). The caps were then tightened and slants

were oriented vertically. The inoculated slants were evaluated for typical colonies at 8 and 16 weeks. Positive slants were rinsed with 200 μl of Tris-EDTA buffer (pH 7.5). DNA was extracted from the slant rinse with a QIAamp DNA mini kit (QIAGEN, Valencia, Calif.) according to the manufacturer's instructions, and the extracted DNA was amplified using IS*900 (12, 18)* and *hspX (7, 18)* primer sets.

TREK Diagnostic Systems culture method. All 1-ml sample aliquots were sent overnight delivery to TREK. Upon receipt, they were stored at 2 to 8°C and set up for testing within 24 h. Samples were recoded with a new random number before processing.

The top of each culture bottle was removed and the following were added to 10 ml of ESP culture media: 1.0 ml of ESP para-JEM growth supplement (TREK), 1.0 ml of ESP para-JEM egg yolk supplement (TREK), and 0.5 ml of ESP para-JEM antibiotic supplement (TREK). The culture bottles were then recapped and mixed by inversion. Each 1-ml sample received from Marshfield Clinic Laboratories was inoculated into the supplemented para-JEM culture bottles using a sterile pipette. The final volume of each culture bottle including both the sample and the additives was 13.5 ml. The culture bottles were recapped, wiped with the mycobacterial disinfectant Amphyl (Unisource Packaging Systems, Janesville, Wis.), and vortexed for 10 to 15 s. An ESP II bottle connector was attached to each culture bottle, and the bottles were placed into the ESP Culture System II instrument in test locations specifically configured for the detection of mycobacteria and were identified with the appropriate sample number. All retail milk samples were tested on the same instrument. Upon completion of ESP II testing, the samples were returned to Marshfield for confirmatory PCR testing. At Marshfield, a 200-μl aliquot of every sample was extracted using the QIAamp DNA mini kit. All TREK cultures had confirmatory PCR assays performed on them because samples were still blind coded and there was no way of knowing which samples were considered positive or negative by culture.

Confirmatory PCR. The extracted DNA from HEYA slant rinses and ESP II samples was assayed by PCR for the presence of MAP genetic material using the following primers (Integrated DNA Technologies, Coralville, Iowa): IS*900* forward CCGCTA ATTGAGAGATGCGATTGG and reverse AATCAACTCCAGC AGCGCGGCCTCG and *hspX* forward GACCGGCTATCTGTG GAAC and reverse CTCGTCGGCTTGCACCTG. The IS*900* primers amplify a portion of an insertion sequence found 15 to 20 times throughout the MAP genome *(12, 18).* The *hspX* primers amplify a portion of a heat shock–like protein found exclusively in MAP *(7, 18).* The amplification mixtures contained the following concentrations of reactants: 20 pM each primer, 1.25 U DNA polymerase (Roche Diagnostic Corp., Indianapolis, Ind.), 0.2 mM nucleotides (Roche), and either 2.5 mM Mg^{2+} for IS*900* or 1.5 mM Mg^{2+} for *hspX.* Each set of reactions included a negative control (in which PCR-grade water was substituted for a sample) and a previously extracted MAP DNA (ATCC 19698) positive control. Amplification conditions for the IS*900* primer set were as follows: 10 min at 94°C; 50 cycles of 1 min at 94°C, 15 s at 65°C, and 1 min at 72°C; and a final 10-min extension at 72°C. Amplification conditions for the *hspX* primer set were as follows: 5 min at 94°C; 50 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C; and a final 10-min extension at 72°C. PCR products were analyzed by agarose gel electrophoresis (1.5% agarose). The electrophoresis buffer contained 5 mg/liter ethidium bromide, and gels were examined for bands using a Gel Doc 2000 (Bio-Rad Laboratories, Hercules, Calif.). A sample was considered positive when amplified product was noted at 229 bp for the IS*900* primer set and at 211 bp with the *hspX* primer set. All products were compared with a standard molecular weight ladder and the positive control to determine size.

Classification of results. Samples were considered positive for viable MAP when they were positive based on either of the two testing methodologies performed at the two laboratories. At Marshfield, milk samples were considered positive for viable MAP based on the following two criteria: (i) typical MAP growth was observed on HEYA and (ii) PCR with DNA extracted from the slant rinse from that sample produced amplified products at the correct molecular weights for the IS*900* and *hspX* primer sets. Aliquots of the milk samples cultured at TREK were considered positive for viable MAP based on the following three criteria: (i) detection of a positive signal on the ESP II instrument, (ii) PCR with DNA extracted from para-JEM media for that sample produced amplification products at the correct sizes for the IS*900* and *hspX* primer sets, and (iii) unblinded samples were cross-referenced to those with typical growth on the original HEYA cultures at Marshfield (TREK samples did not require PCR confirmation of HEYA slant rinse because confirmatory PCR assay of the DNA extracted from para-JEM media was already considered).

The technology of the ESP Culture System II is based upon the detection of changes in headspace pressure within a sealed bottle. The ESP II instrument monitors changes in either gas production or gas consumption due to microbial growth. The system detects mycobacterial growth by automatically monitoring (every 24 min) the change in headspace pressure in the liquid culture due to the consumption of oxygen gas and indicates a positive result when the parameters for the internal algorithms are satisfied.

The criteria used for classifying samples as positive or negative for viable MAP are similar to those followed by most laboratories, including the National Veterinary Service Laboratories (Ames, Iowa). Currently, accepted methods for MAP testing typically consist of culture (HEYA, ESP II, etc.) followed by PCR assay with primer sets IS*900,* IS*1311, hspX,* 16S, or others for confirmation.

Sensitivity. The sensitivity of HEYA, as reported by the manufacturer, is <10 organisms, although results may vary depending on factors such as sample processing. In preliminary PCR experiments on known concentrations of MAP (determined by spectrophotometer absorbance readings and HEYA colony counts), the detection limit was 1 organism per reaction with the IS*900* primer set (data not shown) and 20 organisms per reaction with the *hspX* primer set (data not shown). Data regarding the sensitivity of the ESP II were presented at the 2001 American Association of Veterinary Laboratory Diagnostics meeting. The ESP II was reported to be 100% sensitive for detection of MAP in bovine fecal samples in medium to high shedders (>30 CFU) and 94% in low shedders (<30 CFU). The present study is the first in which the ESP II instrument was used to detect MAP in milk; therefore, sensitivity of this method with milk samples is unknown.

Statistical analysis. A chi-square analysis was used to compare positive sample distribution among states and across year quarters.

RESULTS

Retail pasteurized whole milk samples (233 from California, 234 from Minnesota, and 235 from Wisconsin) were tested for viable MAP. DNA specific to MAP (either

TABLE 1. *Distribution of retail pasteurized whole milk containing viable* Mycobacterium avium *subsp.* paratuberculosis *(MAP) by test location*[a]

	No. of milk samples with viable MAP			
Total no. of samples tested	Detected at both MCL and TREK	Detected only at MCL	Detected only at TREK	Total
702	2	6	12	20 (2.8%)

[a] MCL, Marshfield Clinic Laboratories; TREK, TREK Diagnostic Systems Research and Development.

IS*900* or *hspX* genes) was amplified in 452 (64%) of the samples. Of the 702 retail pasteurized milk samples tested for MAP, 20 (2.8%) were positive by culture (HEYA or ESP II) and by confirmatory PCR (HEYA slant rinse or para-JEM media) for both of the genes IS*900* and *hspX*, which confirms the presence of viable MAP (Table 1).

When the results from the two testing locations were compared, Marshfield identified a total of eight (1.1%) samples as positive for viable MAP using HEYA (colony counts of 1 to 10) and confirmatory slant rinse PCR. TREK identified a total of 14 (2.0%) samples as positive for MAP growth, and these samples were subsequently confirmed by PCR amplification of both IS*900* and *hspX* genes and cross-referenced for typical growth on HEYA culture (colony counts of 1 to 10) from the original isolates at Marshfield. Comparing the positive samples from both systems, a total of two (0.3%) samples tested positive for viable MAP at both Marshfield and TREK (Tables 1 and 2). The number of samples containing viable MAP was similar among states ($P > 0.05$) (Table 3). Of the 20 samples found to contain viable MAP, 7 (35%) were from California, 9 (45%) were from Minnesota, and 4 (20%) were from Wisconsin. There was a seasonal effect on the presence of viable MAP found in retail pasteurized whole milk ($P = 0.05$): more MAP-positive samples were identified during the months of July, August, and September (Fig. 2).

Controls. All retail milk aliquots spiked with 500 μl ($>10^6$ organisms) of log phase MAP (ATCC 19698) were positive with both the Marshfield (HEYA culture produced confluent growth in all positive samples) and TREK culture methodologies and were confirmed as positive by PCR assay with both primer sets. The previously extracted MAP DNA (ATCC 19698) PCR-positive control was positive in all sets of reactions with both IS*900* and *hspX* primer sets. The PCR-negative controls (in which PCR-grade water was substituted for a sample) were negative in all sets of reactions with both IS*900* and *hspX* primer sets.

DISCUSSION

Cows diagnosed with Johne's disease can shed MAP in their milk *(23, 25, 27, 28).* In laboratory settings, MAP can survive conditions simulating commercial pasteurization *(4, 9, 10, 16, 26)* when present in concentrations of 10^2 CFU/ml or higher *(8, 9, 11)*. In previous studies, MAP DNA has been identified with PCR amplification of the

TABLE 2. *Culture and PCR results from Marshfield Clinic Laboratories and TREK for retail whole milk samples containing viable* Mycobacterium avium *subsp.* paratuberculosis[a]

Marshfield ID	TREK ID	Positive results[b]	HEYA	ESP II	HEYA rinse PCR		ESP II PCR	
					IS*900*	*hspX*	IS*900*	*hspX*
1545	8549	TREK	+	+	+	−	+	+
2407	4819	TREK	+	+	−	+	+	+
2528	7436	TREK	+	+	+	−	+	+
3148	7332	TREK	+	+	−	+	+	+
4031	4800	TREK	+	+	+	−	+	+
4167	3908	TREK	+	+	+	−	+	+
4453	4304	TREK	+	+	−	+	+	+
4633	6307	TREK	+	+	+	−	+	+
5232	8074	TREK	+	+	+	−	+	+
6211	6614	TREK, MCL	+	+	+	+	+	+
7128	3644	TREK	+	+	+	−	+	+
7830	9226	TREK, MCL	+	+	+	+	+	+
8669	4227	TREK	+	+	+	−	+	+
8789	7575	TREK	+	+	+	−	+	+
1250	7356	MCL	+	−	+	+	+	−
5454	3118	MCL	+	−	+	+	−	−
5699	2559	MCL	+	−	+	+	−	+
6600	6413	MCL	+	−	+	+	−	−
7286	5080	MCL	+	−	+	+	+	+
9278	4552	MCL	+	−	+	+	+	−

[a] HEYA, Herrald egg yolk agar slants with mycobactin J and amphotericin B, nalidixic acid, and vancomycin; ESP II, para-JEM liquid medium on the TREK ESP Culture System II instrument.

[b] TREK, positive result obtained at TREK Diagnostic Systems, Research and Development using the ESP II, confirmatory PCR, and HEYA slant culture; MCL, positive result obtained at Marshfield Clinic Laboratories using HEYA and confirmatory PCR.

TABLE 3. *Distribution of milk samples positive for viable* Mycobacterium avium *subsp.* paratuberculosis *by brand and state*

State/brand no.[a]	No. of positive samples	No. of samples tested
Wisconsin		
1	0	72
2	1	82
3	1	24
4	1	22
5	1	21
6	0	12
Total	4	233
Minnesota		
2	3	23
6	1	25
7	0	24
8	3	69
9	0	21
10	2	58
11	0	9
12	0	5
Total	9	234
California		
13	0	2
14	1	38
15	1	36
16	1	28
17	0	21
18	2	21
19	0	19
20	1	21
21	1	22
22	0	10
Unknown	0	17
Total	7	235

[a] Twelve (55%) of 22 of brands tested yielded at least one positive sample.

IS*900* gene in retail milk in the United Kingdom *(17)* and Canada *(8)*. Grant et al. *(11)* reported that 1.8% of commercially pasteurized milk in the United Kingdom contained viable MAP. To our knowledge, retail pasteurized whole milk in the United States had not been tested. The purpose of this study was to test retail pasteurized whole milk from three of the top five milk-producing states in the United States for viable MAP. Testing at Marshfield revealed that 1.1% of the milk samples contained viable MAP. Using the criteria described here, TREK identified 2.0% of samples tested as positive for viable MAP. In total, 2.8% of samples tested positive for viable MAP at either Marshfield and/or TREK.

TREK has developed a liquid culture method for use with their detection system. Generally, liquid culture media (BACTEC and ESP II) grow mycobacteria better and more rapidly than do solid media *(32)*, which may be why more positive samples were identified by TREK. Another reason for the discrepancy between test locations may be the difference in the amount of sample cultured in each system.

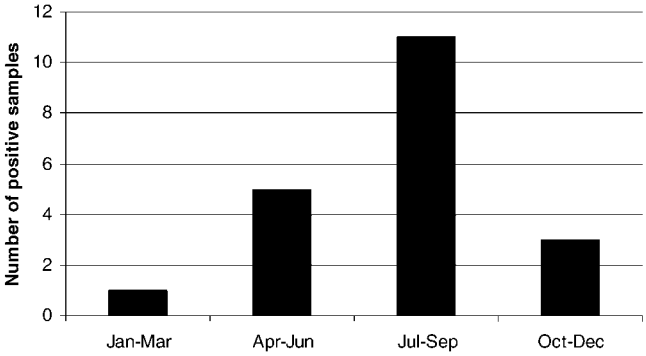

FIGURE 2. *Total number of retail pasteurized whole milk samples that tested positive for viable* Mycobacterium avium *subsp.* paratuberculosis *during each quarter of the collection year. Season significantly affected the occurrence of viable MAP in retail milk (*$P = 0.05$*).*

Each sample (80 ml) was concentrated to 1.5 ml during sample processing. Of the 1.5 ml, 200 μl of each sample was inoculated onto HEYA in duplicate at Marshfield. The remainder of each sample was sent to TREK, and 1 ml of each sample was inoculated into culture on the ESP II instrument. TREK inoculated their cultures with 2.5 times more sample than did Marshfield. If low numbers of MAP were present in the sample, TREK may have had a better chance of detection because of the greater volume used to inoculate the cultures. Mycobacterial cells also have the tendency to clump *(14)*. This unequal distribution of MAP in each sample may explain the inconsistent results for the same sample at the two testing locations. When each sample was split, one laboratory may have received a sample containing a ''clump'' of MAP, and the other laboratory's sample may have contained no MAP. Another explanation of why different samples were identified as positive at TREK and at Marshfield is that the two media used had different growth components and antibiotics and different concentrations of these components. Individual field isolates probably grow better in the presence of some media components compared with others.

Liquid media, such as 7H9, Dubos, MGIT MAP, and para-JEM, are better at supporting the growth of mycobacteria *(32)*, but they also support the growth of unwanted organisms. Although minimal contamination occurred in this study, overgrowth by contaminating organisms in liquid media can present a problem. It is considered good laboratory practice when using a liquid medium to also use traditional solid slants for recovery *(22)*. Because it is impossible to observe typical morphology when using liquid media for growth of mycobacteria, an additional precaution was taken when determining whether TREK culture samples were truly positive. For a TREK culture to be considered positive for viable MAP, the final liquid culture had to test positive for both IS*900* and *hspX* genetic material, and the results had to correspond with positive typical MAP growth on the original solid media (HEYA) at Marshfield after samples were unblinded. When determining whether a TREK sample was positive, only confirmatory PCR results from the para-JEM culture was taken into account; the

PCR results for the HEYA slant rinses were not considered (Table 2).

The percentage of viable MAP present in commercial pasteurized whole milk may have been underestimated in this study because of the requirement of PCR confirmation with two primer sets. The presence of MAP on solid cultures at Marshfield and in liquid cultures at TREK was confirmed using PCR for IS*900* and *hspX*. Some cultures that demonstrated typical MAP growth morphology at Marshfield or a positive signal on the ESP II system may have been falsely reported as negative because PCR analysis revealed the presence of either IS*900* or *hspX* but not both. The detection of only one of the two genetic markers could have resulted from collection of very few organisms during the slant rinsing procedure. The requirement for both IS*900* and *hspX* to be present increases the chances that the DNA was obtained from an intact MAP chromosome rather than fragments but also provides a more conservative estimate of MAP presence.

The presence of MAP genetic material detected with either the IS*900* or *hspX* primer sets in 64% of the retail milk samples was surprising and suggests that viable MAP was at one time present in that milk sample. Thus, in most cases pasteurization was effective at inactivating viable MAP present in the raw milk.

One possible explanation for the presence of viable MAP in retail milk could be postpasteurization contamination. If raw milk containing MAP is brought into a processing plant and sanitation methods are not adequate, MAP could persist in the environment and ultimately contaminate the end product (i.e., pasteurized retail milk). Although no studies have been conducted to investigate the possible survival of MAP in dairy processing plants, studies of soil, grass, water, and sediment indicate that MAP can survive up to 12 months in outside environments *(34, 35)*.

When the number of milk samples containing viable MAP was plotted by the year quarter in which the samples were collected, an increase in the number of milk samples containing viable MAP was noted during the months of July, August, and September. Survival within macrophages is a hallmark of MAP *(6, 29, 31)*. July through September is also the period when national somatic cell counts are highest in U.S. dairy herds *(1, 20)*. The ability of MAP to survive inside macrophages combined with increased shedding of macrophages in the milk could explain the rise in the incidence of viable MAP found in milk during this period.

The goal of this study was to determine whether viable MAP could be isolated from retail pasteurized whole milk in the United States. In this double-blind study conducted at two testing locations with two different culture methods, retail pasteurized whole milk from California, Minnesota, and Wisconsin was tested for viable MAP. The detection of viable MAP in milk was dependent on the culture method used. Viable MAP was detected in more milk samples when using the liquid culture method than when using solid culture method. Overall, based on criteria similar to those used at most laboratories, including the U.S. National Veterinary Service Laboratories, the combined use of both testing methods revealed viable MAP in 2.8% of retail pasteurized whole milk. If a causal relationship between MAP and Crohn's disease is ever established, these findings indicate that retail milk would need to be considered as a transmission vector.

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