

Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in Ileocecal Lymph Nodes and on Hides and Carcasses from Cull Cows and Fed Cattle at Commercial Beef Processing Plants in the United States[†]

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MS 08-471: Received 19 September 2008/Accepted 22 February 2009

ABSTRACT

Clinical associations between Crohn's disease in humans and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) have been suggested but not confirmed. Cattle could be sources for MAP, but little information on MAP prevalence with beef has been reported. Samples of ileocecal lymph nodes and swabs of hides and carcasses from 343 animals at cull cattle slaughtering facilities and 243 animals at fed cattle slaughtering facilities across the United States were analyzed for the presence of MAP. Amplification of genetic sequences detected MAP DNA predominantly on hides and in lymph nodes of samples taken at both types of processing facilities. More than 34% of the cattle at cull cow slaughtering facilities had ileocecal lymph nodes that tested positive for MAP DNA. From these same cattle, hide prevalence was more than twofold greater than the prevalence in ileocecal lymph nodes, suggesting that cross-contamination could be occurring during transport and lairage. The prevalence of MAP DNA decreased during processing, and less than 11% of the carcasses tested positive after interventions in the cull cow processing facilities. Using standard double-decontamination and culture techniques, less than 1% of the postintervention carcasses tested positive for viable MAP at cull cow facilities. In samples from the facilities processing only fed cattle, MAP prevalence of 1% or less was detected for ileocecal lymph node, hide, and carcass samples, and viable MAP was not detected. Based on this study, fed cattle carcasses are unlikely sources of MAP, and carcasses at cull cow plants have only a slight risk for transmitting viable MAP, due to current interventions.

In humans, Crohn's disease was identified as an emerging disease in the 1940s, and the disease has been associated with human populations in developed nations with intensive farming practices (23). In recent years, the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has been implicated as a possible causative agent for Crohn's disease, due to similar etiologies with a disease in ruminants (8, 13, 25). In the ruminant animal, MAP is known to cause Johne's disease, which results in poor animal performance and in extreme weight loss over time. In cattle, Johne's disease is typically associated with intensive management practices where fecal-oral transmission is high (9, 11).

In cattle, MAP infection occurs via ingestion of feces from cattle or free-ranging animals infected and shedding MAP. Incubation of the pathogen can occur asymptotically in the bovine host for 18 to 60 months (9). Johne's disease in dairy cattle decreases milk production, and MAP

has been monitored in these cattle as part of a National Animal Health Monitoring System (NAHMS) study. In the NAHMS 1996 study, MAP infection was present in 10% or more of the animals in approximately one-quarter of the dairy herds tested (34). Contaminated bovine milk has been suggested as a potential route of transmission to humans (14, 31), since MAP has been reported to survive pasteurization (14, 19, 21, 22).

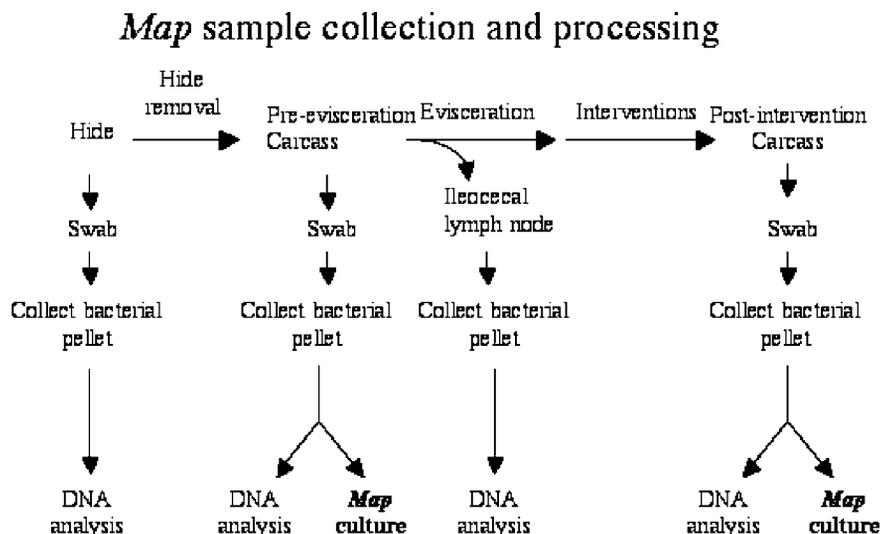
Beef may be an additional source for MAP in humans, but the prevalence of MAP in cattle slaughtered for beef at commercial facilities has not yet been extensively investigated. Culled cows from dairy and beef herds are processed into beef products and may be a source for MAP (12), and based on tests conducted on U.S. farms, the prevalence of MAP infection and Johne's disease in beef herds is less than it is in dairy herds (34). Fed beef cattle feedlots are intensive management systems that concentrate animals from various herds and offer an opportunity for cross-contamination prior to slaughter. In either fed or cull cattle sources, contamination from feces, hide, or carcasses may be potential routes for transmission to beef products. The objective of this study was to determine the baseline prevalence in beef and identify potential sources for MAP. Determining the prevalence of MAP in cattle at slaughter and during processing could establish if and where interventions to decrease MAP transmission need to be applied.

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

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FIGURE 1. Diagram showing animal carcass sample sites during beef processing and the assay systems used for each sample type.



MATERIALS AND METHODS

Sample collection. The schematic of sample collection and processing is presented in Figure 1. Commercial cattle processors of cull cow and/or fed cattle across the United States were visited and samples collected randomly from animals across lots, after stunning. Samples for hide, preevisceration carcasses, post-evisceration carcass, and ileocecal lymph nodes were collected at slaughter from 245 animals on three visits to only fed cattle slaughtering plants and from 341 animals on three visits to cow slaughtering plants. Carcasses were tagged, and samples were taken from the same carcasses through stages in beef processing by using standardized U.S. Meat Animal Research Center (USMARC), Meat Safety and Quality Research Unit techniques (2). Briefly, a hide swab was taken with sterile, premoistened Speci-Sponges (Nasco, Fort Atkinson, WI) on the brisket plate (~1,000-cm² area) from each animal as it was tagged. Preevisceration carcass swabs were taken with two sterile, premoistened sponges (Nasco) from brisket and hindquarters (~8,000-cm² area total) of the tailing side as soon after hide removal as possible and before any interventions. Postintervention (final) carcasses were swabbed again as above from the leading side, using two sterile, premoistened sponges (Nasco). Gastrointestinal tracts were also tagged after evisceration from the tagged carcasses, and ileocecal lymph nodes were removed on the line from cecum–small intestine tissues and bagged separately. In some cases, substitute ileocecal lymph node samples were collected from the next available tract on the line to replace samples lost due to gastrointestinal tract condemnation by inspectors prior to sampling. Due to processing-line speeds and carcass reprocessing, not all samples after hide sampling or tagging were collected from each animal in all cases. All samples were placed in an insulated container containing ice pack and shipped overnight to the USMARC Meat Safety and Quality Research Unit for analyses. Bacteria and bacterial DNA were collected from the samples to determine the presence of MAP.

Sample preparation and analyses. For the hide and carcass swabs, a 10-ml volume of sterile tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) was added to each Speci-Sponge (NASCO) to premoisten before sample collection. After sample collection and shipment to USMARC, bags with sponges were pressed by passage upward through a hand-cranked flat-roller machine (Weston Brand, Cleveland, OH) to remove all liquid from the sponge and allow liquid to collect in the bottom of the col-

lection bag. For each sample type on each collection date, two unused, premoistened sponges were shipped and processed with samples, as negative controls. The liquid volume from each sample was transferred equally between two 15-ml flip-top, conical centrifuge tubes (Nalge Nunc International, Rochester, NY), and bacterial pellets were collected by centrifugation (6,000 × *g* for 15 min at 4°C; model J6-HC, Beckman-Coulter, Fullerton, CA). Pellets from one tube were stored frozen at –20°C for subsequent DNA extraction and analysis. Pellets from the second tube were processed directly for MAP decontamination and enrichment, described below.

For the lymph node samples, the ileocecal lymph nodes were trimmed of fat and intestinal tissue. Trimmed intact nodes were washed by immersion into boiling water for 5 s to surface decontaminate and then placed into a sterile stomacher bag (Nasco). Nodes were crushed with a 2-lb (0.91-kg) rubber mallet and homogenized for 1 min with 10 ml of TSB in a BagMixer 400 laboratory blender (INTERSCIENCE, Hanover, MA). The volume was transferred to sterile 15-ml conical centrifuge tubes (Nalge Nunc International, Rochester, NY), and large tissue debris was removed by low-speed centrifugation (600 × *g* for 5 min; Beckman J6-HC, Beckman Coulter, Palo Alto, CA). The liquid volume was transferred to clean 15-ml tubes, and the bacteria pellet was collected by centrifugation (6,000 × *g* for 15 min at 4°C). Bacterial pellets were stored frozen at –20°C for DNA extraction and analysis.

DNA extraction, amplification, and analysis. Bacterial pellets were thawed on ice, suspended to approximately 180 μl by ultrapure H₂O, and vigorously vortexed for 1 min. A 20-μl volume of proteinase K (1 mg/ml stock) was added to each pellet suspension and incubated at 55°C for 30 min. Total DNA from each hide swab bacterial pellet (approximately 200 μg per pellet) was extracted with the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) per the manufacturer's protocols by using included treatments to remove PCR inhibitors. Total DNA was extracted from each carcass swab bacterial pellet by using the QIAamp DNA Mini Kit (QIAGEN) per the manufacturer's protocols. The filter tubes were processed by using the QIAvac 24 Plus vacuum manifold system, and DNA was eluted from the glass filter columns into sterile microcentrifuge tubes by using 200 μl of sterile ultrapure H₂O. The extracted DNA samples were stored at –20°C for DNA analysis.

Extracted DNA was subjected to amplification using primers

targeting MAP-specific IS900 repeat genome sequences (15). Briefly, each 25- μ l amplification reaction contained 200 nM each IS900 DNA amplification primers (forward, 5'-CCGCTAATTG-AGAGATGCGATTGG-3'; reverse, 5'-AATCAACTCCAGCAG-CGCGGCCTCG-3'), 1.6 mM each deoxynucleoside triphosphate (dNTP), 2.5 mM Mg²⁺, HotStarTaq DNA polymerase buffer and 0.5 U HotStarTaq DNA polymerase (QIAGEN), and sterile ultrapure H₂O. A 5- μ l aliquot from hide samples, a 10- μ l aliquot from carcass samples, and a 2- μ l aliquot from lymph node samples were the volumes of extracted DNA used in the DNA amplification analyses. A positive control (10 ng of MAP ATCC 19698 chromosomal DNA) and a negative control (ultrapure H₂O) were included with each 96-well amplification reaction run. The reactions were amplified with a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA) programmed with an initial denaturing step (10 min at 94°C), 42 amplification cycles (1 min at 94°C, 0.25 min at 65°C, and 2 min 72°C), and a final product extension step (10 min at 72°C), as reported previously (15). Amplified products from the reactions were separated by electrophoresis by 2% agarose gel (Ultrapure Agarose, Invitrogen, Carlsbad, CA) in 89 mM Tris-borate and 2 mM EDTA prepared buffer (pH 8.3; Sigma, St. Louis, MO), stained with ethidium bromide and visualized by UV excitation. Images were captured with the EPI-Chemi Darkroom system (UVP Lab Products, Upland, CA) and NIH Image 1.6 software, and the presence or absence of 229-bp nucleotide product was scored.

MAP enrichment and confirmation. Bacterial pellets from carcass swabs (prepared above) were decontaminated and enriched as described previously (10, 17). Briefly, the bacterial pellets were suspended in 10 ml of 0.7% hexadecylpyridinium chloride (Sigma) and decontaminated for 3 h at room temperature. Decontaminated bacterial pellets were collected by centrifugation (6,000 \times g for 30 min at 20 to 25°C) to remove hexadecylpyridinium chloride. Pellets were suspended in 1 ml of an antibiotic cocktail containing 50 μ g/ml amphotericin-B, 100 μ g/ml naladixic acid, and 100 μ g/ml vancomycin (ANV), and were incubated at 37°C for 22 to 24 h. A 200- μ l aliquot was transferred onto Herrold's egg yolk agar slants with ANV and Mycobactin J (HEYA; BBL, Becton Dickinson, Sparks, MD). The media slants were incubated at 37°C for 8 to 12 weeks until growth appeared. Slant surfaces were scraped, and microbial growth transferred to 200 μ l of sterile H₂O. A 100- μ l volume was removed, and bacterial DNA was extracted with the QIAamp DNA Mini kit, as described above.

Extracted DNA from MAP enrichment tubes (0.5- μ l volumes) and carcass samples (10- μ l volumes) was subjected to amplification by using nested primer sets targeting MAP-specific IS-MAP02 repeat genome sequences (30) for MAP confirmation. Briefly, for the first amplification, each 25- μ l amplification reaction contained 200 nM each ISMAP02 DNA amplification primer (ISMAP02f1, 5'-GCACGGTTTTTCGGATAACGAG-3'; ISMAP02r1, 5'-TCAACTGCGTCACGGTGTCTG-3'), 1.6 mM each dNTP, 2.5 mM Mg²⁺, HotStarTaq DNA polymerase buffer and 0.5 U HotStarTaq DNA polymerase (QIAGEN), and sterile ultrapure H₂O. A positive control (10 ng of MAP ATCC 19698 chromosomal DNA) and a negative control (ultrapure water) were included with each 96-well amplification reaction run. The reactions were amplified by using a PTC-100 programmable thermal controller (MJ Research, Inc.) programmed with an initial denaturing step (10 min at 94°C), 32 amplification cycles (0.75 min at 94°C, 1 min at 58°C, and 2 min 72°C), and a final product extension step (7 min at 72°C). A 1.0- μ l volume of first amplification reaction was subjected to a second reaction (25 μ l total volume) as above except with internal primers (ISMAP02f2, 5'-GGATA-

TABLE 1. Prevalence of *Mycobacterium avium subsp. paratuberculosis*, based on PCR of IS900 genetic sequence in ileocecal lymph node samples and on hides of animals processed at fed cattle-only and cull cow beef processing plants

Type of beef cattle	Ileocecal lymph nodes		Hides	
	No. of samples tested	No. (%) of positive samples	No. of samples tested	No. (%) of positive samples
Fed cattle	232	1 (0.4)	243	3 (1.2)
Cull cow	330	113 (34.2)	343	273 (79.6)

ACGAGACCGTGGATGC-3'; ISMAP02r2, 5'-AACCGACGCC-CCCAATACG-3'). The reactions were amplified with the PTC-100 programmable thermal controller, programmed with an initial denaturing step (10 min at 94°C), 32 amplification cycles (0.75 min at 94°C, 1 min at 60°C, and 2 min at 72°C), and a final product extension step (7 min at 72°C). The amplified products from the reactions were separated by electrophoresis and visualized as described above. The presence or absence of 117-bp nucleotide product was scored.

Data analysis and statistics. Frequency of MAP was determined for each sample type and compared. Statistical analysis was done with Fisher's exact comparative analysis (33) and the GLM procedures in SAS (version 6.12 for Macintosh, SAS Institute, Inc., Cary, NC) to determine if significant ($P < 0.05$) differences existed between fed beef and cull cows relative to the type of sample, between sample types relative to the type of slaughtering facility, and the types of microbial analyses performed.

RESULTS AND DISCUSSION

In recent years, several clinical reports (6, 7, 18, 20, 23, 35) have suggested that Crohn's disease in humans may be associated with MAP infection. This link between Crohn's disease and MAP is circumstantial at present, but the relationship is a concern within the animal agriculture industry. MAP is known to promote Johne's disease, or paratuberculosis, in cattle, and consequently, cattle have been implicated as potential sources for MAP transmission to humans (12). To determine the baseline prevalence in beef and identify potential sources for MAP, six visits to cattle slaughtering plants across the United States were made and samples taken from a total of 586 animals.

MAP preferentially colonizes the intestinal wall of the terminal ileum in cattle, is assimilated by macrophages, and accumulates in the local lymph tissue (7, 8, 11). Previous work has demonstrated a higher prevalence in the gastrointestinal lymph tissue as compared with the fecal samples from the same animal (27). Ileocecal lymph nodes were collected from the gastrointestinal tracts of 562 animals to determine the prevalence of MAP in cattle (Table 1). In fed cattle plants, 0.4% (1 of 232) of the animals were positive for the presence of MAP in ileocecal lymph nodes, as determined by PCR of the IS900 sequence. In comparison, 34.2% (113 of 330) of ileocecal lymph nodes of cattle processed in cow plants were positive for MAP ($P < 0.001$), representing an apparent 76-fold greater level of infection in animals processed at cow slaughtering plants versus fed cattle plants.

Animals slaughtered in the cull cow plants are predominantly older dairy and beef cattle (≥ 4 years of age), whereas the fed cattle are predominantly feedlot finished and slaughtered at 2 years of age or younger. The apparent age differences in relation to MAP prevalence in cattle is not surprising, considering that MAP has an incubation period after initial colonization that can be more than 24 months before animals test positive (9). National Animal Health Monitoring System surveys have documented a significant level of MAP infection (8.6% by fecal culture and 5.5% by serological assay) and Johne's disease in production dairy cows and herds (34), and detection of MAP in retail bovine milk has been observed (14, 31). Less extensive regional surveys in beef cows have reported seroprevalence for MAP (28, 29, 32), and these seroprevalence numbers ranged from 3.0 to 8.0%.

Compared with the animal health surveys for MAP prevalence in animals from dairy and beef cattle herds, we observed three- to fourfold higher levels at the slaughtering plant. Several reasons may account for this difference. The animal health surveys summarized results from fecal and/or serological assays, whereas we analyzed the ileocecal lymph node. MAP preferentially colonizes the intestinal wall of the terminal ileum and accumulates in the local lymph tissue (7, 8, 11). Previous work has demonstrated a higher prevalence in the gastrointestinal lymph tissue as compared with a fecal sample from the same animal (27). A second reason is that chronic MAP infection leads to poor nutrient absorption from the intestine and eventually poor animal performance. Poor animal performance is a major reason for culling from dairy and beef herds. We were not able to trace back age or information on herd health, but the culled cows that we sampled could have been culled for poor performance, or were older and had more time to develop MAP infection than cows observed in an average herd.

Animal hide is a significant source of zoonotic pathogen contamination onto carcasses at beef processing plants (3–5, 26). Based on examination of sample type, animal hides had the highest number of MAP-specific, IS900-positive scores (Table 1). For hides, 1.2% in fed cattle plants and 79.6% in cull cow plants tested positive for the presence of MAP, as determined by PCR of the IS900 sequence ($P < 0.001$). Considering that MAP-infected cattle can shed high numbers of MAP in the feces (9), and only 34% of the animals at cull cow slaughtering plants were potentially MAP infected, based on the lymph tissue tested, the nearly 80% MAP prevalence on hides of these animals indicates that a significant level of shedding and cross-contamination occurred prior to slaughter. This is consistent with the effects of lairage on hide contamination of *Escherichia coli* O157:H7 (1).

Carcasses were sampled after hide removal, but before any antimicrobial interventions, to determine potential transmission from the hide to the carcass (preevisceration samples) (Table 2). Based on the PCR of the IS900 sequence in 583 carcasses, 1.2% of the preevisceration samples in fed cattle plants and 34.6% in cull cow plants were positive for the presence of MAP ($P < 0.001$). After all

TABLE 2. Prevalence of *Mycobacterium avium subsp. paratuberculosis*, based on PCR analyses of samples from preevisceration and postintervention carcasses of animals processed at fed cattle-only and cull cow beef processing plants

Type of beef cattle carcass	No. (%) of MAP positive samples	
	IS900 PCR	ISMAP02 PCR
Preevisceration		
Fed cattle ($n = 245$)	3 (1.2)	2 (0.8)
Cull cow ($n = 338$)	117 (34.6)	77 (22.8)
Postintervention		
Fed cattle ($n = 210$)	4 (1.9)	0 (0.0)
Cull cow ($n = 302$)	30 (9.9)	14 (4.6)

on-line antimicrobial interventions, carcasses were sampled again at the cooler (postintervention samples) (Table 2). Based on PCR of the IS900 sequence, 1.9% of the postintervention samples in fed cattle plants and 9.9% in cow plants were positive for MAP ($P < 0.001$). Because the PCR analysis of IS900 yielded positive results, we repeated PCR analysis with another MAP-specific primer set, ISMAP02 (30), to confirm the potential presence of MAP. Using ISMAP02-PCR, 0.8% of the preevisceration samples in fed cattle plants and 22.8% in cow plants were positive for the presence of MAP (Table 2). Similar analysis with the postintervention samples yielded that 0% of the carcasses in fed cattle plants and 4.6% of the carcasses in cow plants were positive for the presence of MAP (Table 2).

As expected, the type of PCR analysis does appear to influence the results. In the case of genetic analysis using DNA amplification of MAP-specific IS900 and ISMAP02 DNA, the IS900 analysis appeared to be more sensitive. Both DNA elements are repeated numerous times in the MAP chromosome (30), but the IS900 repeats are more than twofold more plentiful and, as a consequence, may have yielded a higher number of positives for both preevisceration (1.5-fold greater) and postintervention (2.4-fold greater) samples. The IS900 primers have been known to also amplify environmental mycobacteria (15); therefore, one could speculate that this could account for higher numbers of mycobacteria when using IS900 as compared with using ISMAP02. However, the protocol changes reported by Ellingson et al. (15) minimize this artifact. It also should be noted that we did not get a large number of positives in the younger fed cattle with the IS900 analysis, and we would have expected environmental mycobacteria to be present on those hides. However, the PCR analyses only target the MAP DNA sequence and provide no information pertaining to an intact or even viable bacterial cell.

Bacterial culture is the "gold standard" to test for Johne's disease in cattle, and all carcass samples were also analyzed with HEYA-ANV culturing after a two-step decontamination process (10, 14). Although MAP is an extremely slow-growing microorganism, and the assay is time-consuming, the culture provides additional information as to the viable status of MAP in the processed samples. More important is that the selective growth in the HEYA-ANV culture system allowed for analysis of 20- to 40-fold more sample volume

TABLE 3. Determination of viable *Mycobacterium avium* subsp. *paratuberculosis* by using double-decontamination and HEYA-ANV culture of beef carcass samples from animals processed at fed cattle-only and cull cow beef processing plants

Type of beef cattle	Preevisceration carcasses		Postintervention carcasses	
	No. tested	No. (%) of cultures positive	No. tested	No. (%) of cultures positive
Fed cattle	245	0 (0.0)	210	0 (0.0)
Cull cow	338	172 (50.9)	302	3 (1.0)

(200 versus 5 to 10 μ l for the direct PCR analysis for carcass samples), and is potentially four- to eightfold more sensitive when corrected for volume of the initial sample. Based on the MAP cultures (Table 3), none of the preevisceration or postintervention carcass samples from fed cattle plants were positive for viable MAP. In the cull cow plant carcass samples, 50.9% of preevisceration samples ($P < 0.001$, compared with fed cattle plants) and 1.0% of postintervention samples ($P > 0.05$, compared with fed cattle plants) were culture positive for viable MAP.

In the cull cow plant samples, the culture-based method did provide the highest numerical prevalence with the preevisceration samples as compared with the PCR-based assays, and we believe this reflects the greater sensitivity of the culture method. Comparatively in the cull cow plant samples, postintervention samples always had lower prevalence than preevisceration samples had, as determined by PCR of IS900 (9.9 versus 34.6%, $P < 0.001$), PCR of ISMAP02 (4.6 versus 22.8%, $P < 0.001$), and HEYA-ANV culture (1.0 versus 50.9%, $P < 0.001$). Interventions were in place to reduce bacterial pathogen contamination on carcasses at each plant. Although the interventions may have differed from plant to plant, they were effective against MAP on the beef carcasses. In postintervention carcasses, we did observe smaller reduction for prevalence of PCR IS900 positives as compared with the MAP culture positives; however, the more than 50-fold reduction in MAP culture positives provides some confidence that interventions reduced the viable MAP bacterial on the beef carcasses. Similar differences between IS900 PCR analysis (65% positive) and culture-based MAP analysis (3% positive) were observed in pasteurized retail milk (14).

In this study, we observed 212 of 583 preevisceration carcasses and 35 of 512 postintervention carcasses that tested positive for MAP by either PCR or culture (Table 4). In the preevisceration positive samples, 39.1% (83 of 212) of these samples were positive by both PCR and culture, 18.9% (40 of 212) were positive only by PCR, and 42.0% (89 of 212) were positive only by culture. In contrast, for the postintervention positive samples, 8.6% (3 of 35) of these samples were positive by both PCR and culture, 91.4% (32 of 35) were positive only by PCR, and no sample (0 of 35) was positive only by culture. Although direct analysis with PCR may provide more timely results for pathogen identification at a lower cost (14, 16, 30), analysis of only the DNA results may have implied a higher prev-

TABLE 4. Comparative breakdown of *Mycobacterium avium* subsp. *paratuberculosis* PCR- and culture-positive results across beef carcass samples before and after interventions, based on technique^a

Carcass sample	No. of samples positive by ^b :		
	PCR	HEYA-ANV culture	Both techniques
Preevisceration (<i>n</i> = 212)	40	89	83
Postintervention (<i>n</i> = 35)	32	0	3

^a Total numbers of preevisceration and postintervention carcasses were 583 and 512, respectively.

^b Cumulative sample number positive by PCR was 123; cumulative sample number positive by culture was 172.

alence on finished carcasses than found with culture-based methods, which are more sensitive. This study was not designed to specifically compare the PCR methods with culture methods on finished beef carcasses, but as shown with pasteurized milk (14), the differences in the results between the methods are such that the potential for misleading interpretations of PCR-only analysis must be acknowledged.

The association between MAP and Crohn's disease in humans has not been definitively proven (18). However, the potential for disease association in humans still exists. Ruminant animals, in particular cattle, are susceptible to MAP infection and have been implicated as potential sources. Beef especially has been suggested as a source for MAP infection in humans (http://www.crohns.org/MAP_food/beef.htm). Based on this study, very few postintervention carcasses from fed cattle tested positive (0 to 2%) for MAP, and fed cattle are unlikely sources, possibly due to their younger age and unlikelihood to have developed a significant level of infection. We detected a very small proportion of postintervention carcasses at cull cow plants as positive for viable MAP (1%), and these animals represent a slight risk as sources for MAP. These results indicate that antimicrobial interventions during processing were working to reduce the carcass contamination. A recent survey of ground beef by using PCR found no MAP positives (24), and in combination with our results on whole beef carcasses, suggests that the potential for MAP transmission from beef to humans is very low.

ACKNOWLEDGMENTS

This project was funded in part by The Beef Checkoff. The authors acknowledge the assistance with the lymph node and hide collections provided by Drs. Michael Guerini and Dayna M. Brichta-Harhay, and the dedicated technical support provided by Dee Kucera, Jane Long, and Greg Smith. Purified DNA from MAP was kindly provided by Dr. Judith R. Stabel at the U.S. Department of Agriculture, National Animal Disease Center, for our molecular assay developments and confirmations.

REFERENCES

- Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaria. 2007. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on

- hides and carcasses of beef cattle at processing. *J. Food Prot.* 70:280–286.
2. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.
 3. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
 4. Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
 5. Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen, and M. Koohmaraie. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* 68:265–272.
 6. Bull, T. J., E. J. McMinin, K. Sidi-Boumedine, A. Skull, D. Durkin, P. Neild, G. Rhodes, R. Pickup, and J. Hermon-Taylor. 2003. Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. *J. Clin. Microbiol.* 41:2915–2923.
 7. Chiodini, R. J. 1989. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. *Clin. Rev. Microbiol.* 2:90–117.
 8. Chiodini, R. J., and C. A. Rossiter. 1996. Paratuberculosis: a potential zoonosis? *Vet. Clin. North Am. Food Anim. Pract.* 12:457–467.
 9. Chiodini, R. J., H. J. Van Kruiningen, and R. S. Merkal. 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Vet.* 74:218–262.
 10. Clark, D. L., Jr., J. L. Anderson, J. J. Koziczkowski, and J. L. Ellingson. 2006. Detection of *Mycobacterium avium* subspecies *paratuberculosis* genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. *Mol. Cell. Probes* 20:197–202.
 11. Cocito, C., P. Gilot, M. Coene, M. de Kesel, P. Poupard, and P. Vannuffel. 1994. Paratuberculosis. *Clin. Microbiol. Rev.* 7:328–345.
 12. Collins, M. T. 1997. *Mycobacterium paratuberculosis*: a potential food-borne pathogen? *J. Dairy Sci.* 80:3445–3448.
 13. Collins, M. T., G. Lisby, C. Moser, D. Chicks, S. Christensen, M. Reichelderfer, N. Hoiby, B. A. Harms, O. O. Thomsen, U. Skibsted, and V. Binder. 2000. Results of multiple diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* in patients with inflammatory bowel disease and in controls. *J. Clin. Microbiol.* 38:4373–4381.
 14. Ellingson, J. L., J. L. Anderson, J. J. Koziczkowski, R. P. Radcliff, S. J. Sloan, S. E. Allen, and N. M. Sullivan. 2005. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. *J. Food Prot.* 68:966–972.
 15. Ellingson, J. L., J. C. Chevillie, D. Brees, J. M. Miller, and N. F. Chevillie. 2003. Absence of *Mycobacterium avium* subspecies *paratuberculosis* components from Crohn's disease intestinal biopsy tissues. *Clin. Med. Res.* 1:217–226.
 16. Ellingson, J. L., J. J. Koziczkowski, and J. L. Anderson. 2004. Comparison of PCR prescreening to two cultivation procedures with PCR confirmation for detection of *Mycobacterium avium* subsp. *paratuberculosis* in U.S. Department of Agriculture fecal check test samples. *J. Food Prot.* 67:2310–2314.
 17. Ellingson, J. L., J. R. Stabel, R. P. Radcliff, R. H. Whitlock, and J. M. Miller. 2005. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in free-ranging bison (*Bison bison*) by PCR. *Mol. Cell. Probes* 19:219–225.
 18. Feller, M., K. Huwiler, R. Stephan, E. Altpeter, A. Shang, H. Furrer, G. E. Pfyffer, T. Jemmi, A. Baumgartner, and M. Egger. 2007. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. *Lancet Infect. Dis.* 7:607–613.
 19. Gao, A., L. Mutharia, S. Chen, K. Rahn, and J. Odumeru. 2002. Effect of pasteurization on survival of *Mycobacterium paratuberculosis* in milk. *J. Dairy Sci.* 85:3198–3205.
 20. Gitnick, G., J. Collins, B. Beaman, D. Brooks, M. Arthur, T. Imaeda, and M. Palieschesky. 1989. Preliminary report on isolation of mycobacteria from patients with Crohn's disease. *Dig. Dis. Sci.* 34:925–932.
 21. Grant, I. R., H. J. Ball, and M. T. Rowe. 2002. Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. *Appl. Environ. Microbiol.* 68:2428–2435.
 22. Grant, I. R., E. I. Hitchings, A. McCartney, F. Ferguson, and M. T. Rowe. 2002. Effect of commercial-scale high-temperature, short-time pasteurization on the viability of *Mycobacterium paratuberculosis* in naturally infected cows' milk. *Appl. Environ. Microbiol.* 68:602–607.
 23. Hermon-Taylor, J., T. J. Bull, J. M. Sheridan, J. Cheng, M. L. Stelakakis, and N. Sumar. 2000. Causation of Crohn's disease by *Mycobacterium avium* subspecies *paratuberculosis*. *Can. J. Gastroenterol.* 14:521–539.
 24. Jaravata, C. V., W. L. Smith, G. J. Rensen, J. Ruzante, and J. S. Cullor. 2007. Survey of ground beef for the detection of *Mycobacterium avium paratuberculosis*. *Foodborne Pathog. Dis.* 4:103–106.
 25. Mishina, D., P. Katsel, S. T. Brown, E. C. Gilberts, and R. J. Greenstein. 1996. On the etiology of Crohn disease. *Proc. Natl. Acad. Sci. USA* 93:9816–9820.
 26. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and enterobacteriaceae on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
 27. Pavlik, I., L. Matlova, J. Bartl, P. Svastova, L. Dvorska, and R. Whitlock. 2000. Parallel faecal and organ *Mycobacterium avium* subsp. *paratuberculosis* culture of different productivity types of cattle. *Vet. Microbiol.* 77:309–324.
 28. Pence, M., C. Baldwin, and C. C. Black III. 2003. The seroprevalence of Johne's disease in Georgia beef and dairy cull cattle. *J. Vet. Diagn. Invest.* 15:475–477.
 29. Roussel, A. J., M. C. Libal, R. L. Whitlock, T. B. Hairgrove, K. S. Barling, and J. A. Thompson. 2005. Prevalence of and risk factors for paratuberculosis in purebred beef cattle. *J. Am. Vet. Med. Assoc.* 226:773–778.
 30. Stabel, J. R., and J. P. Bannantine. 2005. Development of a nested PCR method targeting a unique multicopy element, ISMAP02, for detection of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples. *J. Clin. Microbiol.* 43:4744–4750.
 31. Sweeney, R. W., R. H. Whitlock, and A. E. Rosenberger. 1992. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. *J. Clin. Microbiol.* 30:166–171.
 32. Thorne, J. G., and L. E. Hardin. 1997. Estimated prevalence of paratuberculosis in Missouri, USA, cattle. *Prev. Vet. Med.* 31:51–57.
 33. Uitenbroek, D. G. 1997. Fisher exact. Available at: <http://www.quantitativeskills.com/sisa/statistics/fisher.htm>. Accessed 14 September 2008.
 34. U.S. Department of Agriculture, National Animal Health Monitoring System. 1997. Johne's disease on U.S. dairy operations, N245.1097. U.S. Department of Agriculture, National Animal Health Monitoring System, Fort Collins, CO.
 35. Wall, S., Z. M. Kunze, S. Saboor, I. Soufleri, P. Seechurn, R. Chiodini, and J. J. McFadden. 1993. Identification of spheroplast-like agents isolated from tissues of patients with Crohn's disease and control tissues by polymerase chain reaction. *J. Clin. Microbiol.* 31:1241–1245.