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Characterization of *Mycobacterium avium* subspecies *paratuberculosis* disseminated infection in dairy cattle and its association with antemortem test results

María C. Antognoli [a,*], Franklyn B. Garry [b], Heather L. Hirst [b], Jason E. Lombard [b,c], Michelle M. Dennis [d], Daniel H. Gould [d], Mo D. Salman [a]

[a] *Animal Population Health Institute, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1681, USA*
[b] *Integrated Livestock Management, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1678, USA*
[c] *United States Department of Agriculture, Animal and Plant Health Inspection Services, Veterinary Services, Center of Epidemiology and Animal Health, Fort Collins, CO 80526, USA*
[d] *Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1619, USA*

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) disseminated infection in dairy cattle affects animal health and productivity and is also a potential public health concern. The study objectives were to characterize MAP disseminated infection in dairy cattle and to determine the role of antemortem tests in detecting cattle with disseminated infection. Forty culled dairy cows representing a variety of serum enzyme-linked immunosorbent assay (ELISA) results and body conditions were selected for the study. The physical condition of the cows was assessed via clinical examination prior to euthanasia and blood and feces were collected and tested by serum ELISA and fecal culture, respectively. Fifteen tissues were aseptically collected from each cow during necropsy and cultured for isolation of MAP. Disseminated infection was diagnosed when MAP was isolated in tissues other than the intestines or their associated lymph nodes (LNs) and was distinguished from infection found only in the gastrointestinal tissues and from absence of infection. Of the 40 cows in the study, 21 had MAP disseminated infection. Results showed that 57% (12/21) of cows with disseminated infection had average to heavy body condition and no diarrhea. Cows with disseminated infection had no to minimal gross pathologic evidence of infection in 37% (8/21) of cases. Only 76% (16/21) of cows with disseminated infection had positive historical ELISA results and only 62% (13/21) had a positive ELISA at slaughter.

* Corresponding author. Tel.: +1 970 491 6894; fax: +1 970 491 1889.
E-mail address: Maria.Antognoli@colostate.edu (M.C. Antognoli).

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Thus, antemortem evidence of MAP infection was lacking in a high proportion of cows where MAP disseminated infection was confirmed.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis*; Disseminated MAP infection; Antemortem diagnostic tests; Dairy cattle

1. Introduction

Johne's disease (JD), or Paratuberculosis, is a chronic bacterial disease of domestic and wild ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Clinical disease in cattle is characterized by intermittent to continuous unresponsive diarrhea, weight loss, and inter-mandibular edema. The economic losses to the dairy industry resulting from JD are attributed to decreased milk production (Buergelt and Duncan, 1978; Wilson et al., 1993; Benedictus et al., 1997; Hendrick et al., 2005; Lombard et al., 2005), decreased value of culled cows (Johnson-Ifearulundu and Kaneene, 1997; Johnson-Ifearulundu et al., 1999), and increased replacement costs (Ott et al., 1999).

The detection of cattle capable of transmitting the disease to susceptible herd mates is frequently based on the results of serum enzyme-linked immunosorbent assay (ELISA), which has a high sensitivity for detecting cattle that are shedding large amounts of MAP in feces (Collins et al., 1991; Sweeney et al., 1995; Whitlock et al., 2000; Collins et al., 2005). The organism has occasionally been isolated from milk, colostrum, semen, and placenta of infected animals (Larsen and Kopecky, 1970; Seitz et al., 1989; Sweeney et al., 1992; Streeter et al., 1995; Sweeney, 1996). There is limited knowledge about how frequently MAP transmission occurs via these alternative routes, but these studies demonstrated that the organism is distributed outside the gastrointestinal tract in some infected animals. Other studies have reported disseminated infection with MAP in tissues other than the intestines and associated lymph nodes (LNs) (Hines et al., 1987; Wilson et al., 1993; Van der Giessen et al., 1995; Whitlock et al., 1996; Pavlik et al., 2000; Ayele et al., 2004). These studies, however, looked for MAP disseminated infection in cows that either showed overt clinical disease or were previously confirmed as fecal shedders by fecal culture. No studies have reported how frequently MAP disseminated infection occurs in those with no evident signs of clinical disease and those where MAP has not been detected in feces (i.e subclinically infected cattle). In addition, little is known about which tissues outside of the gastrointestinal tract are predisposed to MAP infection, when during the course of infection these tissues become infected, or how MAP disseminates inside the host. No previous studies have reported the relationship between MAP disseminated infection and the results of rapid antemortem tests and/or the physical condition or appearance of the animals.

The objectives of this study were to characterize MAP disseminated infection in dairy cattle with a variety of physical conditions and to determine the role of antemortem tests and clinical assessments in detecting cattle with MAP disseminated infection at slaughter.

2. Materials and methods

2.1. Herds and cattle

Forty culled dairy cows from four dairy farms located in northern Colorado, USA, were included in this study. Infected cattle were present on all four dairies as previously determined by isolation of MAP from feces and necropsy specimens. The seroprevalence of infection in these dairies ranged between 2% and 11%. The majority of the study animals were selected based on prior positive ELISA results. Thus, the selection of cattle was targeted to assure the inclusion of a substantial number of infected animals. Six cows with negative historical ELISA results and normal physical appearance were included to ensure that a variety of physical conditions and antemortem test results were represented in the study. The study animals were either purchased from or donated by the dairy owners and transported to the Colorado State University Veterinary Diagnostic Laboratory where

they were subjected to a physical examination and blood/fecal sample collection followed by euthanasia.

A body condition score (BCS) on a 1–5 scale was assigned to each cow based on the amount of body fat coverage (Ferguson et al., 1994). These routine BCS assignments were then collapsed to develop a simplified JD clinical score on a scale of 1–3 to more easily categorize cows as fat, normal or thin, with the thin cows possibly suffering from weight loss due to JD. Clinical score 1 was assigned to cows with BCS $\geq$ 3.5, representing well to over-conditioned cows. Clinical score 2 was assigned for cows with BCS between 2.5 and 3.25, typical of healthy, lean, high-producing dairy cows. Clinical score 3 was assigned to cows with BCS $<$ 2.5, suggestive of weight loss and excessive negative energy balance.

Humane euthanasia was performed using a captive bolt followed by rapid intravenous injection of a saturated solution of potassium chloride. The severity of the gross pathologic lesions located in the gastrointestinal tract was evaluated during necropsy and categorized using a scale of 1–4. Gross lesion score 1 was assigned to animals with normal appearing gastrointestinal tract. Gross lesion score 2 was assigned to animals having slight thickening of the mucosa of the ileum and normal appearing associated lymphatics. Gross lesion score 3 was assigned to animals with moderately thickened mucosa of the ileum, enlargement of the associated mesenteric LN and few enlarged serosal lymphatic vessels. Gross lesion score 4 was reserved for animals with severe thickening of the mucosa of the ileum, notable enlargement of the associated mesenteric LN, notable thickening of the mucosa of the jejunum, and many white colored serosal lymphatic vessels with pronounced thickening. All procedures affecting animals that were used in this research protocol were approved by the Colorado State University Animal Care and Use Committee.

2.2. Collection of samples

Blood and fecal samples were collected prior to euthanasia from all cows to determine ELISA and fecal culture status. Blood samples were collected from the jugular vein into 10 ml vacutainer tubes with 1 in., 14 gauge needles. Fecal samples were collected from the rectum with single-use, clean obstetric sleeves and immediately transferred to sterile whirl pack bags. Liver and rectal tissue samples (simulating biopsies) from 38 and 14 cows, respectively, were preserved in 10% buffered formalin for histopathological examination. Samples of 15 tissues were collected during necropsy using aseptic technique, and immediately submitted for bacteriologic culture. Tissues sampled included ileum, jejunum, ileocecal LN, mesenteric LN, hepatic LN, liver, kidney, lung, supramammary LN, retropharyngeal LN, prescapular LN, popliteal LN, heart muscle, longissimus colli muscle, and extensor carpi radialis muscle. The last two muscles, which provide support to the spinal column and forelimb, respectively, were included because their trims are often included in ground beef. All instruments used for tissue collection were cleaned with 70% ethanol and flame sterilized between tissues. All non-gastrointestinal tissue samples were collected first to prevent potential cross contamination with gastrointestinal contents. Samples from gastrointestinal tissues were collected last, after the esophagus and the rectum were cut and all organs from the gastrointestinal were removed from the carcass.

2.3. Serologic testing

Blood samples were centrifuged and serum was separated on the day of blood collection. Serum samples were stored at -70 °C until the collection of serum from all 40 cows was completed. Serologic testing was performed with a commercial ELISA for antibodies against MAP according to the manufacturer's instructions (Herdcheck, IDEXX Laboratories Inc., Westbrook, ME, USA). All samples were tested in duplicate with the same test kit lot and by the same individual to minimize kit lot and operator variability.

Results for the ELISA were calculated and reported as S/P ratios using the following equation: (OD of sample $-$ OD of negative control)/(OD of the positive control $-$ OD of negative control). Test results were classified as positive if the S:P ratio $\geq$ 0.25 or negative if the S:P ratio was $<$0.25 as per manufacturer's recommendations.

2.4. Mycobacterial culture of feces and tissues

Conventional culture of feces and tissues for isolation of MAP was conducted at the Colorado State

University Veterinary Diagnostic Laboratory. Two grams of tissues previously cut and minced with sterile scissors or 2 g of feces were mixed with 35 ml of sterile water, shaken vigorously, and placed in a mechanical rocker for at least 30 min. The suspension was then allowed to stand at room temperature for another 30 min, after which 5 ml of supernatant were mixed with 0.75% hexadecylpyridinium chloride in brain heart infusion broth and incubated overnight at 35–37 °C for decontamination. The samples were then centrifuged at 900 × *g* for 30 min and the pellet resuspended in 1 ml of antibiotic mix containing amphotericin B (100 mg/ml), nalidixic acid (100 mg/ml), and vancomycin (50 mg/ml), and incubated overnight at 35–37 °C for final fungal decontamination. All samples were inoculated in four tubes of Herrold egg yolk medium (HEYM), three with Mycobactin J and one without Mycobactin J. All four tubes were incubated at 37 °C in a slanted position with loose caps to allow the surface of the medium to dry. Caps on the slants were tightened after 1–2 weeks and cultures were examined every other week for the first 6 weeks for signs of contamination. Contaminated samples were re-cultured. All cultures without signs of colony growth were held for 16 weeks before results were determined. Colonies appearing after 6 weeks were evaluated for mycobactin dependence along with morphology and acid-fast staining. Samples with positive culture results were reported as number of colonies per tube of HEYM; negative results were reported as ''no growth''; and samples that were contaminated after a single re-culture attempt were reported as ''contaminated''. The presence of MAP was also confirmed by BACTEC radiometric liquid culture and IS900 PCR in some gastrointestinal tissues that were submitted to an independent laboratory.

2.5. Histopathology

Rectal and liver samples of approximately 3 mm × 6 mm were collected using a skin biopsy punch during post mortem examination and immersed in 10% neutral buffered formalin. Formalin-fixed, paraffin-embedded tissue samples were sectioned at 3–5 μm and were prepared with hematoxylin and eosin and Ziehl–Nielsen acid fast stains for histologic evaluation. These tissue samples were chosen as potential antemortem histological assessments of MAP infection because they are easily accessible in live cattle and could be obtained with minimal invasion and risk to the animal. In the particular case of liver biopsy, the tissues could be used not only for JD diagnosis but also for other diagnostic purposes and liver tissue has been reported to frequently yield positive culture results in cattle with disseminated MAP infection (Rossiter and Henning, 2001). Results were reported as ''Diagnostic for Johne's disease'' when foci of granulomatous hepatitis with intralesional acid fast bacteria were evident upon microscopic examination of the tissue, ''Suggestive of Johne's Disease'' when foci of granulomatous hepatitis were identified without the presence of acid fast bacteria, and ''no evidence of Johne's disease'' when granulomatous hepatitis and acid fast bacteria were not detected.

2.6. Characterization of cattle

Cattle were classified into three groups according to their JD status: ''Disseminated Infection'', ''Gastrointestinal Infection'', and ''No Infection''. Cattle were included in the group ''Disseminated Infection'' if they had MAP isolated in tissues other than intestine and associated LNs. Cattle were included in the category ''Gastrointestinal Infection'' if organism isolation was limited to feces, intestinal tissues or associated LN. Cattle were included in the ''No Infection'' group if all tissues and feces were culture negative for MAP.

2.7. Analysis

The associations between JD clinical scores, presence or absence of diarrhea, categorical results of historical and pre-slaughter ELISAs, results of conventional fecal culture, and the JD status of the cows were determined by a chi square test for association or by Fisher's exact test. Similarly, a chi square test was used to determine the association between JD status and type of tissue infected with MAP. McNemar's chi square test for paired data was used to determine if there were differences in the proportions of positive results between the historic ELISA and the ELISA performed prior to slaughter. A chi square test was used to determine if the proportion of positive conventional fecal culture results was different among cattle groups. The differences in historical and pre-slaughter ELISA

S:P ratios among groups of cows with different JD status were determined by one-way analysis of variance and Scheffe's test for multiple comparisons (PROC ANOVA, version 9.1, SAS Institute Inc., Cary, NC). The significance level chosen for all statistical analysis was 0.05.

3. Results

3.1. Characterization of MAP disseminated infection

Twenty one out of the 40 cows included in the study had disseminated infection as determined by the isolation of MAP from extra intestinal tissues. Infection limited to the gastrointestinal tract and associated LN was found in 7 of 40 cows. Twelve cows showed no evidence of MAP infection by the culture methods used in this study. The average age of the cows was 5 ± 1.6 years old among those with disseminated infection, 4.4 ± 1.4 years among those with gastrointestinal infection, and 5.2 ± 2.2 years among cattle with no infection.

Table 1 shows a detailed description of clinical score, gross lesion score, and presence or absence of diarrhea among cattle with ''Disseminated Infection'', ''Gastrointestinal Infection'', and ''No Infection''. Our results indicate that approximately half of the cows with MAP disseminated infection (12/21) had JD clinical scores of 1 or 2 (normal or high BCS) while only 43% (9/21) of cows with disseminated infection had clinical scores of 3, suggesting negative energy balance and weight loss. All of the cows in the category ''Gastrointestinal Infection'' had normal to high body condition. Only one of the 12 not infected cows had a clinical score of 3, indicating substantial weight loss that could not be attributed to any other disease based solely on gross pathology findings or clinical history of the animal. The association between JD status (''Disseminated Infection'', ''Gastrointestinal Infection'' or ''No Infection'') and JD clinical scores was not statistically significant ($p = 0.21$).

Approximately 40% (8/21) of the cattle with disseminated infection had minimal or no grossly observable lesions in the intestines. Fewer than half (9/21) of the cows with disseminated infection showed evidence of diarrhea at the time of euthanasia. No statistically significant association was found between JD status and the presence of diarrhea ($p = 0.29$).

3.2. Tissues affected in cattle with MAP disseminated infection

The proportion of MAP positive samples among cattle with disseminated infection is shown in

Table 1
Clinical score, gross lesion score, and presence of diarrhea among cattle with disseminated infection, gastrointestinal infection, and no infection

Characteristics	Disseminated infection (%), $N = 21$	Gastrointestinal infection (%), $N = 7$	No infection (%), $N = 12$	Overall (%), $N = 40$
Clinical score 1	38.1	71.4	58.4	50.0
Clinical score 2	19.0	28.6	33.3	25.0
Clinical score 3	42.9	0.0	8.3	25.0
Gross lesion score 1	23.8	42.8	58.3	37.5
Gross lesion score 2	14.3	28.6	25.0	20.0
Gross lesion score 3	33.3	28.6	16.7	27.5
Gross lesion score 4	28.6	0	0	15.0
Diarrhea absent	57.1	100	83.3	72.5
Diarrhea present	42.9	0	16.7	27.5

Clinical score 1 = high body fat, body condition ≥ 3.5.
Clinical score 2 = normal to lean body fat, body condition scores between 2.50 and 3.25.
Clinical score 3 = cattle with low body fat, body condition score < 2.5.
Gross lesion score 1 = normal appearing gastrointestinal tract.
Gross lesion score 2 = slight thickening of the ileum without obvious lymphatic involvement.
Gross lesion score 3 = moderate thickening of the ileum and enlargement of associated LN, few enlarged lymphatic vessels.
Gross lesion score 4 = severe thickening of the ileum, enlargement of LN, obvious diffuse (oral–aboral) thickening of the jejunum, and many white thickened lymphatic vessels.

Table 2
Proportion of cattle with disseminated infection that cultured positive by tissue type

Tissue	Proportion of culture positives	Tissue	Proportion of culture positives
Hepatic LN	17/21	Lung	4/21
Ileocecal LN	17/21	Retropharyngeal LN	4/21
Ileum	18/21	Popliteal LN	3/21
Jejunum	16/21	Heart	2/21
Mesenteric LN	16/21	Prescapular LN	1/21
Liver	10/21	Longissimus colli	0/21
Kidney	6/21	Extensor c. radialis	0/21
Suprammamary LN	6/21		

Table 2. The bacteria were most commonly isolated from intestinal tissue, LNs associated with the gastrointestinal tract, and hepatic LN of cows with disseminated infection. Bacteria were also isolated from liver, heart, kidney, lung, and supramammary, retropharyngeal, popliteal and prescapular LNs. Liver was the most commonly infected non-gastrointestinal tissue in cows with disseminated infection (10/21), and 14 LNs not associated with the gastrointestinal tract were colonized in a total of six cows with disseminated infection. Only two skeletal muscle tissues were explored in this study (longissimus colli and extensor carpi radialis) and neither were culture positive for MAP in any of the study cows.

3.3. Antemortem tests

The results of the historic ELISA, final ELISA, rectal biopsy, liver biopsy, and fecal culture in the ''Disseminated Infection, ''Gastrointestinal Infection'' and ''No Infection'' cattle groups are provided in Table 3. The mean S:P ratio for the historical ELISA was 1.7 (±6.26) in the group with disseminated infection, 0.66 (±0.58) in the group with gastrointestinal infection, and 0.27 (±0.74) among cattle with no infection. The mean S:P ratio for the final ELISA was 0.91 (±0.87) in the group with disseminated infection, 0.64 (±0.64) in the group with gastrointestinal infection, and 0.20 (±0.51) in the group with no infection.

Table 3
Results of antemortem tests (ELISA, liver biopsy, rectal biopsy, and fecal culture) among cattle with disseminated infection, gastrointestinal infection, and no infection

Type of test	Test result	Disseminated infection	Gastrointestinal infection	No infection	Overall
Historic ELISA	Positive	16/21	4/7	7/11[a]	27/39
	Negative	5/21	3/7	4/11[a]	12/39
Mean S:P		1.7 ± 6.26	0.66 ± 0.58	0.27 ± 0.74	1.59 ± 4.65
Final ELISA	Positive	13/21	4/7	1/12	18/40
	Negative	8/21	3/7	11/12	22/40
Mean S:P		0.91 ± 0.87	0.64 ± 0.64	0.20 ± 0.51	0.65 ± 0.79
Liver histopathology	Suggestive	7/19	0/7	0/12	7/38
	No evidence	12/19	7/7	12/12	31/38
Rectal histopathology	Suggestive	0/8	0/1	0/5	0/14
	No evidence	8/8	1/1	5/5	14/14
Conventional fecal culture	Positive	18/21	3/7	1/12[b]	22/40
	Negative	3/21	4/7	11/12	18/40

[a] One animal with no historical ELISA test results (2-year-old heifer).

[b] Although only one CFU was found in one of four tubes of HEYM this animal was classified as not infected based on test negative results to BACTEC fecal culture, culture of all intestinal and extraintestinal tissues, and ELISA. The presence of one acid fast colony in one of the HEYM tubes was considered as a case of ''pass through'' where the bacterium went through the intestines without establishing infection.

Statistically significant differences were found in the proportion of positive results between the historic ELISA and the one performed prior to slaughter ($p = 0.03$). No statistically significant differences in historical ELISA mean S:P ratios were found among cows with different JD status via one-way analysis of variance. In contrast, statistically significant differences in the mean S:P ratio for the final ELISA between ''Disseminated Infection'' and ''No Infection'' groups were detected with Scheffe's test for multiple comparisons.

Histopathologic findings from liver biopsy were suggestive of JD in 7/19 cows with disseminated infection. None of the evaluated liver biopsies were diagnostic for JD. The detection of granulomatous hepatitis was unique to cattle with disseminated infection; no cows of the ''Gastrointestinal Infection'' and ''No Infection'' groups were found to have this lesion. Neither granulomatous proctitis nor acid fast bacteria were ever identified through microscopic examination of rectal mucosa tissue samples from 14 cows (eight cows in the ''Disseminated Infection'' category, five in the ''Gastrointestinal Infection'' category, one cow in the ''No Infection'' category).

4. Discussion

The presence of cattle with MAP disseminated infection in dairy herds can have a negative impact on the progress and results of a program to control JD because such animals can transmit the infection through routes other than the traditional fecal–oral route. In this study most cattle with disseminated infection were also fecal culture positive to MAP by conventional culture, and thus would have been identified as contagious animals if such testing were performed. However, serum ELISA results from either historical testing or prior to slaughter were negative in one quarter to one third of the animals affected with MAP disseminated infection, respectively, which means that a large percentage of these animals would have escaped detection using serum ELISA alone.

A large volume of scientific publications suggest evidence of an association between MAP infection and human Crohn's disease, which implies that cattle infected with MAP may be a source of zoonotic spread and a risk to human health (Chiodini et al., 1984; Hermon-Taylor et al., 2000; Harris and Lammerding, 2001; Greenstein and Collins, 2004). The increased participation of dairy farms in the Voluntary Bovine Johne's Disease Control Program may lead to increased numbers of MAP infected cows being sent to slaughter. Cattle with disseminated infection have been found to have MAP in tissues that ultimately could have the potential to end up in ground beef products (Hines et al., 1987; Whitlock et al., 1996; Rossiter and Henning, 2001). Results from our study substantiate this finding since several peripheral LNs that are commonly included in ground meat product were culture positive for MAP. The Food Safety and Inspection Service's current inspection process at slaughter plants would fail to identify MAP infected cattle except for those animals with advanced clinical disease. Given concerns about a potential zoonotic role for MAP and the fact that there are not existing means to prevent MAP infected tissue from entering the food supply, it seems crucial to evaluate antemortem resources for identification of cattle with disseminated infection at slaughter and to determine which tissues are most frequently colonized by MAP.

Previous studies reported that MAP disseminated infection was found in thin market cows and in cattle with advanced clinical JD (Whitlock et al., 1996; Pavlik et al., 2000; Rossiter and Henning, 2001). In our study, however, we included cows with subclinical infection and found that 57% of cattle with MAP disseminated infection showed no overt clinical signs of disease (diarrhea or poor body condition). These results suggest that common indicators used in the field to identify and isolate or cull cattle affected with JD, such as poor body condition and presence of unresponsive diarrhea, would fail to detect a significant proportion of cattle with MAP disseminated infection. Although our sample group was targeted towards cattle with previous positive ELISA results, we assured that cattle with a variety of clinical presentations were included in the study and also that ELISA-negative cattle were included for comparison. Future studies should examine the prevalence and characteristics of MAP disseminated infection in a larger sample of cattle from farms with varying levels of infection.

Knowing which organs or tissues are more frequently colonized with MAP in cattle with disseminated infection could enhance the application of hazard analysis and critical control point-based

approach to minimize the likelihood of contamination of human food products with this infectious agent. Our results indicated that MAP is most frequently isolated from feces, intestinal tissues, and associated LNs, as well as in the hepatic LN and hepatic tissues. However, we also found that LNs not associated with the gastrointestinal tract such as the supramammary, retropharyngeal, popliteal, and prescapular, as well as several other organ tissues could be infected with MAP in cattle with disseminated infection. These findings have several implications: (1) if the bacterium is located in a LN that drains muscles it may be possible to find the bacterium in red meats, (2) peripheral LNs are frequently trimmed off the carcass and included in ground meat for human consumption, increasing the risk of human exposure to MAP, and (3) the presence of MAP in tissues such as kidney, retropharyngeal or supramammary LNs suggests the potential for routes of bacterial transmission other than the traditional fecal–oral route. Although MAP was found in neither of the two skeletal muscles tested in this study, we cannot conclude that skeletal muscle can not be colonized with MAP. Future studies with a larger sample size and exploring a larger number of tissues, including blood and tissues intended for consumption, are needed to more accurately determine the risk of MAP entering the food chain. In addition, a more sensitive pathogen detection technique may be needed when assessing the risk of MAP contamination of muscle tissues in future studies. The role of the currently available antemortem tests in detecting cattle with disseminated infection was found to be limited in this study. Serum ELISA performed within a year prior to slaughter could not discriminate between cattle with disseminated infection, gastrointestinal infection or no infection. Additionally, the final ELISA could not differentiate between cattle with disseminated infection and those with gastrointestinal infection, and failed to identify almost 40% of the cattle with disseminated infection. Granulomatous hepatitis was only detected in the liver biopsies from cattle in the ''Disseminated Infection'' group; however, the overall detection ability of liver biopsy analysis was poor, as it failed to identify 63% of the animals in this category. The rectal tissue sampling was included in a late phase of the study after the suggestion that it might be a useful antemortem test to identify advanced cases of clinical disease and potential disseminated infection. However, rectal histopathology failed to identify any of the cows with disseminated infection or infection limited to the gastrointestinal tract. Thus, the commonly used rapid methods of identifying cattle with MAP infection, including clinical signs, serum ELISA testing, and tissue biopsy were inadequate in identifying MAP disseminated infection. While fecal culture detected contagious animals, and most of the cattle with disseminated infection fit that description, this test cannot produce results in a timeframe less than several months.

5. Conclusions

Our results document the occurrence of MAP disseminated infection in cows with subclinical infection, contrary to the notion that MAP disseminated infection is a manifestation of late-stage disease characterized by poor body condition and unresponsive diarrhea. Furthermore, our results indicate that currently available rapid antemortem tests such as the serum ELISA, liver, and rectal biopsy have limited value in detecting cattle with MAP disseminated infection.

The presence of MAP in LNs and tissues not associated with the gastrointestinal tract from cows with disseminated infection suggest that this bacterium has the potential to be included in ground meat for human consumption and to be a source of human exposure to MAP. Further research is needed to quantitate the risks for human exposure to this agent through consumption of animal tissues, particularly if MAP is determined to be a causative agent of human Crohn's disease. Our findings demonstrate the need to explore new methods for antemortem detection of cattle with MAP disseminated infection and MAP infected tissues, in order to prevent their inclusion in human food products.

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