Brief Communication Communication brève

Exposure of young dairy cattle to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) through intensive grazing of contaminated pastures in a herd positive for Johne's disease

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Abstract — This study investigated the susceptibility of 1- to 2-year-old cattle to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) on pasture previously grazed by infected cattle. The exposure of yearling cattle to pastures contaminated with MAP resulted in infection with MAP, showing that age resistance to infection can be overcome by pressure of infection.

Résumé — Exposition des jeunes bovins laitiers à *Mycobacterium avium* ssp. *paratuberculosis* (MAP) par un broutage intensif de pâturages contaminés chez un troupeau positif pour la maladie de Johne. Cette étude a examiné la susceptibilité des bovins âgés de 1 an jusqu'à 2 ans à *Mycobacterium avium* ssp. *paratuberculosis* (MAP) dans des pâturages fréquentés antérieurement par des bovins infectés. L'exposition des bovins âgés d'un an aux pâturages contaminés par MAP s'est traduite par une infection à MAP, montrant que la résistance de l'âge à l'infection peut être subjuguée par la pression d'infection.

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Paratuberculosis or Johne's disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Control of JD often focuses on preventing transmission from adult cattle shedding the MAP organisms in feces to young replacement stock. Traditionally, newborn calves have been considered most at risk for contracting the infection, and control measures have been aimed primarily at calving areas and newborn calf management (1–3). However, the susceptibility of older replacement stock (1 to 2 years of age) to infection following exposure to MAP is uncertain.

With increased interest in organic farming and animal welfare, less intensive production systems that employ pasture grazing have gained in popularity. While it has been presumed that cattle could be exposed to MAP by grazing where infected animals have defecated, it is also possible that grazing behavior could lead to avoidance of fecal pads, which could minimize exposure. The herd in this study was managed by an intensive grazing practice known as "leader-follower" system in which lactating cows graze lush grass as their primary forage (4,5). The entire milking herd is turned out into small partitioned portions of the pasture, and rotated to another partition after each milking. Yearling cattle trail 1 day behind the milking herd, eating the leftover grass from the previous day. The risk of exposure to MAP for the "follower" animals in this management system is not known.

The primary objective of this study was to document the exposure of yearling animals to MAP through grazing of pasture previously occupied by lactating cattle infected with MAP. An additional objective was to determine the extent of MAP infection in the tissues of yearling cattle following exposure to MAP by grazing on contaminated pastures.

The study animals originated from a herd of 80 lactating Jersey cows. The herd had been assembled in 1997 with purchase of cows and calves of unknown JD status. In 1999, the first clinical case of JD in the herd was detected. Serological prevalence of MAP infection within the herd ranged from 4% in 1999 to 16% in 2001, and decreased to 5.2% in 2005 following implementation of rigorous management changes and culling of the highest shedders. At the time the study was implemented in April 2006, the adult herd was sampled using culture and real-time polymerase chain reaction (RT-PCR) of pooled fecal samples (5 cows/pool). Of the 17 fecal pools, 8 were positive on culture and 15 were positive by RT-PCR. Individual fecal cultures were obtained from cows that contributed feces to

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Table 1. Fecal real-time polymerase chain reaction (RT-PCR) and fecal culture results over time, and results of MAP culture on tissue harvested at slaughter

	Sample 1			Sample 2			Sample 3			Sample 4			Tissue MAP	
Steer	CFU[a]/ steer	Ct[b] 1	Ct 2	CFU/ steer	Ct 1	Ct 2	CFU/ steer	Ct 1	Ct 2	CFU/ steer	Ct 1	Ct 2	CFU/ steer[c]	Number positive[d]
1	4	34.2	34.4	0	34.5	34.9	0	50.0	38.4	0	50.0	50.0	263	14 (50%)
2	0	36.1	36.1	3	32.3	31.5	0	38.3	37.7	1	38.8	38.5	843	17 (57%)
3	0	38.1	36.6	0	34.6	34.9	0	38.7	50.0	0	41.0	41.4	2464	17 (57%)
4	0	35.9	36.4	3	33.6	33.5	0	37.3	38.7	0	38.0	50.0	68	5 (18%)
5	0	37.1	37.3	2	33.1	32.5	0	39.9	39.4	0	50.0	50.0	0	0
6	0	36.4	37.6	7	32.9	33.4	0	36.7	36.3	0	50.0	50.0	0	0
7	0	33.6	34.5	5	33.5	33.1	0	38.3	38.6	4	37.2	37.2	9337	23 (83%)
8	0	36.2	35.3	1	37.1	37.9	0	37.2	36.5	1	44.3	50.0	25	7 (25%)
9	0	36.7	35.9	0	36.1	36.4	0	50.0	36.2	0	50.0	50.0	0	0
Ct Avg		36.0	36.0		34.2	34.2		38.1	37.7		39.8	39.0		

[a] Represents the number of colony forming units (CFU) of MAP totaled for 4 culture tubes.
[b] Represents the number of cycles to positive threshold in duplicate samples.
[c] Represents the total CFU from 28 tissue samples per steer cultured for *Mycobacterium avium* subsp. *paratuberculosis* (MAP).
[d] Represents the number of tissues with at least 1 CFU of MAP.

positive pools. Fifteen cows were positive, with 4 cows identified as heavy shedders. Eleven of 14 fecal samples collected from a recently grazed pasture, and 5 of 10 grass samples, were RT-PCR positive for MAP.

The seasonal lactating herd was maintained on pasture, with access to free-stall housing during the winter months. Calving areas consisted of maternity pens that could contain 2 to 4 cows at one time. Calves were separated from the dam immediately after birth and were fed single source colostrum from the farm's enzyme-linked immunosorbent assay (ELISA)-negative animals, followed by milk replacer for 6 wk. Calves were housed in individual hutches until weaning, at which time they were kept in small age-matched groups on pastures that had not been grazed by adult cattle, nor had been fertilized with manure from adult cattle for several years. When they reached yearling age, the replacement animals were allowed to follow the adult herd on pasture.

A total of 9 Jersey steers 15 months of age at the start of the study period were included. These steers were born and raised on the dairy (using the same practices as the heifer calves). In April 2006, at 15 months of age, they began following the adult herd on pasture. This was practiced until November 2006 at which time the steers were housed in an outdoor pen, and received a diet of free choice hay and mineral supplemented with monensin (Rumensin; Elanco Animal Health, Greenfield, Indiana, USA) at 200 mg/head/d. The steers again followed the adult herd on pasture from April 2007 until they were sent to slaughter in May 2007. The steers were 28 months of age at the end of the study period. The animals were slaughtered in a USDA-FSIS approved facility, according to the guidelines for the slaughter of animals for human consumption.

Fecal cultures and fecal RT-PCR testing were performed on the steers on 3 occasions throughout the first pasture season, and at slaughter. Samples 1, 2, and 3 were obtained at 4, 5, and 7 mo, respectively after introduction of the steers to a contaminated pasture. Sample 4 was obtained at slaughter. At slaughter, blood was obtained from each animal and processed for MAP antibody detection using ELISA (Parachek; BIOCOR Animal Health, Omaha, Nebraska, USA). For each animal, 28 separate tissue samples were collected for MAP culture. Tissue samples included liver, hepatic lymph node, duodenum, 8 separate samples of jejunum, 8 adjacent mesenteric lymph nodes, proximal, middle, and distal ileum, 3 ileocecal lymph nodes, ileocecal valve, cecum, and spiral colon. Samples of ileum and ileocecal lymph node were saved in 10% buffered formalin and examined for histological lesions of paratuberculosis and presence of acid-fast staining organisms. Fecal samples were cultured for MAP on Herrold's egg yolk medium (HEYM) using a centrifugation-double incubation method previously described (6). Tissue samples were also cultured for MAP on HEYM, using a method previously described (7). After 16 wk of incubation, the number of colony-forming units (CFU)/tube was recorded. Fecal samples were also tested for MAP by RT-PCR using a commercially available kit according to the manufacturer's instructions (VetAlert; Tetracore, Rockville, Maryland, USA; SmartCycler; Cepheid, Sunnyvale, Calfornia, USA). The number of cycles to positive threshold (Ct) was recorded in duplicate samples. A Ct value of ≥ 42.0 indicated a negative result. A test was considered positive if either sample was positive.

For each steer, the number of tissues that were culture-positive, and the sum of the CFU for all positive tissues (that is, total colonies recovered per steer) were recorded.

The serum ELISA results for MAP antibodies were negative for all 9 steers. Table 1 illustrates the results of the fecal cultures and fecal RT-PCR performed throughout the pasture season (samples 1, 2, and 3), and at slaughter (sample 4). Fecal RT-PCR was positive in all 9 steers for samples 1, 2, and 3. At slaughter (sample 4), 4 steers had positive fecal RT-PCR. The Ct values correspond to low levels of shedding, near the detection limit for culture, which accounts for the fewer number of steers with positive fecal culture.

Six of the 9 steers had at least 1 tissue sample culture-positive for MAP organisms (Table 1). For the positive steers, the number of positive tissue samples ranged from 5 (18%) to 23 (82%; median, 16 samples). Culture-positive sites were uniformly restricted to intestinal or mesenteric lymph node samples, as MAP was never isolated from liver and hepatic lymph nodes. The organism was most frequently isolated from the mid to distal jejunum segments and their respective mesenteric lymph nodes; the proximal, mid, and distal ileum; and the ileocecal

lymph nodes. The distribution of MAP in the tissues of these naturally infected steers was similar to that reported in short-term experimental infections (8).

At postmortem examination, there were no grossly visible lesions in any of the steers. Six of the 9 steers (numbers 3–6, 8, and 9) had no histological evidence of MAP infection; these included the 3 which were culture-negative. The remaining 3 steers had mild histological evidence of early MAP infection, including individual Langhans type giant cells in the paracortex of the mesenteric lymph node or lamina propria of the ileum, or macrophages and small giant cells suggesting littoral cells draining from the ileum. Acid-fast staining organisms were not detected. As in previous studies, culture appeared to be a more sensitive means for detection of MAP in tissues than direct microscopic examination for acid-fast staining organisms (8,9). This is perhaps due in part to the amount of tissue cultured (2 g) versus the thin slice of tissue subjected to histological examination.

All fecal samples from yearling steers were RT-PCR positive at 3 sampling times during the pasture season suggesting that these animals were positive for MAP in their feces as the organism was "passed through" following oral consumption of MAP from contaminated pasture. The fact that MAP can be isolated from feces of uninfected cattle subsequent to ingestion of feces from cows infected with MAP has previously been documented in experimental studies (10). Thus, we consider the presence of MAP in the feces of these steers while on pasture to be a marker of oral consumption of MAP from the contaminated pasture, rather than "active shedding" due to neonatal infection. The authors acknowledge that testing of fecal samples prior to pasture exposure would have been useful to document this fact, but the steers were already at pasture when they were made available for testing. However, evidence that the shedding was "passive" due to oral consumption, rather than active includes the following: 1) the uniformity of shedding level (Ct values) from fecal samples in 15-month-old animals. Active shedding, all at the same level, from natural infection would be unusual in this number of yearling animals, given the typical long incubation period of JD and the variable shedding patterns early in infection. 2) If the steers were infected prior to being exposed to pasture and were "actively" shedding at the time of sample 1, we would not have expected the drop in fecal shedding that was observed between samples 2 and 3, or 3 and 4. Between samples 2 and 3, several heavy shedder cows were culled from the herd, which most likely resulted in a decrease in pasture contamination and subsequently lower levels of "passive" shedding in the steers. Additionally, 5 of the steers were not shedding at slaughter, again suggesting that the earlier positive fecal results were from "passive" shedding, as "active shedding" would be expected to continue. 3) Of the 32 heifers from the same age cohort that were raised with the steers until they were 12 mo old, and which did not follow the adult herd on pasture, 31 remained negative on fecal RT-PCR during the period of this study (data not shown). 4) The lack of histological lesions in 6 animals and the very mild lesions of early infection in 3 animals suggest that the fecal shedding that was detected 9 mo earlier (sample 1) was not due to active infection, but was "pass through" due to oral consumption of contaminated forage. Based on this evidence, it was concluded that the presence of MAP in the feces of the steers while on pasture is a marker of oral exposure to MAP deposited on the pasture by the adult herd. Clearly, deposition of feces in distinct pads does not prevent contamination of the nearby grass consumed by the steers.

Based on this evidence, the yearlings were not infected with MAP when they were placed on the pasture, and MAP detected in the feces at that time was most likely due to passive shedding. However, the exposure that resulted in passive shedding ultimately resulted in infection in 6 of those steers. Thus it may be concluded that the early infection found in the tissues of 6 steers at the time of slaughter occurred through exposure to MAP while grazing the contaminated pasture, and yearling animals should not be considered resistant to infection via grazing.

This study has documented that cattle grazing pasture with fecal pads from infected adults will consume MAP organisms. Exposure of yearling cattle to pastures contaminated with MAP, therefore, can result in infection with MAP, and age resistance to infection can be overcome by pressure of infection.

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