Dynamics of endemic infectious diseases of animal and human importance on three dairy herds in the northeastern United States

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ABSTRACT

Endemic infectious diseases in dairy cattle are of significant concern to the industry as well as for public health because of their potential impact on animal and human health, milk and meat production, food safety, and economics. We sought to provide insight into the dynamics of important endemic infectious diseases in 3 northeastern US dairy herds. Fecal samples from individual cows and various environmental samples from these farms were tested for the presence of major zoonotic pathogens (i.e., *Salmonella*, *Campylobacter*, and *Listeria*) as well as commensal bacteria *Escherichia coli* and enterococci. Additionally, the presence of *Mycobacterium avium* ssp. *paratuberculosis* was tested in fecal and serum samples from individual cows. Test results and health and reproductive records were maintained in a database, and fecal, plasma, DNA, and tissue samples were kept in a biobank. All bacteria of interest were detected on these farms and their presence was variable both within and between farms. The prevalence of *Listeria* spp. and *L. monocytogenes* in individual fecal samples within farm A ranged from 0 to 68.2% and 0 to 25.5%, respectively, over a period of 3 yr. Within farm B, continuous fecal shedding of *Salmonella* spp. was observed with a prevalence ranging from 8 to 88%; *Salmonella* Cerro was the predominant serotype. Farm C appeared less contaminated with *Salmonella* and *Listeria*, although in the summer of 2005, 50 and 19.2% of fecal samples were positive for *Listeria* and *L. monocytogenes*, respectively. The high prevalence of *E. coli* (89 to 100%), *Enterococcus* (75 to 100%), and *Campylobacter* (0 to 81%) in feces suggested they were ubiquitous throughout the farm environment. Fecal culture and ELISA results indicated a low prevalence

of *Mycobacterium avium* ssp. *paratuberculosis* infection in these farms (0 to 13.6% and 0 to 4.9% for culture-positive and ELISA-positive, respectively), although the occasional presence of high shedders was observed. Results have major implications for food safety and epidemiology by providing a better understanding of infectious disease dynamics on dairy farms. Comprehensive understanding of these infections may lead to better farm management practices and pathogen reduction programs to control and reduce the on-farm contamination of these pathogens and to prevent their further entry into the food-chain.

Key words: infection dynamics, dairy herd, longitudinal study, epidemiology and food safety

INTRODUCTION

Infectious diseases are of increasing concern on dairy farms because of their potential impact on animal and human health, milk and meat production, food safety, and economics. Dairy farms are recognized as important reservoirs of foodborne pathogens. Isolation of bacterial pathogens with similar biotypes from dairy farms and from outbreaks of human disease substantiates the hypothesis of a constantly maintained reservoir of foodborne pathogens in the dairy environment that can affect humans by direct contact, ingestion of beef, raw contaminated milk and cheese, or cross-contamination during processing of milk products (Oliver et al., 2005). Although common infectious diseases such as bovine viral diarrhea, bovine rhinotracheitis, and contagious mastitis typically do not pose human health threats, some diseases found in dairy animals are caused by zoonotic pathogens (i.e., salmonellosis, campylobacteriosis, listeriosis) or potentially zoonotic pathogens (i.e., Johne's disease; Grant, 2005). Dairy cattle and their environment may harbor pathogens that pose potential human health hazards (Nam et al., 2004).

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Contamination of foods with pathogens such as *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. is a major public health concern worldwide as these pathogens can cause diseases with significant debilitating effects (Nam et al., 2004). Ruminants are natural reservoirs of these microorganisms and frequently appear nonsymptomatic while shedding these bacteria into the environment (Gansheroff and O'Brien, 2000). Pathogens such as *Salmonella* can persist on dairy farms with few or no clinical manifestations (Van Kessel et al., 2007). Shedding of *Salmonella* occurs with the presence of clinical illness or in the absence of clinical signs (Edrington et al., 2004). *Listeria monocytogenes* is another bacterial pathogen that causes significant morbidity and mortality in humans and animals and can be shed in the fecal material of clinically infected animals; however, healthy animals can be latent carriers of *L. monocytogenes* (Borucki et al., 2004; Nightingale et al., 2004). Raw milk, meat, and raw foods of plant origin that were cross-contaminated by manure from infected or shedding animals represented direct links between infected humans and *L. monocytogenes* in farm animals and farm environments (Borucki et al., 2004; Nightingale et al., 2004). Healthy cattle may be reservoirs for several *Campylobacter* species such as *Campylobacter jejuni* and *Campylobacter coli* (Wesley et al., 2000; Harvey et al., 2004). Because of their increasing resistance to antibiotics, the commensal bacteria *Escherichia coli* (Lundin et al., 2008) and enterococci have become a focus of attention (Franz et al., 1999). *Mycobacterium avium* ssp. *paratuberculosis* (MAP), a slow-growing bacterial pathogen of ruminants, causes paratuberculosis (Johne's disease), an incurable chronic disease of ruminants (Gollnick et al., 2007), resulting in animal welfare and economic issues. On US dairy farms, herd-level prevalence of MAP infection based on serologic testing (ELISA) was at least 22%, with a prevalence of at least 40% for herds over 300 animals (Wells and Wagner, 2000). Within each infected herd <10% of animals typically test positive for MAP (Mitchell et al., 2008).

Zoonotic pathogens were isolated from dairy cattle and from various locations within dairy farm environments such as manure, water, feed, bird droppings, feral animals, flies, and bedding materials (Murinda et al., 2004; Van Kessel et al., 2007). Therefore, understanding the persistence of these organisms in dairy cattle and their environment is essential to understanding their sources, possible reservoirs, and further dissemination, and for evaluating on-farm control strategies. Food safety, animal health, and productivity have a common need for improved understanding of the herd-level dynamics of bacterial infection. Many investigations have focused on single point-in-time

sampling or multiple samples over a short period of time on a large number of herds. The objective of this paper was to describe longitudinal sampling and provide insight into the dynamics of important endemic infectious diseases in 3 well-managed northeastern US dairy herds.

MATERIALS AND METHODS

Description of the Research Program and Dairy Farms

The dairy farms from which samples were collected participate in a multi-state research program conducted under a cooperative research project between the Regional Dairy Quality Management Alliance and the USDA-ARS. The Regional Dairy Quality Management Alliance comprises stakeholders in 10 states in the northeastern and mid-Atlantic areas of the United States and its mission is to ensure a healthful and safe food supply by advocating the adoption of Best Management Practices in northeast dairies. This project emphasizes longitudinal data collection of endemic infectious diseases of public health and animal health concern in dairy herds.

All data comprising information about farm management surveys, herd and cow level information such as laboratory test results, health and reproductive records was maintained in a database at Cornell University (Ithaca, NY). Biological samples were kept in a secure biobank at New Bolton Center, University of Pennsylvania, to allow for future research endeavors that require samples collected over the lifetime of cows in dairy herds with a known infectious status. This biobank contains a repository of well-characterized fecal and plasma replicates from each cow during each sampling time, DNA samples from each cow, and tissue samples from the culled cows. Bacterial isolates were stored indefinitely at each laboratory location.

Three commercial dairy farms participated in this study: farm A in New York State, farm B in Pennsylvania, and farm C in Vermont. Farms were chosen to represent dairy farms in their respective region and state. Each of these farms participated in the dairy quality assurance program in their state and had DHIA membership with monthly milk testing, Farm Animal Identification and Records implementation, and computerized on-farm disease recording. The milking herds consisted of approximately 330, 110, and 140 cows on farms A, B, and C, respectively. Farm stalls were fitted with comfort mattresses and bedded with bedding materials such as sand and wood products. Replacement animals were primarily from within the herd, although replacements were occasionally purchased.

Sampling, Sample Collection, and Sample Handling

Sampling commenced in February, March, and November 2004 on farms A, B, and C, respectively. The project design included biannual collection of individual fecal samples and quarterly collection of blood serum from all milking and nonlactating cows. Environmental samples were collected quarterly from several sources, including source water, water from drinking troughs of cows and heifers, standing water or slurry in freestall barns, feed from different pens of cows and calves, manure composites from milking cows, nonlactating cows, periparturient cows, and calf pens as well as the milking alleyways, calf bedding, corn silage, bird droppings, flies, and insects. Environmental sampling sites were mapped at the initial visit and, as much as possible, the same location was sampled over time. In special interest situations, sampling frequency was increased and more samples at shorter time intervals were collected; these samplings were identified as special samplings. For example, special samplings from the milking parlor, milking equipment, and other environmental sources were done from farm A to track the source of *Listeria* contamination, as the milking equipment appeared contaminated with *Listeria*. Similarly, the occurrence of salmonellosis on farm B prompted more intensive sampling of environmental sources and individual cows for fecal samples to track the course of the outbreak (Van Kessel et al., 2007).

Fecal samples were collected directly from the rectum using individual disposable gloves and were aseptically transferred into sterile vials (50-mL plastic tube, BD Falcon propylene screw top, BD Biosciences, San Jose, CA). After collection, fecal tubes labeled with the corresponding sample numbers were placed into large Ziploc bags (approximately 20 fecal tubes into each bag), which were then placed into coolers with ice packs. Environmental samples were collected in sterile containers that were appropriate for the sample such as Whirl-Pak bags (Nasco, Ft. Atkinson, WI) for feed samples, centrifuge tubes for fecal slurry (BD Biosciences), and sterile bottles (500 mL or 1 L) for water samples. On farm A, during special samplings from the milking parlor, samples were collected using a Bacti-Sponge kit (Hardy Diagnostics, Santa Maria, CA) dampened with 10 mL of neutralizing buffer (Difco, BD Diagnostics, Sparks, MD) and sterile cotton swabs. Two full blood tubes (2 heparin tubes) were collected from each cow using the tail vein. The blood samples were placed in test tube racks, double wrapped in plastic wrap, and packed in the cooler with ice packs.

All coolers containing collected samples were transported overnight to the 3 laboratories for further analysis. Fecal and environmental samples were sent to the

Environmental Microbial Safety Laboratory of the ARS (Beltsville, MD) and the Bacterial Epidemiology and Antimicrobial Resistance laboratory of Russell Research Center (Athens, GA) for testing of foodborne pathogens such as *Salmonella*, *Listeria*, and *Campylobacter*, as well as the commensal bacteria, generic *E. coli*, and *Enterococcus*. Blood and fecal samples were sent to the Johne's Research Laboratory (New Bolton Center at the University of Pennsylvania) for MAP testing.

Microbial Analysis

For detection of *Salmonella* and *Listeria* spp., upon arrival at the Environmental Microbial Safety Laboratory, approximately 25 g of feces or composite manure sample was weighed into a filtered stomacher bag (GSI Creos Corporation, Tokyo, Japan), diluted (2:1, wt/wt) with 1% buffered peptone water (BPW) and pummeled in an automatic bag mixer (BagMixer, Interscience Laboratories Inc., Weymouth, MA) for 2 min. Typically, larger aliquots of feed samples (40 to 60 g) were used and when the samples were of low moisture content, larger volumes of BPW were required for extraction. For detection of *E. coli*, *Enterococcus*, and *Campylobacter* at the Bacterial Epidemiology and Antimicrobial Resistance laboratory, 1-g fecal samples were diluted 1:9 (wt/vol) in sterile PBS (0.1 M, pH 7.2), and 100- μ L aliquots were inoculated into selective enrichment media. For feed samples, 10 g was placed in a Whirl-Pak bag into which 90 mL of 1 $\times$ BPW was added and incubated at 37°C for 24 h. For water, 100 mL of 10 $\times$ BPW was added to 900 mL of sampled water and incubated at 37°C for 24 h.

***Listeria* spp.** Fecal samples were collected from all cows; however, microbial analysis for *Listeria* was done on approximately every fifth randomly selected fecal sample for most of the samplings. In some cases microbial testing was done for all fecal samples collected. For enrichment of *Listeria* spp., 5 mL of fecal filtrate was added to 5 mL of double-strength modified *Listeria* enrichment broth (BD Diagnostics). Water (250 μ L) was plated in triplicate directly onto modified Oxford medium (MOX) agar (Difco Laboratories, Detroit, MI) using an Autoplate 4000 spiral-plater (Spiral Biotech, Gaithersburg, MD). Plates were incubated at 37°C and scored for presumptive *Listeria* spp. colonies (esculin hydrolysis; dimpled, flat, silver colonies) at 24 and 48 h. For enrichment of *Listeria*, water (100 mL) was filtered through sterile 0.45- μ m cellulosic filters (47 mm; Osmonics Inc., Westborough, MA) with suction, and the filters were placed in 10 mL of modified *Listeria* enrichment broth (BD Diagnostics).

For all samples, enrichment tubes were incubated at 37°C for 48 h and the enriched broth was streaked

(10 μ L) onto MOX (BD Diagnostics). Cycloheximide-supplemented MOX (50 μ g/mL; **C-MOX**) was used for fecal samples to inhibit fungal growth. Plates were incubated at 37°C and scored for presumptive *Listeria* colonies as above at 24 and 48 h. Isolated, presumptive *Listeria* colonies were patched (biomass transfer) from MOX or C-MOX plates onto MOX, polymyxin acriflavin lithium-chloride ceftazidime esculin mannitol (**PALCAM**, BD Diagnostics), trypticase soy agar with 0.6% yeast extract, and a chromogenic plating medium, BCM *Listeria* (Biosynth International Inc., Naperville, IL). Colonies that exhibited the *Listeria* phenotype (as described above on MOX, gray-green with esculin hydrolysis on PALCAM) were preserved for future analysis. Colony biomass was transferred from the PALCAM plates to 1.5 mL of tryptic soy broth and incubated at 37°C for 48 h. The culture was centrifuged (16,000 $\times$ g) and the supernatants were discarded. The bacterial pellet was resuspended in 0.5 mL of 1 $\times$ freezing medium, and the isolates were stored at -80°C. Hemolytic activity of select presumptive *L. monocytogenes* isolates (blue colonies on BCM *Listeria*) was determined by stabbing blood agar (Columbia with 5% sheep blood; Remel, Lenexa, KS) and incubating at 37°C for 48 h. The Christie, Atkins, Munch-Peterson test was performed on select isolates using *Staphylococcus aureus* Beta Lysin Disks (Remel) and *Rhodococcus equi* (ATCC 6939; American Type Culture Collection, Manassas, VA) on sheep blood agar.

Salmonella. For enrichment of *Salmonella*, 5 mL of fecal filtrate was added to 5 mL of double-strength tetrathionate broth (BD Diagnostics). For all samples, enrichment tubes were incubated at 37°C for 18 to 24 h after which 10 μ L of the broth was streaked onto xylose lysine tergitol 4 (**XLT4**) agar (XLT4 agar base with XLT4 supplement, BD Diagnostics). Plates were incubated at 37°C and scored at 24 and 48 h for presumptive *Salmonella* (black colonies). Isolated, presumptive *Salmonella* colonies (at least 6 randomly chosen isolates per sample) were transferred from XLT4 plates onto XLT4, brilliant green, and L-agar (Lennox broth base with 1.5% agar; Gibco Laboratories, Long Island, NY) and incubated at 37°C for 24 h. Colonies that exhibited the *Salmonella* phenotype (black on XLT4 and pink on brilliant green agar) were preserved for future analysis. The isolates were stored at -80°C. Water samples were filtered (100 mL) through sterile 0.45- μ m cellulosic filters (47 mm; Osmonics Inc.) and filters were placed in 10 mL of tetrathionate broth and incubated for 18 to 24 h at 37°C. Enrichments were streaked and preserved as above. Water samples were directly spiral-plated (250 μ L) onto XLT4 agar using an Autoplate 4000 spiral-plater (Spiral Biotech), incubated at 37°C, and scored at 24 and 48 h for *Salmonella* as above.

Salmonella isolates were serotyped as described previously (Van Kessel et al., 2007). Briefly, in the initial phases of the study, *Salmonella* isolates were sent to the National Veterinary Services Laboratories (Ames, IA) for serotyping. Once the herd prevalence rate increased to >40%, isolate serotypes were determined with repetitive sequence-based PCR using the enterobacterial repetitive intergenic consensus primers. An isolate with an enterobacterial repetitive intergenic consensus-PCR pattern that was similar to a pattern of an isolate serotyped by National Veterinary Services Laboratories was considered of that serotype.

***E. coli*, *Enterococcus* spp., and *Campylobacter*.** Microbial analysis for these microorganisms was done on approximately every randomly selected fourth fecal sample. For *E. coli* detection, 100- μ L aliquots of fecal dilutions (1:9 wt/vol, in PBS) and 100- μ L aliquots of other enriched samples (in BPW) were streaked for isolation onto CHROMagar EEC (Hardy Diagnostics, Santa Maria, CA) plates. The plates were incubated for 18 to 24 h at 37°C. For detection of *Enterococcus*, 100- μ L aliquots of fecal dilutions (1:9 wt/vol, in PBS) and 100- μ L aliquots of other enriched samples (in BPW) were inoculated into 24-well tissue culture plates (BD Biosciences) containing 1 mL of enterococcosel broth (BD Diagnostics) per well. The enrichment broth was incubated for 18 to 24 h at 37°C, followed by streaking for isolation onto enterococcosel agar (BD Diagnostics). Isolates were identified by species using a multiplex PCR (Jackson et al., 2004). For *Campylobacter* spp., 100- μ L aliquots of fecal dilutions (1:9 wt/vol, in PBS) and 100- μ L aliquots of other enriched samples (in BPW) were inoculated into 24-well tissue culture plates (BD Biosciences) containing 1 mL of Bolton broth enrichment media (BD Diagnostics) per well. The enrichment broth was incubated for 36 to 48 h at 42°C under microaerobic conditions (5% O₂, 10% CO₂, and 85% N₂), followed by streaking for isolation onto Campy-Cefex agar plates (Stern et al., 1992). Agar plates were incubated for 36 to 48 h at 42°C under microaerobic conditions (as described above). Presumptive *Campylobacter* colonies were selected by observation of cellular morphology and motility using a wet mount under phase-contrast microscopy. Isolates were identified by species using a commercial multiplex PCR (BAX PCR, DuPont Qualicon, Wilmington, DE). For *E. coli*, *Enterococcus*, and *Campylobacter* isolation, environmental samples other than manure composites were enriched in BPW (1:9 wt/vol, 18 to 24 h at 37°C) and then were struck for isolation onto CHROMagar EEC, enterococcosel agar, and Campy-Cefex, respectively. Agar plates were incubated as described for fecal samples.

MAP. Upon receipt at the laboratory, blood tubes were centrifuged for 10 min at 900 $\times$ g and the plasma

was separated. Harvested plasma was placed in three 1.5-mL screw capped vials (with a rubber O-ring seal) labeled with farm identification, cow identification, and collection date and stored in a -20°C freezer or stored in a refrigerator for up to 3 d when they could not be processed immediately. Stored plasma samples were taken from the freezer and allowed to come to room temperature before they were processed for ELISA testing. The sample vial was inverted several times to ensure complete mixing. Plasma samples were evaluated with the ParaCheck (Prionics USA Inc., La Vista, NE; formerly CSL/Biocor) ELISA to monitor serologic status of the cows.

For fecal samples, 2 g was placed in a 50-mL plastic tube containing 35 mL of water (fecal-water tube). The contents were shaken vigorously and placed on a mechanical shaker for a minimum of 30 min. Following mixing, the sample was allowed to stand at room temperature for 30 min. A 5-mL sample from the top portion of the fecal-water tube was transferred to a second 50-mL plastic centrifuge tube containing 25 mL of 0.9% hexadecylpyridinium chloride in half-strength brain heart infusion broth solution (final concentration of hexadecylpyridinium chloride = 0.75%). Next, tubes were incubated at 35 to 37°C for 18 to 24 h (decontamination or germination step). Following the germination, tubes were centrifuged for 30 min at $900 \times g$, the supernatant was discarded, and the pellet was resuspended by adding 1 mL of antibiotic brew (1 L

of half-strength brain heart infusion broth, 18.5 g/L; amphotericin B, 50 mg/L; nalidixic acid, 100 mg/L; vancomycin, 100 mg/L) followed by vortexing. In the next step (incubation step), the resuspended pellet was incubated overnight or up to a maximum of 3 d at 35 to 37°C . Following incubation, 4 tubes of Herrold's egg yolk media [2 in-house and 2 commercial (BD Diagnostics)] were inoculated with 0.2 mL per tube and then incubated in a slanted position at 37°C . The tubes were read every 2 wk with the final reading at 16 wk. Slightly raised white-yellow colonies were evaluated for typical acid fastness and morphological appearance of MAP. Each culture with colony growth was subcultured for mycobactin dependency before reporting the culture positive for MAP.

RESULTS

Listeria spp.

The prevalence of *Listeria* spp. and *L. monocytogenes* in individual fecal samples on 3 farms is given in Table 1. The percentage of *Listeria* spp. and *L. monocytogenes* in all environmental samples is shown in Figure 1. Three out of 10 environmental samples (i.e., 2 barn water samples and 1 calf manure composite sample) were positive for *Listeria* spp. and *L. monocytogenes* during the first farm A sampling in February 2004. On the second sampling in June 2004, 6 samples (3 manure

Table 1. Prevalence¹ of *Listeria* spp. and *Listeria monocytogenes* in fecal samples collected from dairy cows on 3 farms over the study period

Farm	Sampling date	Samples collected (n_c) ²	Samples tested (n_t) ³	<i>Listeria</i> spp.	<i>Listeria monocytogenes</i>
A	17-Feb-2004	308	72	25.0	4.2
	5-Oct-2004	326	65	0.0	0.0
	12-Apr-2005	335	66	21.2	10.6
	3-Oct-2005	308	63	7.9	1.6
	1-May-2006	327	327	20.5	3.7
	9-Oct-2006	333	0	NA ⁴	NA
	16-Apr-2007	333	110	68.2	25.5
B	22-Mar-2004	103	12	0	0.0
	13-Sep-2004	110	10	0	0.0
	6-Jun-2005	111	111	0	0.0
	4-Dec-2005	108	0	NA	NA
	5-Jun-2006	104	1	0	0.0
	4-Dec-2006	103	0	NA	NA
	5-Jun-2007	111	0	NA	NA
C	15-Nov-2004	125	26	0.0	0.0
	9-May-2005	134	26	50.0	19.2
	14-Nov-2005	132	132	0.0	0.0
	15-May-2006	168	0	NA	NA
	13-Nov-2006	141	141	7.8	0.0
	21-May-2007	147	0	NA	NA

¹Prevalence is calculated as the number of positive samples divided by the number of samples tested (n_t) expressed as percentage. The positive samples for *Listeria* spp. also include *L. monocytogenes* positive samples.

² n_c is the total number of individual fecal samples collected.

³ n_t is the total number of samples used for microbial tests.

⁴Samples were not tested.

composite, 1 feral fecal, 1 insect, and 1 standing water sample) were positive for *Listeria* spp. and 1 of these (feral fecal) was positive for *L. monocytogenes*. In a special sampling on June 29, 2004, 2 fly samples and 1 insect sample were collected, and all 3 were positive for *Listeria* spp., 1 of which (fly sample) was positive for *L. monocytogenes* (Figure 1). During a special farm A sampling in May 2007, samples were collected from a total of 40 sites from milking equipment, milk meters, teat cups and liners, milk filter, bulk tank, and different locations in the milking parlor and storage area including floors and drains. *Listeria* spp. was isolated from 14 sampling sites (35%), 6 (15%) of which were positive for *L. monocytogenes*.

The prevalence of targeted bacteria in different types of environmental samples collected from the dairy facilities on the 3 farms is presented in Table 2. The environmental samples from which *Listeria* spp. and *L. monocytogenes* were isolated in farm A included manure composites, drinking water from water troughs of heifer, lactating, and nonlactating cow pens, TMR, and flies. *Listeria* spp., including *L. monocytogenes*, were never detected in the farm source water on any of the 3 farms (Table 2). The prevalence of *Listeria* spp. and *L. monocytogenes* in drinking water on the 3 farms is greater than manure composites based on samples tested on each sample type (Table 2). On farm A, 61.7 and 29.8% of the total drinking water samples tested

(n = 47) were positive for *Listeria* spp. and *L. monocytogenes*, respectively. The prevalence of *Listeria* spp. and *L. monocytogenes* was 24.8 and 2.3%, respectively, of the total manure composite samples tested (n = 129) on farm A.

Environmental samples containing *Listeria* spp. and *L. monocytogenes* on farm B included manure composites, drinking water from different troughs, lactating cow concentrate (feed), and flies. The percentage of *Listeria* spp. and *L. monocytogenes* was 21.7 and 3.8%, respectively, of the total drinking water samples tested (n = 106) on farm B. Of the total manure composites (n = 224), 4 and 0.9% were positive for *Listeria* spp. and *L. monocytogenes*, respectively, on farm B. On farm C, samples from which *Listeria* spp. and *L. monocytogenes* were detected included fecal droppings from feral animals, bird droppings, manure composites, and drinking water. *Listeria* spp. and *L. monocytogenes* were recovered from 74.3 and 17.1%, respectively, of the total drinking water samples (n = 35) on farm C. *Listeria* and *L. monocytogenes*-positive manure composites represented 8.3 and 0.6% of the total manure composite samples tested (n = 168) on farm C.

***Salmonella* spp.**

The prevalence of *Salmonella*-positive fecal samples is in Table 3. During the first full herd sampling in

Figure 1. Prevalence of *Listeria* spp. and *Listeria monocytogenes* in environmental samples from 3 farms. The positive samples for *Listeria* spp. also include *L. monocytogenes* positive samples. A) farm A: total samples = 347 (including 40 samples on special sampling day, 16-May-2007), average number of samples per sampling = 23, study period from 17-Feb-2004 to 16-May-2007; B) farm B: total samples = 555, average number of samples per sampling = 28, study period from 22-Mar-2004 to 4-Dec-2006; C) farm C: total samples = 277, average number of samples per sampling = 31, study period from 15-Nov-2004 to 13-Nov-2006.

February 2004 in farm A, 1 of 10 fecal samples cultured was positive for *Salmonella* Senftenberg. The only culture-positive sample in the second full herd sampling at this farm on October 2004 was determined to contain *Salmonella* Mbandaka. On farm B, at the first full herd sampling in March 2004, only 1 sample was found positive with *Salmonella* Typhimurium var. Copenhagen. At the next full herd sampling in September 2004, *Salmonella* Kentucky was found in 1 individual animal fecal sample and *Salmonella* Cerro was found in 45 individual animal fecal samples. A more frequent full herd sampling schedule was implemented at this farm to capture the course of the *Salmonella* outbreak. Cerro was the predominant serotype identified and 98% of *Salmonella*-positive animals were shedding *Salmonella* Cerro.

The prevalence of *Salmonella* in various environmental samples is in Figure 2. None of the 10 environmental samples collected in March 2004 from farm B (beginning of the study for this farm) were positive for *Salmonella*. Beginning in June 2004, 45 to 50 environmental samples were collected from farm B weekly for 4 wk in response to the presence of clinical salmonellosis. During the first week, *Salmonella* Typhimurium var. Copenhagen (4 samples) was recovered, whereas in the second week, *Salmonella* Typhimurium var. Copenhagen (2 samples) and *Salmonella* Kentucky (1 sample) were recovered. By the third week, *Salmonella* Typhimurium var. Copenhagen (2 samples) and *Salmonella* Kentucky (10 samples) were recovered, and during the final week, only *Salmonella* Kentucky was isolated from 1 sample. The isolates came primarily from composite manure samples. The prevalence of different serovars of *Salmonella* isolated from samples in the summer of 2004 as well as from the second full herd sampling in September 2004 is presented in a separate box in Figure 2. Manure composites and drinking water were the major nonfecal sources from which *Salmonella* was isolated on farm B, and source water from this farm was never positive for *Salmonella* (Table 2). On farm B, 58.1% (193 of 332) of manure composites and 40.4% (59 of 146) of drinking water samples were positive for *Salmonella*. Additionally, results from fecal and environmental samples from this farm indicated that *Salmonella* Typhimurium var. Copenhagen and *Salmonella* Kentucky were initially recovered, whereas *Salmonella* Cerro persisted in the herd at high prevalence. *Salmonella* was not isolated from farm A environmental samples, except for 1 manure composite sample in June 2004 and another manure composite sample from the pit in October 2004. On farm C, *Salmonella* was isolated at only 2 sampling times: November 2006 and May 2007 (Figure 2), and 7.5% (15 of 199) of the total manure composites were *Salmonella*-positive.

Table 2. Prevalence¹ of targeted bacteria in environmental samples collected from the dairy facilities on 3 farms during the study period

Bacteria	Farm	Manure composite	Drinking water	Feed	Birds or bird droppings	Flies	Source water	Others ²	Total
<i>Listeria</i> spp.	A	24.8 (129)	61.7 (47)	7.3 (55)	18.2 (11)	50.0 (16)	0 (13)	33.3 (36)	28.3 (307)
	B	4.0 (224)	21.7 (106)	2.9 (103)	16.7 (12)	20.0 (15)	0 (17)	14.1 (78)	9.2 (555)
<i>Listeria monocytogenes</i>	C	8.3 (168)	74.3 (35)	22.2 (36)	71.4 (7)	28.6 (7)	0 (9)	26.7 (15)	21.3 (277)
	A	2.3 (129)	29.8 (47)	1.8 (55)	0 (11)	12.5 (16)	0 (13)	5.6 (36)	7.2 (307)
<i>Salmonella</i> spp.	B	0.9 (224)	3.8 (106)	1.0 (103)	0 (12)	6.7 (15)	0 (17)	1.3 (78)	1.6 (555)
	C	0.6 (168)	17.1 (35)	0 (36)	28.6 (7)	0 (7)	0 (9)	6.7 (15)	3.6 (277)
<i>Escherichia coli</i>	A	1.6 (126)	0 (47)	0 (46)	0 (11)	0 (11)	0 (13)	0 (33)	0.7 (287)
	B	58.1 (332)	40.4 (146)	4.0 (151)	16.7 (12)	47.1 (17)	0 (24)	15.1 (93)	36.4 (775)
<i>Enterococcus</i> spp.	C	7.5 (199)	0 (42)	0 (43)	0 (8)	0 (7)	0 (11)	5.6 (18)	4.9 (328)
	A	95.4 (131)	66.7 (48)	56.1 (57)	50.0 (4)	66.7 (3)	21.4 (14)	74.4 (43)	76.0 (300)
<i>Campylobacter</i> spp.	B	94.1 (102)	100 (1)	38.8 (85)	100 (1)	NS ³	12.5 (16)	85.7 (21)	66.8 (226)
	C	96.3 (215)	61.4 (44)	60.5 (38)	25.0 (8)	100 (9)	0 (12)	60.9 (23)	80.8 (349)
<i>Campylobacter</i> spp.	A	92.4 (131)	77.1 (48)	71.9 (57)	75 (4)	100 (3)	21.4 (14)	83.7 (43)	81.3 (300)
	B	99.0 (102)	0 (1)	76.5 (85)	100 (1)	NS	25.0 (16)	76.2 (21)	82.7 (226)
<i>Campylobacter</i> spp.	C	96.7 (215)	84.1 (44)	84.2 (38)	62.5 (8)	66.7 (9)	8.3 (12)	73.9 (23)	87.7 (349)
	A	40.5 (131)	0 (48)	0 (57)	0 (4)	0 (3)	0 (14)	2.3 (43)	18.0 (300)
<i>Campylobacter</i> spp.	B	33.3 (102)	0 (1)	0 (85)	0 (1)	NS	0 (16)	0 (21)	15.0 (226)
	C	17.2 (215)	0 (44)	0 (38)	0 (8)	0 (9)	0 (12)	4.4 (23)	10.9 (349)

¹Prevalence, expressed as percentage, is calculated as the number of positive samples divided by the number of samples (given in parentheses) tested for each sample type.

²Includes miscellaneous samples such as standing water or slurry, feral feces, milk filters, swab samples, bedding, insects, mud puddles, dirt, and so on.

³No sample tested.

Table 3. Prevalence¹ of *Salmonella* spp. in fecal samples collected from dairy cows on 3 farms over the study period

Farm	Sampling date	Sample collected (n _c) ²	Sample tested (n _t) ³	<i>Salmonella</i> spp.
A	17-Feb-2004	308	10	10.0
	5-Oct-2004	326	1	100.0
	12-Apr-2005	335	0	NA ⁴
	3-Oct-2005	308	0	NA
	1-May-2006	327	0	NA
	9-Oct-2006	333	24	0.0
	16-Apr-2007	333	0	NA
B	22-Mar-2004	103	20	5.0
	13-Sep-2004	110	110	41.8
	27-Oct-2004	109	109	68.8
	15-Dec-2004	108	108	59.3
	24-Jan-2005	109	109	68.8
	14-Mar-2005	111	111	66.7
	25-Apr-2005	113	113	57.5
	6-Jun-2005	111	111	29.7
	1-Aug-2005	108	108	8.3
	27-Sep-2005	102	102	28.4
	4-Dec-2005	108	108	88.0
	31-Jan-2006	122	122	72.1
	27-Mar-2006	103	103	59.2
	5-Jun-2006	104	104	83.7
	7-Aug-2006	104	104	70.2
	25-Sep-2006	102	102	68.6
	4-Dec-2006	103	103	44.7
	29-Jan-2007	110	110	72.7
	27-Mar-2007	109	109	66.1
	30-Apr-2007	112	112	67.9
C	5-Jun-2007	111	111	64.9
	15-Nov-2004	125	26	0.0
	9-May-2005	134	0	NA
	14-Nov-2005	132	0	NA
	15-May-2006	168	0	NA
	13-Nov-2006	141	141	2.8
	21-May-2007	147	147	2.0

¹Prevalence is calculated as the number of positive samples divided by the number of samples tested (n_t) expressed as percentage.

²n_c is the total number of individual fecal samples collected.

³n_t is the total number of samples used for microbial tests.

⁴Samples were not tested.

E. coli, *Enterococcus* spp., and *Campylobacter* spp.

The percentage of *E. coli*, *Enterococcus*, and *Campylobacter* in individual fecal samples is in Table 4. The prevalence of generic *E. coli* in fecal samples from these farms varied between 89 and 100% and enterococci between 75 and 100%. The average prevalence of *Campylobacter* spp. in fecal samples from farm C was lower compared with the other 2 farms, although *Campylobacter* was not isolated from any fecal sample during 2 sampling times (April 2005 and May 2006) on farm A and 1 sampling time (June 2007) on farm B. The prevalence of *E. coli*, *Enterococcus*, and *Campylobacter* in environmental samples is in Figure 3; a similar pattern can be observed for these bacteria in each of the 3 farms. *Campylobacter* spp. was not isolated in any of the environmental samples during 2, 5, and 3 sampling times on farm A, B, and C, respectively. In addition to feces, these 3 organisms were detected mostly in manure

composite samples collected from different locations in each farm. *Campylobacter* spp. was never detected in the source water collected from the 3 farms (Table 2).

MAP

The prevalence of MAP in individual dairy cows for the 3 study herds is in Table 5. On farm A, prevalence of MAP-positive cows ranged from 1.5 to 3.2% and from 0.9 to 3.1% as obtained from fecal culture and ELISA analyses, respectively. Cows that were MAP-positive for both fecal culture and ELISA tests ranged from 0.6 to 1.6% in farm A. Fecal culture analysis showed that 6.8% of cows on farm B were positive for MAP in March 2004 followed by a decrease in prevalence to <1% for the remainder of the study period. Cows that were MAP-positive by ELISA (seropositive) ranged from 0 to 2.9% and 0 to 1.9% of cows were positive for both tests on farm B. On farm C, MAP-positive cows

Figure 2. Prevalence of *Salmonella* spp. in environmental samples from 3 farms. A) farm A: total samples = 287, average number of samples per sampling = 22, study period from 17-Feb-2004 to 16-Apr-2007; B) farm B: total samples = 775, average number of samples per sampling = 29, study period from 22-Mar-2004 to 5-Jun-2007; C) farm C: total samples = 328, average number of samples per sampling = 30, study period from 15-Nov-2004 to 21-May-2007.

ranged from 1.7 to 13.6% and from 1.4 to 4.9% from fecal culture and ELISA results, respectively. Cows that were MAP-positive for both tests ranged from 0.7 to 3.0% on farm C. Correlation between the number of seropositive and culture-positive animals was $r = 0.61$. Six animals (ID 1078, 1085, 1148, 1160, 1181, and 1200) on farm A and 5 animals (ID 66, 106, 114, 120, and 164) on farm C that were culture-positive and seronegative became seropositive in subsequent visits. One animal each on farm A (ID 1637) and farm C (ID 216) that was identified as seropositive and culture-negative became culture-positive later in the study. On farm B, none of the animals that were culture-positive and seronegative became seropositive or vice versa in subsequent visits.

For farm A, out of 10 culture-positive animals at visit 1, 4 (40%) were already culled at visit 2, 4 (40%) were identified as culture-positive, and 2 (20%) were

culture-negative. On farm B, among 7 culture-positive animals at visit 1, 1 (14.3%) was already culled at visit 2, 1 (14.3%) was culture-positive, and 5 (71.4%) were culture-negative. Similarly, on farm C, out of 17 culture-positive cows during visit 1, 3 (17.6%) animals were already culled at visit 2, 4 (23.5%) were culture-positive, and 10 (58.8%) were culture-negative.

Based on fecal culture and ELISA results it was found that animal 1180 on farm A was consistently MAP-positive from the February 2004 sampling until the October 2005 sampling, with a maximum fecal concentration of 1.68×10^5 cfu of MAP/g. This animal was eventually culled on January 17, 2006, because of clinical Johne's disease. Similarly, animal 1179 from farm A was MAP-positive from the October 2004 sampling to July 2006, and was subsequently culled on July 31, 2006. In the first 3 samplings at farm B, animal 51 was positive for MAP and was continuously shedding

Table 4. Prevalence¹ of *Escherichia coli*, *Enterococcus* spp., and *Campylobacter* spp. in fecal samples collected from dairy cows on 3 farms over the study period

Farm	Sampling date	Sample collected (n _c) ²	Sample tested (n _t) ³	<i>E. coli</i>	<i>Enterococcus</i> spp.	<i>Campylobacter</i> spp.
A	17-Feb-2004	312	78	94.9	78.2	57.7
	5-Oct-2004	326	82	93.9	95.1	57.3
	12-Apr-2005	335	84	92.9	91.7	0.0
	3-Oct-2005	329	83	98.8	80.7	32.5
	1-May-2006	327	82	96.3	93.9	0.0
	9-Oct-2006	333	84	100.0	91.7	26.2
	16-Apr-2007	333	84	97.6	95.2	44.0
B	22-Mar-2004	103	26	88.5	88.5	61.5
	13-Sep-2004	109	28	100.0	100.0	53.6
	13-Dec-2004	108	28	89.3	75.0	53.6
	6-Jun-2005	111	28	92.9	89.3	21.4
	5-Dec-2005	108	26	96.2	100.0	50.0
	5-Jun-2006	101	26	100.0	92.3	80.8
	4-Dec-2006	103	26	96.2	80.8	76.9
	4-Jun-2007	112	28	92.9	92.9	0.0
	15-Nov-2004	125	32	100.0	100.0	9.4
C	9-May-2005	133	34	97.1	91.2	8.8
	14-Nov-2005	133	34	97.1	100.0	29.4
	15-May-2006	169	44	95.5	100.0	13.6
	13-Nov-2006	142	36	100.0	97.2	13.9
	21-May-2007	146	37	97.3	100.0	29.7

¹Prevalence is calculated as the number of positive samples divided by the number of samples tested (n_t) expressed as percentage.

²n_c is the total number of individual fecal samples collected.

³n_t is the total number of samples used for microbial tests. Samples collected from about every 4th cow were used for the microbial test; that is, about 25% of total fecal samples collected were used for microbial analysis.

high numbers of MAP in the feces with a maximum of 7.42×10^5 cfu/g at the September 2004 sampling. This animal was subsequently culled on October 27, 2004. On farm C, animal 66 tested positive for MAP based on both fecal culture and ELISA tests from February 2005 to May 2006 and was culled on June 15, 2006. Fecal samples from this animal showed a maximum concentration of 2.38×10^5 cfu of MAP/g at the May 2006 sampling.

The birth dates of MAP-positive cows in the 3 farms (positive at least once during the stay period in the farm) are shown in Figure 4 in a time-line fashion. This graph provides an indication of birth-cohort infections. As an example, the cow identifications and birth dates for 2 such clusters are highlighted in 2 dotted boxes for farm A. Cows 1179, 1180, 1181, and 1182 were all positive for MAP and were all born in November 2000. Two cows, 50 and 51 on farm B, were born on the same day (December 01, 1999) and both were positive for MAP. Similarly, on farm C, 2 cows (493 and 495) were positive for MAP and were both born on January 01, 2000.

DISCUSSION

Our results indicate that endemic infectious disease agents with zoonotic potential are common in dairy animals and their environments. The data show that

major bacterial pathogens of animal and human importance associated with these zoonotic diseases were found on our study farms, but their presence was variable both within and between farms. The prevalence of *Listeria* spp. and *L. monocytogenes* in individual animal fecal samples ranged from 0 to 68.2% and 0 to 25.5%, respectively, on farm A over a period of 3 yr (longitudinal study is still in progress). It was reported that fecal samples from healthy cattle frequently contain *L. monocytogenes* and its prevalence ranges from 3.1 to 51% (Skovgaard and Morgen, 1988; Nightingale et al., 2004). Further, cattle may contribute to amplification and dispersal of *L. monocytogenes* into the farm environment. The bovine farm ecosystem frequently maintains a high prevalence of *L. monocytogenes* (Nightingale et al., 2004).

In our study, continuous shedding of *Salmonella* in individual fecal samples was observed on farm B with a prevalence ranging from 8 to 88% after the identification of a *Salmonella* Cerro outbreak in September 2004. In this farm, prevalence of *Salmonella* Typhimurium var. Copenhagen declined within 3 mo, whereas that of *Salmonella* Kentucky increased. Cerro ultimately supplanted these 2 serotypes in individual animals and the environment and persisted in the herd at high prevalence (Van Kessel et al., 2007). The prevalence of *Salmonella* in the United States as estimated from the 1996 and 2002 National Animal Health Monitoring

Figure 3. Prevalence of *Escherichia coli*, *Enterococcus*, and *Campylobacter* spp. in environmental samples from 3 farms. A) farm A: total samples = 300, average number of samples per sampling = 21, study period from 17-Feb-2004 to 9-Jul-2007; B) farm B: total samples = 226, average number of samples per sampling = 14, study period from 22-Mar-2004 to 4-Jun-2007; C) farm C: total samples = 349, average number of samples per sampling = 29, study period from 15-Nov-2004 to 20-Aug-2007.

Systems (NAHMS) dairy surveys was 27.5 and 31%, respectively (USDA-APHIS, 2005). Additionally, in a multi-state survey, *Salmonella* spp. were isolated from >90% of dairy farms, 4.8% of fecal samples, and 5.9% of environmental samples from 110 organic and conventional dairy farms in Minnesota, Wisconsin, Michigan, and New York State (Fossler et al., 2004).

Although *Campylobacter* spp. can be considered commensal organisms (Wesley et al., 2000) or normal flora in cattle, they may cause abortion, infertility, and embryonic death in cows and lead to diarrhea in newborn calves. Foodborne transmission of *Campylobacter* can occur through fecal contamination of milk or water or of carcasses at slaughter (USDA-APHIS, 2003). The

prevalence rates for *Campylobacter* in cattle vary from 5 to 89.4% (Harvey et al., 2004) and *Campylobacter jejuni* and *Campylobacter coli* in dairy cattle range from 5 to 53% (Wesley et al., 2000), depending on methods of isolation (direct plating or enrichment), age of animal (calf or adult), season, and sample analyzed (feces or intestinal contents). The prevalence of *Campylobacter* spp. on the 3 study farms was different, with average prevalence higher on farm B (0 to 81%) and lower on farm C (9 to 30%). The percentage of *Campylobacter*-positive fecal samples on farm A was as high as 58% for 2 sampling times in 2004. Previous estimates of *C. jejuni* in dairy cows range from 0.8 to 100% (Wesley et al., 2000). The NAHMS Dairy 1996 study determined that 80.6 and 19.4% of dairy operations were positive for *C. jejuni* and *C. coli*, respectively, by a multiplex PCR assay. In the same study, approximately 38% of dairy cows had fecal samples positive for *C. jejuni*, and 1.8% were positive for *C. coli*. Results from NAHMS Dairy 2002 showed that the percentage of cows shedding *Campylobacter* spp. and the percentage of dairy herds with at least 1 cow shedding *Campylobacter* were 51.4 and 97.9%, respectively, as determined through fecal cultures (USDA-APHIS, 2003). Wesley et al. (2000) detected *C. jejuni* and *C. coli* in 37.7 and 1.8% of fecal samples, respectively, by using PCR assays.

Different species of *Enterococcus* were isolated from feces of ruminants including beef cattle and dairy cows (Franz et al., 1999). *Escherichia coli* were isolated from a total of 95.8% of the samples and 100% (n = 97) of dairy operations (Lundin et al., 2008). As expected, the high prevalence of these commensal bacteria, *E. coli* and enterococci, in the 3 study farms indicated their widespread nature.

Murinda et al. (2004) collected swab, liquid, and solid dairy farm environmental samples from 4 dairy farms in 2002 to 2003 (165 to 180 per farm, a total of 691 samples). *Campylobacter jejuni*, *Salmonella* spp., and *L. monocytogenes*, respectively, were isolated from 5.06, 3.76, and 6.51% of samples evaluated. The observation of a higher percentage of individual fecal and environmental samples positive for *Listeria* spp. on farm A and for *Salmonella* on farm B indicated their consistent presence and possible persistence on these farms, further emphasizing the implementation of necessary on-farm pathogen control approaches.

Five to 10% of animals typically test positive for MAP on infected farms (Benedictus et al., 2008), whereas shedding of high numbers of the organism (>100 cfu/g of feces) was observed in <20% of animals (Mitchell et al., 2008). Fecal culture and ELISA results from the 3 study farms indicated a low prevalence of MAP carriage. Nevertheless, culture results showed the occasional presence of animals that shed very high

numbers of MAP (>300 cfu/g). These animals were all eventually culled. Producers were aware of test results and followed the policy of culling high shedding (MAP) animals as they were no longer economical for milk production; no specific action was taken for low shedding animals. Future studies to determine the relationship between MAP shedding levels and maintenance in herds will be helpful to further elucidate the MAP bio-burden (culture loads) in the herds and the duration of MAP-positive cows staying in the herds. This type of longitudinal study would provide health status information of individual cows in a herd that would help the producers in their decision-making process of managing the farm animals and their environment.

The sensitivity of the ELISA tests depend on the population (stage of infection) of cattle tested (Collins et al., 2005). Despite low sensitivity of serum antibody diagnostic tests (Collins et al., 2005), these low-cost tests are most effective for commercial dairy herds, provided appropriate measures are taken based on ELISA results. The results obtained for this study, which employed a single, commercially available ELISA test (ParaCheck), may not be same for other assays that employ different antigens or otherwise differ in performance. Nonetheless, in Collins et al. (2005), agreement between the ELISA test used in this study and 4 other ELISA assays [HerdCheck (IDEXX Laboratories, Westbrook, ME), Pourquier (Institut Pourquier, Mont-

Table 5. Prevalence¹ of *Mycobacterium avium* ssp. *paratuberculosis*-positive cows on 3 dairy farms over the study period

Farm	Sampling date	Sample tested (n _t)	Fecal culture positive, n (%)	ELISA positive, n (%)	Fecal culture and ELISA positive, n (%)
A	17-Feb-2004	312	10 (3.21)	7 (2.24)	5 (1.60)
	17-Jun-2004	313	— ²	8 (2.56)	
	5-Oct-2004	326	10 (3.07)	10 (3.07)	5 (1.53)
	11-Jan-2005	345	—	9 (2.61)	
	12-Apr-2005	335	7 (2.09)	5 (1.49)	2 (0.60)
	11-Jul-2005	324	—	6 (1.85)	
	3-Oct-2005	329	10 (3.04)	4 (1.22)	3 (0.91)
	20-Feb-2006	321	—	5 (1.56)	
	1-May-2006	327	6 (1.83)	4 (1.22)	2 (0.61)
	10-Jul-2006	336	—	4 (1.19)	
	9-Oct-2006	333	5 (1.50)	3 (0.90)	3 (0.90)
	8-Jan-2007	329	—	NA ³	
	16-Apr-2007	333	10 (3.00)	5 (1.5)	4 (1.20)
B	22-Mar-2004	103	7 (6.80)	3 (2.91)	2 (1.94)
	28-Jun-2004	104	—	2 (1.92)	
	12-Sep-2004	109	2 (1.83)	1 (0.92)	1 (0.92)
	15-Dec-2004	108	0 (0)	0 (0)	
	15-Mar-2005	111	—	0 (0)	
	6-Jun-2005	111	1 (0.9)	0 (0)	
	26-Sep-2005	102	—	0 (0)	
	5-Dec-2005	108	0 (0)	0 (0)	
	27-Mar-2006	103	—	0 (0)	
	5-Jun-2006	102	1 (0.98)	0 (0)	
	25-Sep-2006	104	—	0 (0)	
	4-Dec-2006	103	1 (0.97)	1 (0.97)	0 (0)
	26-Mar-2007	112	—	NA	
	4-Jun-2007	112	1 (0.89)	0 (0)	
C	15-Nov-2004	125	17 (13.6)	2 (1.60)	2 (1.6)
	14-Feb-2005	123	—	2 (1.63)	
	9-May-2005	133	7 (5.26)	4 (3.01)	4 (3.01)
	22-Aug-2005	142	—	7 (4.93)	
	14-Nov-2005	133	5 (3.76)	4 (3.01)	3 (2.26)
	6-Feb-2006	143	—	3 (2.10)	
	15-May-2006	175	3 (1.71)	5 (2.86)	2 (1.14)
	21-Aug-2006	160	—	3 (1.88)	
	13-Nov-2006	142	7 (4.93)	2 (1.41)	1 (0.70)
	12-Feb-2007	154	—	NA	
	21-May-2007	147	5 (3.4)	2 (1.36)	1 (0.68)

¹Prevalence is calculated as the number of positive samples divided by the total number of samples tested (n_t) expressed as percentage. All samples collected were tested for *Mycobacterium avium* ssp. *paratuberculosis*.

²No fecal samples were collected for fecal culture test; only blood samples were collected for ELISA testing on that sampling day.

³Samples were not tested.

Figure 4. Timeline showing birth dates of *Mycobacterium avium* ssp. *paratuberculosis* (MAP)-positive cows as obtained from fecal culture results of 3 farms: A) farm A: a total of 38 positive cows; B) farm B: a total of 11 positive cows; and C) farm C: a total of 33 positive cows during the study period as presented in Table 5.

pellier, France), SERELISA ParaTB (Synbiotic Corp., San Diego, CA), and AntelBio (Antel Biosystems Inc., Lansing, MI)] for diagnosis of bovine paratuberculosis was 77.4 to 92.3%. Diagnosis of Johne's disease was dif-

ficult because many infected animals (up to 90%) may show no signs at the time of sample collection and there is a lack of sensitive and specific assays that detect MAP early during infection (Bannantine et al., 2008).

In contrast to prevailing dogma, it was suggested that tests for MAP-specific antibody such as ELISA-based assays that detect antibodies to lipoarabinomannan antigen, may provide early diagnosis of MAP-infected cattle (Bannantine et al., 2008). Additionally, these tests may be more sensitive than currently available commercial diagnostic tests. The test results obtained from experimentally infected cattle (Bannantine et al., 2008) after further evaluation with naturally infected animals in the field trials may be very helpful for incorporation into a diagnostic test for Johne's disease.

Considering the number of cows that were fecal culture-positive for MAP at least once and the total number of cows that were born during a 6-yr period from the start of 1998 to the end of 2003, a rough calculation of expected probability of fecal shedding on farms A, B, and C was 7, 6, and 13%, respectively. Given the number of births per month, these probabilities lead to significant clusters when 3 or more MAP shedders are born in a given month on farm A and more than 1 shedder is born in a given month on farms B and C. These birth clusters are in Figure 4. In the period preceding the onset of clinical symptoms, infected animals may intermittently shed large quantities of MAP in their feces, milk, and colostrum. This subclinical shedding is generally thought the main factor in maintaining MAP infection in farms in addition to environmental contribution. *Mycobacterium avium* ssp. *paratuberculosis* can survive for up to a year in the environment and can persist for a limited period of time in the absence of susceptible individuals (Schukken et al., 2007).

Previous studies (Wesley et al., 2000; Murinda et al., 2004) on preharvest prevalence of foodborne pathogens focused on one-time-point sampling. Although these investigations provided point prevalence of the pathogens, longitudinal studies conducted concurrently in multiple farms in different geographical areas, such as the one described here, are necessary to provide insight into the dynamics of endemic infectious diseases in dairy herds. It is recognized that endemic infectious diseases are continuously present in the population and elimination of these diseases is not feasible, and in some cases, may be very difficult or impossible (Schukken et al., 2007). Nevertheless, a detailed understanding of the infection process and mechanism for persistence is essential to controlling these infections and lowering their incidence and prevalence in the dairy herds. Intensive longitudinal data are indispensable either to describe precisely the outbreak and subsequent endemic infection of a pathogen in dairy farms such as *Salmonella* in a Pennsylvania dairy farm (Van Kessel et al., 2007) or to describe the transmission dynamics and evaluate the persistence of pathogens in dairy farms through mathematical models (Benedictus et al., 2008; Mitchell et al., 2008). Bene-

dictus et al. (2008) used the data from a unique set of animals within one well-managed dairy herd that was followed over a period of approximately 20 yr to study the transmission dynamics of MAP infection, which is a disease of slow progression. These longitudinal data indicated that calves born shortly following the calving of an infected dam and calves growing up with a future high shedder were more likely infected compared with calves without this risk profile.

In the present study, using similar sampling and culture techniques on multiple farms, there was a difference in the prevalence (lower vs. higher levels) of zoonotic pathogens (including MAP) between farms. The reasons for variations in the prevalence of these pathogens from farm to farm are currently unknown, but may be related to many factors involving farm management, genetics, and nutrition (i.e., exposure, feed types, housing, hygiene condition, and manure management practices). Most bacteria were isolated directly from individual fecal samples; however, composite manure samples, drinking water as presented to cattle, feed, and other vectors (e.g., flies, insects, birds, and feral animals) constituted other areas of concern on these dairy farms. Some of these sources may be defined as critical control points on the dairy farms where pathogen reduction programs can be introduced. The use of preharvest intervention strategies at multiple critical points could control the presence of pathogens in dairy farms.

These data showing the prevalence of pathogens are relevant for pathogen control or eradication through better management and mitigation strategies. Callaway et al. (2003) discussed several potential preharvest intervention strategies (e.g., vaccination, competitive exclusion, the exploitation of the physiology of specific pathogens, and the use of antibiotics) to reduce foodborne pathogens in food animals. The presence of pathogens in the environmental samples suggests interventions in the drinking water troughs at the pen level, good manure management practices, including control of feral animals and in ensuring dairy farm hygiene to prevent further dissemination into the food-chain.

Although infected animals and contaminated environments rarely appear to directly cause human infections, animal-derived food products that are not processed before consumption such as raw milk and other products that appear contaminated during processing, such as meats, represent direct links between human infections and pathogens in farm animals and farm environments. Borucki et al. (2004) compared the pulsed-field gel electrophoresis restriction enzyme digestion profiles of *L. monocytogenes* dairy farm-associated strains, milk, environmental, and bovine, to human sporadic and epidemic disease strains and found that 23% of human

sporadic strains had electrophoresis patterns identical to farm isolates. Dairy farms may serve as a source of pathogens that are inadvertently introduced into food-processing environments and may subsequently be transferred into foods through postprocessing contamination.

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