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Survey of animal-borne pathogens in the farm environment of 13 dairy operations

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ABSTRACT

A survey was conducted on 13 dairies to determine the occurrence of 5 animal-borne pathogens (*Salmonella enterica*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Mycobacterium avium* ssp. *paratuberculosis*, and *Cryptosporidium parvum*) and their distributions across farm elements (feces, bedding, milk filters, stored manure, field soil, and stream water). Presence of *C. parvum* was measured only in feces and stored manure. All but one farm were positive for at least one pathogen species, and 5 farms were positive for 3 species. *Escherichia coli* O157:H7 was detected on 6 farms and in all farm elements, including milk filters. *Mycobacterium avium* ssp. *paratuberculosis* was detected on 10 of 13 farms and in all farm elements except for milk filters. *Salmonella enterica* and *C. jejuni* were detected at lower frequencies and were not identified in soil, stream water, or milk filters on any of the 13 farms. *Cryptosporidium parvum* was detected in feces but not in stored manure. Stored manure had the highest occurrence of pathogens (73%), followed by feces (50%), milk filters, bedding, soil, and water (range from 23 to 31%). Association of pathogen presence with farm management factors was examined by *t*-test; however, the small number of study farms and samples may limit the scope of inference of the associations. Pathogens had a higher prevalence in maternity pen bedding than in calf bedding, but total pathogen occurrence did not differ in calf compared with lactating cow feces or in soils with or without manure incorporation. Herd size and animal density did not appear to have a consistent effect on pathogen occurrence. The extent of pathogen prevalence and distribution on the farms indicates considerable public health risks associated with not only milk and meat consumption and direct animal contact, but also potential dissemination of the pathogens into the agroecosystem.

Key words: dairy environment, animal-borne pathogen, *Escherichia coli* O157:H7, *Mycobacterium avium* ssp. *paratuberculosis*

Short Communication

Food- and waterborne illnesses place a substantial burden on public health. The Centers for Disease Control and Prevention (2011a,b) estimate annual foodborne illnesses in the United States to number 48 million, with additional tens of thousands of cases of waterborne disease. In many instances, zoonotic pathogens from animal production systems that move into the human food chain and water supply are implicated as the source of contamination leading to illness. Pathogens of farm animal origin typically disseminate into the agroecosystem through land-spreading of manures as nutrient sources for growing crops. Once spread with manure to agricultural land, pathogens can survive for extended periods (Nyberg et al., 2010; Toth et al., 2011), leading to the opportunity for contamination of food production and water supply systems (Jamieson et al., 2002).

Dairy cattle can be reservoirs for several pathogens, including *Salmonella* (Blau et al., 2005; Huston et al., 2002), *Escherichia coli* O157:H7 (Murinda et al., 2002; Cho et al., 2006), *Campylobacter* (Colles et al., 2003; Kwan et al., 2008), and the parasite *Cryptosporidium* (Uehlinger et al., 2006; Dixon et al., 2011). Additionally, dairy herds harbor *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), the causative organism for Johne's disease (Whittington et al., 2004), a chronic intestinal disease in ruminants that may be associated with Crohn disease in humans (Over et al., 2011). To gain a comprehensive understanding of relevant prevalence of these pathogens and their distributions in the farm environment, we surveyed 13 dairy operations in southeastern and south-central Pennsylvania for the presence of *Salmonella enterica* (hereafter *Salmonella*), *Escherichia coli* O157:H7, *Campylobacter jejuni* (hereafter *Campylobacter*), MAP, and the protozoan parasite *Cryptosporidium parvum* (hereafter *Cryptosporidium*). Our objectives were (1) to determine the

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relative frequency of the 5 animal-borne pathogens and assess their distribution across different on-farm environmental sample types and locations; and (2) examine if certain farm management parameters (lactating herd size, animal density per unit cropland, maternity pen or calf bedding, cow or calf feces, manure incorporation in crop fields) affected pathogen presence and distribution.

All 13 herds were either exclusively Holstein-Friesian breed or predominantly Holstein with a few Jersey cows on one farm. Herds were selected as a convenience sample from farms participating in an ongoing research study. Herd size and farm management parameters are presented in Table 1. Previous history of pathogen presence on the study farms is unknown, except for farm 2, on which *Salmonella* was identified as recently as 2008 but had not been found since then. On-farm sample collection was performed from April through July 2011. Composite samples, 5 per sample type, were aseptically obtained from lactating cow and calf feces, 1 composite of cow and 1 composite of calf fecal material per farm collected immediately following excretion; calf bedding and maternity pen or close-up cow bedding where available; milk filters (1 each from morning and afternoon milkings, which were combined for analysis); stored manure; crop field soils; and flowing surface water downstream of crop fields (1 sample each were from tile underdrainage and from stagnant water in a seasonal streambed). Samples were transported on ice to the laboratory and analyzed for the presence of the 5 pathogen species (*Salmonella*, *E. coli* O157:H7, *Campylobacter*, MAP, and *Cryptosporidium*). For *Cryptosporidium*, only fecal and manure storage samples were submitted for analysis. *Salmonella*, *E. coli* O157:H7, and *Campylobacter* were detected using real-time PCR with appropriate species-specific commercial primers used to determine pathogen presence. DNA was extracted by using a MagMax total nucleic acid isolation kit (Applied Biosystems Inc., Foster City, CA) with a KingFisher 96 magnetic particle processor (Thermo Scientific, Vantaa, Finland), with 300 μL of sample, 200 μL of lysis/binding solution, 150 μL of isopropanol, 300 μL of wash 1, 450 μL of wash 2, and 75 μL of elution buffer. *Salmonella* was identified with a MicroSEQ *Salmonella* spp. detection kit (Applied Biosystems) used with RapidFinder Express on a 7500 Fast Realtime PCR (Applied Biosystems). Samples positive for *Salmonella* by PCR analysis were cultured on xylose-lysine-deoxycholate agar, and serovars of *Salmonella* isolates were determined using Kauffman-White surface antigen typing (Kauffmann, 1972). *Escherichia coli* O157:H7 was detected with a MicroSEQ *E. coli* O157:H7 detection kit used with RapidFinder Express on a 7500 Fast Realtime PCR. *Campylobacter* were identified with a TaqMan *Campylobacter jejuni* detection kit (Applied BioSystems) used with RapidFinder on a 7500 Realtime PCR. The samples were tested for MAP using the PCR method described by Aly et al. (2010). Extracted DNA was suspended in sterile water. To perform PCR, the suspension was centrifuged and then processed for amplification with a VetAlert Johne's Real-Time PCR kit (Tetracore Inc., Rockville, MD) using bead-beating, chaotropic DNA extraction, and a SmartCycler thermal cycler (Cepheid Inc., Sunnyvale, CA), with a threshold of $\leq$42 cycles for positive identification by PCR. Concurrently, culture confirmation of MAP presence was performed on PCR-positive samples by amplifying an aliquot of the original suspension in brain-heart infusion broth with 0.75% hexadecyl pyridinium chloride. Inoculum was added to Herrold egg yolk medium agar (Becton Dickinson Microbiology, Sparks, MD) and incubated at 37°C for up to 16 wk. Colonies were subcultured on Herrold egg yolk medium agar without mycobactin to confirm MAP identification (Aly et al., 2010). *Cryptosporidium* oocysts were detected by spreading fecal or manure storage sample material on a glass slide, staining with kinyoun carbol fuchsin, washing with ethanol followed by water, and then counterstained with Loeffler methylene blue. *Cryptosporidium* oocysts stain bright red when viewed under magnification (NCCLS, 1997).

Degree of association of selected farm management parameters with pathogen presence was tested using the Wilcoxon-Mann-Whitney nonparametric *t*-test with a probability level of 5% in SAS (SAS Institute, 2008). Total pathogen, MAP, or *E. coli* O157:H7 percentage positive values per farm were the independent variables, and the categories herd size and animal density on cropland, lactating cow versus calf fecal, calf versus maternity pen bedding, and manure incorporation in the field by tillage were dependent variables.

All except one farm had at least one sample that tested positive for one or more pathogens (Table 2). Of the 13 farms, 5 tested positive for 1 pathogen species, 5 tested positive for 3 species, and 2 farms were positive with 2 pathogen species (Table 2). Of all samples (a total of 120), 32.5% were positive for 1 species, 5.0% positive for 2 species, and 1.7% positive for 3 species. Type of samples with pathogen frequency from highest to lowest had the order of stored manure (73%) > lactating cow or calf feces (50%) > field soil (31%) > bedding (29%) > milk filters (26%) > stream water (23%; Table 3).

Of the 5 pathogen species, *E. coli* O157:H7 and MAP occurred at the highest frequencies and with the widest distribution across farm locations (Table 2, Figure 1). *Escherichia coli* O157:H7 was identified at least once in each of the 6 sample types (Figure 1), was the only