Short communication: 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 μ L of sample, 200 μ L of lysis/binding solution, 150 μ L of isopropanol, 300 μ L of wash 1, 450 μ L of wash 2, and 75 μ L 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 Kauffmann-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 ≤ 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

Table 1. Farm management parameters

Farm ID	Lactating herd size	Lactating herd housing ¹	Feeding method ²	Manure storage type	Manure incorporation	Water source sampled	Stocking rate (lactating cows/ha of cropland)
Farm 1	275	Freestall	TMR	Lagoon/bedded pack	Yes	Stream	1.92
Farm 2	170	Freestall	TMR	Lagoon/solids	Sometimes	Seasonal stream	1.65
Farm 3	70	Tiestall	TMR	Slurry tank	No	Tile outflow	1.27
Farm 4	41	Tiestall	Component	Slurry tank	Yes	Stream	1.84
Farm 5	125	Freestall	TMR	Slurry tank	No	Stream	1.27
Farm 6	55	Tiestall	TMR	Slurry tank	Yes	Stream	2.20
Farm 7	43	Tiestall	TMR/topdress	Slurry tank	No	Stream	0.64
Farm 8	61	Tiestall	TMR	Slurry tank	Yes	Stream	0.61
Farm 9	54	Tiestall	TMR	Slurry tank	No	Stream	1.10
Farm 10	104	Freestall	TMR	Slurry tank	No	Stream	3.71
Farm 11	71	Tiestall	TMR	Slurry tank	Yes	Stream	0.12
Farm 12	89	Tiestall	TMR	Slurry tank	No	Stream	0.48
Farm 13	77	Tiestall	TMR	Daily haul	No	Stagnant pool	2.85

¹Tiestall refers to the animals tied to the stall rails, generally with a lead and neck collar. In the freestall condition, cows were allowed to roam freely through stall sections of the dairy barn, often divided into areas of similar milk production.

²Total mixed rations contained all components of a dairy diet—forages, concentrates, plus protein, mineral, and vitamin supplements—blended in a mixer. These blended rations could be tailored to cows' milk production levels and prevent sorting of less-desirable components during ingestion. Component feeding involved offering different types of forages separately, generally with the remaining feed ingredients added as a topdressing.

species detected in samples from milk filters, and was the most frequently occurring pathogen in bedding materials (6 of the 9 total pathogen-positive samples). Detected on 6 of the 13 farms, *E. coli* O157:H7 had a very high presence on the 2 largest farms in terms of lactating cow numbers (farms 1 and 2), with 9 of 11 and 9 of 12 samples testing positive, respectively (Table 2). Reports in the literature suggest a prevalence of toxigenic *E. coli* in individual cow fecal samples in the range of 1 to 4% (Hancock et al., 1997; Murinda et al., 2002; USDA-APHIS, 2003), but a high proportion of herds with at least one animal shedding (up to 75%; Hancock et al., 1997). *Escherichia coli* O157:H7 has

been shown to survive for periods of up to 3 mo in manure-amended soils and a dairy effluent lagoon (J. D. Toth, unpublished data). The widespread prevalence of *E. coli* O157:H7 on dairy operations and its extended viability in the environment indicate a serious risk of movement of the pathogens into the food production system and water supplies and potential deleterious outcomes on human and animal health.

Mycobacterium avium ssp. *paratuberculosis* was found in all on-farm locations except milk filters, and was by far the most common pathogen in stored manure, accounting for 11 of the 15 individual positive samples (Figure 1). This pathogen was also detected on 10 of the

Table 2. Numbers of positive samples for each pathogen species per farm

Farm ID	Total samples	No. of samples positive ¹	<i>Salmonella enterica</i>	<i>Escherichia coli</i> O157:H7	<i>Campylobacter jejuni</i>	MAP ²	<i>Cryptosporidium parvum</i> ³
Farm 1	11	10	3	9	0	2	0
Farm 2	12	10	0	9	1	2	0
Farm 3	9	4	0	2	3	2	0
Farm 4	7	4	0	1	1	2	0
Farm 5	9	1	0	0	0	1	0
Farm 6	9	3	0	0	0	3	0
Farm 7	8	4	1	0	0	3	1
Farm 8	9	2	0	1	0	1	0
Farm 9	10	0	0	0	0	0	0
Farm 10	9	1	0	1	0	0	0
Farm 11	8	1	0	0	0	1	0
Farm 12	9	4	0	0	0	3	0
Farm 13	10	2	0	0	1	0	2
Total		46	4	23	6	20	3

¹Samples positive for any pathogen. Total may not equal the sum of the 5 pathogens because some samples were positive for more than one species.

²*Mycobacterium avium* ssp. *paratuberculosis*.

³*Cryptosporidium parvum* was tested in fecal and manure storage samples only.

Table 3. Prevalence of pathogens (*Salmonella enterica*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Mycobacterium avium* ssp. *paratuberculosis*, and *Cryptosporidium parvum*), by location on farm

Farm location	Total samples	Total farms positive	Total samples positive	Percentage of samples positive
Bedding	21	4	6	29
Feces	26	8	13	50
Stored manure	15	10	11	73
Milk filters	19	5	5	26
Soil	26	8	8	31
Stream water ¹	13	3	3	23

¹Water samples were collected from areas downstream from crop fields; in one case each sample was from tile lines draining a crop field or from a stagnant pool in a seasonal streambed.

13 farms and in 3 of 26 fecal samples. Previously, Raizman et al. (2004) tested 28 Minnesota dairy herds for MAP and found that feces from 77% of cow alleyways and 68% of stored manure samples were positive. On a within-herd prevalence basis, Pradhan et al. (2009) found MAP at much lower levels, 0 to 13.6% of fecal samples from 3 herds in the northeastern United States tested positive over a 3-yr period. Although MAP is an obligate pathogen unable to reproduce outside the host animal, the organism is known to display extended viability in the environment, surviving for up to 55 wk in manure applied to soil (Whittington et al., 2004).

Campylobacter and *Salmonella* were identified in, and *Salmonella* was isolated from, bedding, fecal samples, and stored manure but not in soil, water, or milk filters (Figure 1). Four of the 5 fecal samples positive for *Campylobacter* were from calf feces, and 1 was from lactating cows. For *Salmonella*, 2 serovars were identified, Cerro and Montevideo. Three of the positive samples occurred on farm 1, in lactating cow feces, maternity pen bedding, and lagoon effluent. The lagoon effluent contained both serovars. Earlier studies have identified

a wide variety of *Salmonella* serovars in dairy herds in the United States, including Cerro (Van Kessel et al., 2008) and Montevideo (Blau et al., 2005), with the serovar and prevalence varying by geographic region, season of sampling, and other factors (Edrington et al., 2004). Newport and Typhimurium, the most common *Salmonella* serovars detected in feces from lactating cows in the northeastern United States suspected of having clinical salmonellosis (Cummings et al., 2009), were not isolated in the present study. *Cryptosporidium* was present in 3 of 26 fecal samples but not in stored manure; note that other types of samples (bedding, milk filters, soil, stream water) were not tested for this parasite. Dixon et al. (2011), summarizing previous research in the United States on *Cryptosporidium* occurrence in dairy cattle feces, reported a prevalence of 8 to 49% in calves and 6 to 13% in adult cows.

The 2 largest herds (farms 1 and 2) had the highest percentage of total pathogen-positive samples (83 to 92%; Table 2). Previous studies have reported that larger herds tend to have higher pathogen prevalence (Cho et al., 2006; Cummings et al., 2009; Ruzante et al., 2010). However, Dodson and LeJeune (2005) observed a strong association between herd size and *Salmonella* prevalence but not for prevalence of *E. coli* O157:H7 or *Campylobacter*. Fossler et al. (2005) reported no association between herd size and *Salmonella* occurrence across 129 sampled farms.

We used the nonparametric Wilcoxon-Mann-Whitney *t*-test to evaluate the degree of association between herd size and *E. coli* O157:H7 or total pathogens. Given the convenience sampling and small numbers of study farms and samples per farm, the statistical tests have a relatively low power to detect differences and restricted scope of inference. Results may be viewed as supportive of conclusions but not definitive. In the present study, herd size did not appear to have a consistent significant effect on pathogen prevalence. With an arbitrary number set at 100 lactating cows, *E. coli* O157:H7 occurred more frequently in the larger-farm group than in the smaller-farm group, but the difference was not significant at the 5% level ($P = 0.054$);

Figure 1. Relative distribution of the 5 pathogens [*Salmonella enterica*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Mycobacterium avium* ssp. *paratuberculosis* (MAP), and *Cryptosporidium parvum*] studied across farm sampling locations.

the same lack of significant herd-size effect was noted if the cutoff was set at 65 cows ($P = 0.24$; 65 cows being the average herd size in Pennsylvania). In neither case was a herd-size difference observed in total pathogens ($P = 0.35$ and 0.38 for herd-size groupings of 100 or 65 lactating cows, respectively). On the assumption that higher animal density may increase environmental loading of pathogens following land application of contaminated manures (Atwill et al., 2012), we used animal density (number of lactating cows per hectare of cropland) as the test variable for total pathogens and *E. coli* O157:H7 occurrence. Animal density did not have a significant influence on total pathogens or *E. coli* O157:H7 ($P = 0.14$ and 0.092 , respectively, for animal densities greater and less than 1.5/ha). Previous research has examined effects of grazing beef cattle stocking rate on pathogens in fecal samples (Hoar et al., 2001), but comparable studies have not been conducted on dairy cow density and pathogen occurrence. Also, the Wilcoxon-Mann-Whitney test results suggested a higher total pathogen prevalence in maternity pen bedding than in calf bedding ($P = 0.038$), but total pathogens did not differ between calf and lactating cow feces ($P = 0.39$). Regarding manure application methods in the field, incorporation of manure into the soil may protect manure-borne pathogens from UV insolation as well as drying, hence prolonging pathogen survival (Hutchison et al., 2004). In the present study, the Wilcoxon-Mann-Whitney test suggested no difference regarding the presence of *E. coli* O157:H7, MAP, or total pathogens in soils ($P = 0.11$, 0.46 , and 0.17 , respectively) as a function of manure incorporation.

Previous pathogen prevalence studies have mostly focused on one or a few pathogen species in individual animals or herds (except for Pradhan et al., 2009). The present work enabled us to examine 5 pathogen species and their distribution patterns across different farm elements: animals (feces) and their bedding, manure in storage, field soil, stream water, and milk filters. The high prevalence of pathogens in animal feces and in stored manure and the extent of pathogen occurrence across farm elements have public health implications. Dissemination of the pathogens into the agroecosystem through land application of manure and potentially also through milk and meat consumption and direct animal contact may pose a threat to human and animal health if pathogens move off-farm and into food and water supplies.

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