Research Paper

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Food safety considerations in integrated organic crop–livestock systems: prevalence of *Salmonella* spp. and *E. coli* O157:H7 in organically raised cattle and organic feed

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Abstract

Availability of organic foods has been increasing in the United States due to consumer demand for food produced with a lower environmental footprint. Surveys show that organic consumers view organic food as safer and of higher quality, but a number of food-borne outbreaks have been linked to organic produce in recent years. This outcome suggests that improved food safety protocols are needed to ensure food safety along organic food chains. In this study, model integrated crop–livestock organic systems were established in three states (Iowa, Minnesota and Pennsylvania) to evaluate biological performance and food safety aspects. Organically raised cattle and their feed/forage, feces, hide and meat were examined for the presence of two pathogens, *Escherichia coli* O157:H7 and *Salmonella* spp. Results confirmed that *E. coli* O157:H7 was not isolated on any hide or meat samples. Across all sites, the prevalence rate for *E. coli* O157:H7 on feed/forage and fecal matter was 9.43 and 7.26%, respectively. *Salmonella* spp. was isolated from 1.89, 3.33 and 18.6% of feed, fecal and hide samples, respectively. *Salmonella* spp. was not detected on any meat samples. While *Salmonella* spp. rates on hides were greater than those in feed and fecal samples, the prevalence rate of both pathogens on cattle hides was lower than in previous studies. The use of an organic fly repellent containing essential oils in this study would need to be investigated further to determine any correlation between its use and lower pathogen rates on hides. Feed/forage was more likely to test positive for *E. coli* O157:H7 from June through August, indicating the importance of strict sanitary measures to prevent feed contamination during summer months. These results suggest that careful adherence to food safety management strategies can help reduce the risk of bacterial contamination in integrated crop–livestock systems.

Introduction

Market research shows consumers believe organic foods are safer and healthier than conventional foods (Sofos, 2008; Umberger *et al.*, 2009). Comparisons of organic and conventional foods have shown greater concentrations of beneficial polyphenolic cancer-fighting compounds and a lower incidence of pesticide residues in organic *vs* conventional foods (Barański *et al.*, 2014). However, some organic foods have been linked to food-borne illness outbreaks and microbial safety protocols for organic foods may need further evaluation to minimize the risk of food-borne outbreaks (Harvey *et al.*, 2016). *Escherichia coli* O157:H7 and *Salmonella* spp. are the major pathogens associated with organic foods and responsible for 33 and 44% of all outbreaks, respectively (Harvey *et al.*, 2016). These two pathogens are particularly economically damaging and were estimated to cost the industry over $12 billion in 2010 in the United States alone (Scharff, 2012). From 1998 to 2008, there were a total of 7246 viral- or bacterial-vectored food-borne outbreaks (Crowe *et al.*, 2015), with 16 of the last 18 total bacterial and viral outbreaks associated with organic foods occurring between 2005 and 2015 (Harvey *et al.*, 2016). More recently, studies have shown equal or lower contamination in organic foods compared with conventional counterparts (Gad *et al.*, 2018; Merlini *et al.*, 2018; Becker *et al.*, 2019).

Since 2005, the overall rate of organic sales has increased, with a 29.3% increase in meat and poultry sales (Sustainable Food News, 2016). This trend is expected to continue to into the future (Scaglia, 2017), and studies showing greater nutritional quality in organic *vs* conventional beef (Średnicka-Tober *et al.*, 2016; Ribas-Agusti *et al.*, 2019) may bolster sales. However, cattle are known reservoirs for *E. coli* and *Salmonella* spp. and often carry them asymptomatically, thus making beef an excellent entry point for pathogens into the food supply (Hsi *et al.*, 2015). It is therefore imperative to determine the prevalence of these pathogens

in cattle-based systems and develop effective intervention protocols to ensure food safety compliance.

Since synthetic fertilizers are prohibited in organic systems (USDA-AMS-NOP, 2018), raw livestock manure is often applied to enhance soil fertility. However, manure can harbor *E. coli* O157:H7 and *Salmonella* spp. as a vehicle for transmission of enteric pathogens (Kudva *et al.*, 1998; Islam *et al.*, 2004; Franz *et al.*, 2005; Holley *et al.*, 2007). The survival rate of these pathogens is highly variable and depends on climatic factors (Muirhead, 2009). Contaminated manure has been shown to effectively transfer pathogens to vegetation (Holley *et al.*, 2007; Jacobsen and Bech, 2012). Thus, the application of manure can increase the risk of contamination events, particularly as vegetation may provide an oral pathogen inoculation for grazing cattle.

Livestock feed ingredients such as dried forage, grains, hay and silage have been shown to be a source of both *E. coli* O157:H7 and *Salmonella* spp. (Davies and Wales, 2013). Under supportive environmental conditions these organisms can undergo replication, and, thus, should be monitored in the farm environment (Krytenburg *et al.*, 1998; Lynn *et al.*, 1998; Dargatz *et al.*, 2005; Wales *et al.*, 2010). Examples of food-borne outbreaks associated with animal feed include *Salmonella enterica* subtype Agona that was traced to contaminate fish meal that was fed to cattle (Clark *et al.*, 1973). Davis *et al.* (2003) found that strains of *Salmonella* spp. and *E. coli* O157:H7, isolated from feed, could be found in environmental and fecal samples as well.

Recently, interest in integrating crops and livestock on organic farms has generated concern for adherence to food safety rules related to manure application and contamination in the United States (USDA-FDA, 2018). In one study evaluating the effectiveness of the USDA-NOP 120-day raw manure set-back rule, Patterson *et al.* (2018) found low concentrations of *E. coli* in soil 84–111 days after sheep grazing in an integrated system. In our study, a model integrated crop–livestock system, consisting of annual rotations between crops and forage/pasture systems, was established in three states to measure crop and animal performance, in addition to food safety aspects. The objectives of the study described here were to determine the prevalence of *E. coli* O157:H7 and *Salmonella* spp. in feed, fecal, hide and meat samples from cattle raised in these integrated systems in Iowa, Minnesota and Pennsylvania from August 2015 to March 2017. While the original hypothesis was that there would not be any contamination issues in the integrated system, the alternative hypothesis of differences in *E. coli* and *Salmonella* spp. prevalence rates between months of the study and sample types within these systems would be useful in developing site-specific on-farm and processing facilities' food safety protocols.

Materials and methods

Experimental design and sampling overview

Model integrated systems were established as observational study sites at the University of Minnesota West Central Research and Outreach Center (Morris, MN), the Rodale Institute (Kutztown, PA) and an on-farm site in Iowa (Erlandson Farm, Greenfield, IA). Specific details of the model system design and grazing regime are described in Phillips *et al.* (2017). Briefly, 3-year integrated system rotations were established within 10- to 50-ha sites in Fall 2015–Spring 2016 that included small grain forages of rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) for grazing the first year, followed by row crops of soybeans (*Glycine max*

L.) and corn (*Zea mays* L.), and then a mixed-species pasture of fescue (*Festuca arundinacea* L.), orchardgrass (*Dactylis glomerata* L.), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) (Fig. 1). The Iowa site began with the pasture year followed by a second year of small grain forage, due to land constraints. The study began in August 2015 with 6 to 7-month-old cattle (organic, mixed dairy breed herd consisting of Holstein, Montbeliarde, Viking Red, Normande and Jersey genetics). Each site selected local, organic herds to enhance the site specificity of the project. Sampled herd sizes were 4, 12 and 11 head for Iowa, Pennsylvania and Minnesota, respectively. Cattle grazed on forages/pasture beginning in April when forages were at least 15 cm tall until slaughter in October 2016 in Minnesota and Iowa, and from April to October 2016 in Pennsylvania (cattle not harvested until 2017). Crops and cattle were raised according to USDA-AMS organic regulations as set forth in the National Organic Program (NOP) rules (USDA-AMS-NOP, 2018). Pastures (IA site) and small grain forages (MN and PA sites) received no fertilization, outside of manure deposits, during the study. If pasture/forage was insufficient for cattle nutritional needs, certified organic commercial feed was provided to the cattle based on recommendations from an organic livestock specialist at the University of Minnesota. Cattle had access to minerals and water ad libitum throughout the study.

Feed and forage sampling

Commercial bagged feed and live forage/pasture samples were obtained from each site and tested for presence of *E. coli* O157:H7 and *Salmonella* spp. For feed samples, 100 g of feed were randomly sampled with sterile equipment from feed bags. Forage/pasture matter was clipped at the base of the plant, within a square-foot quadrat, in five random locations of the forage/pasture. Forage samples included plant foliage, along with incidental roots and soil matter. Feed consisted of organic grains, or an organic mixed ration with mineral supplements. Feed and forage/pasture were sampled throughout the season and when steers were moved onto new pastures. Samples were placed in sterile collection bags with cold packs that did not come into direct contact with the samples to avoid influencing microbial analysis. Samples were shipped overnight to the Department of Food Science and Human Nutrition, Iowa State University (Ames, IA) for analysis. Samples were stored in a refrigerator (4°C) and processed within 12 h of arrival. The number of samples taken was based on the number of different feed types provided to the cattle in the study.

Collection of fecal samples

Fecal samples were collected from freshly deposited cow pats at the start of the study at each site, before harvest, and, whenever there was a change in feed. Sterile sample collection cups were utilized to store and transport fecal samples. Fecal sample cups were shipped with cold packs as described above to the Department of Food Science and Human Nutrition at Iowa State University and stored and processed as described above. The number of samples taken was based on the number of cattle present at that specific location.

Hide and meat sampling

Hide surface samples were collected prior to harvest and meat steaks were collected after harvest, using a $10 \times 10\text{-cm}^2$ template with an EZ Reach™ sponge sampler (World Bioproducts, IL,
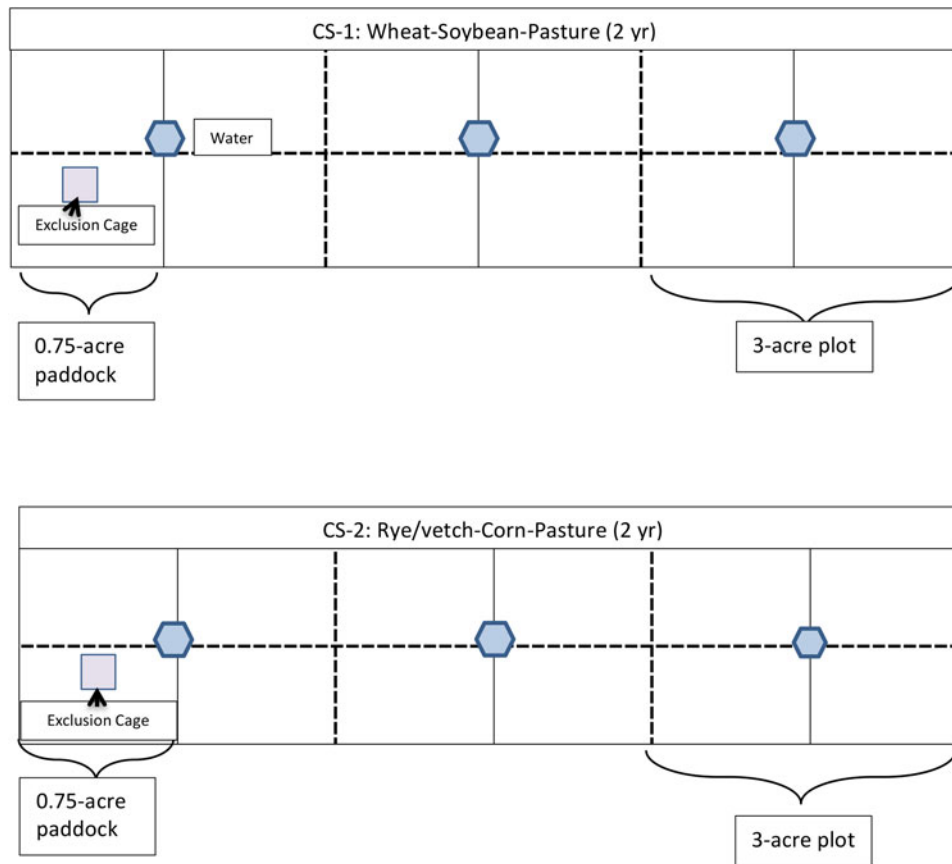
Fig. 1. Experimental design showing the two crop sequence (CS) treatments (rotations) replicated three times as 3-acre plots. The rotations consisted of wheat or rye for grazing in Yr 1, followed by corn or soybean in Yr 2 and ending with a return to pasture in Yr 3–4. Each 3-acre plot was sub-divided into four 0.75-acre paddocks during the grazing phases of the study.

USA). The rump area was sampled for hide and a loin section was swabbed on both sides for the meat samples. All samples were shipped with cold packs as described above to the Department of Food Science and Human Nutrition at Iowa State University and stored and processed as described above. The number of samples taken was based on the number of cattle present at that specific location.

Microbial analysis

Sub-samples (25 g) of feed/forage samples from each site were analyzed for microbial content. Fecal samples were analyzed as sub-samples of 10 g each. For meat swabs, 1 ml of sampling buffer was extruded and sampled. Samples were then selectively enriched in parallel for *E. coli* O157:H7 or *Salmonella* spp. and analyzed using miniVidas® kits (bioMerieux® SA, Marcy-l'Etoile, France) and processed as per manufacturer's instructions. Samples that tested positive were then subjected to confirmatory methods as prescribed by USDA and FDA BAM standards (USDA-FDA, 2017).

E. coli O157:H7 colonies were identified by plating onto Sorbitol MacConkey® agar (Difco®, BD, Franklin Lakes, NJ, USA) with Cefixime® and Tellurite® (CT-SMAC) (Oxiod Microbiological Products, Hampshire, UK) as colorless colonies. Colorless colonies were considered typical and 3–4 colonies per plate, if present, were selected at random to be analyzed using a Dry Spot® latex kit for *E. coli* O157 (Oxiod Microbiological

Products, Hampshire, UK). Agglutination with the latex reagents was considered as positive for *E. coli* O157:H7.

Samples that were presumptively positive for *Salmonella* spp. were streaked in duplicate onto Brilliant green sulfa agar (BGS) (Difco®, BD, Franklin Lakes, NJ, USA) and xylose lysine tergitol™ 4 agar (XLT-4) (Difco®, BD, Franklin Lakes, NJ, USA). Typical colonies were selected as per manufacturer's instructions. Colonies were then used to inoculate butts of triple sugar iron and lysine-iron agar which were then incubated with loosened caps at $35 \pm 2°C$ for 24 h, and examined as a set, for positive responses in accordance with the manufacturer's guidelines.

Statistical analysis

Data were analyzed by the type of sample (feed/forage, fecal, hide and meat), month and location using PROC FREQ and ANOVA in SAS/STAT® 9.3. Percentages displayed in Results are the number of positive or negative samples over the total number of samples obtained from that subset of data.

Results

Prevalence of pathogens in feed/forage samples

The weather during the study period across the three locations was typical of each state's climate, which is categorized as a humid continental climate. Warm to hot and humid weather

Table 1. Weather conditions in 2016 at the three sites of the 'Integrated Organic Crops and Livestock' project

Month	Greenfield, IA		Morris, MN		Kutztown, PA	
	Monthly average air temperature (°C)	Monthly precipitation total (mm)	Monthly average air temperature (°C)	Monthly precipitation total (mm)	Monthly average air temperature (°C)	Monthly precipitation total (mm)
January	−6.71	17.27	−10.74	7.39	−2.03	86.61
February	−1.33	38.37	−5.93	16.79	0.53	124.46
March	6.73	33.03	2.75	15.50	7.05	24.89
April	10.41	113.54	6.32	47.01	9.40	57.40
May	15.41	128.02	14.27	50.55	14.88	135.38
June	23.70	21.08	19.89	48.01	20.82	66.04
July	23.00	137.42	20.88	250.96	24.24	124.21
August	22.54	223.77	20.55	70.88	24.01	96.52
September	19.97	149.61	16.19	42.68	19.99	116.08
October	13.64	32.01	8.86	100.09	12.74	41.15
November	7.73	33.28	4.43	34.04	6.79	55.63
December	−5.03	34.04	−9.48	19.32	1.14	99.82

occurred during the main grazing period of May to September, with the warmest months from June to August (Table 1).

A total of 53 feed/forage samples across all locations was tested for the presence of *E. coli* O157:H7 and *Salmonella* spp. over the course of the study. Overall, five samples tested positive for *E. coli* O157:H7 and one sample was positive for *Salmonella* spp. (Tables 2 and 3). No *E. coli* O157:H7 or *Salmonella* spp. were detected in feed samples from Iowa. Three of sixteen forage samples from Minnesota tested positive for *E. coli* O157:H7, which were obtained from pastures in June and July 2016, 2 months prior to cattle harvest. Two of the twenty feed samples from Pennsylvania tested positive for *E. coli* O157:H7 from the mixed ration feed in August. The sole positive *Salmonella* spp. sample in feed was from Pennsylvania (Table 2) and also was obtained in August from the mixed ration feed. All positive *Salmonella* spp. samples were isolated in the warmer months of June, July and August.

Prevalence of pathogens in fecal samples

A total of 124 fecal samples across all sites was analyzed throughout the study. Nine samples tested positive for *E. coli* O157:H7 and four tested positive for *Salmonella* spp. (Tables 2 and 3). All positive samples for *Salmonella* spp. were from Iowa in August, prior to cattle harvest. *E. coli* O157:H7 was isolated from one sample from Minnesota, obtained during the first sampling period in August at the post-weaning stage. Eight fecal samples from Pennsylvania tested positive for *E. coli* O157:H7 (Table 3). While all Iowa fecal samples tested negative for *E. coli* O157:H7 (Table 3), the Pennsylvania samples that tested positive, collected prior to cattle harvest, caused a significant difference between months sampled, due to low numbers from the other sites (Table 5).

Prevalence of pathogens in hide and meat samples

Sampling of hides occurred prior to cattle harvest. Forty-three hide samples were obtained in July and August 2016 from Minnesota and in August 2016 from the Iowa herd. There were no hide samples from Pennsylvania because harvesting did not occur until the next year when cattle market prices were higher. *E. coli* O157:H7 was not detected on any hide samples, while *Salmonella* spp. was detected on eight samples obtained in August (Tables 2 and 3). Minnesota and Iowa cattle each had four positive samples for *Salmonella* spp. The prevalence rate for *Salmonella* spp. was significantly greater on hides compared with other sample types (Table 4) and greater prevalence occurred in August compared with the other months sampled (Table 5).

Neither *E. coli* O157:H7 nor *Salmonella* spp. was detected from any meat sample obtained from study animals (Tables 2 and 3). Again, no meat analysis occurred in Pennsylvania due to the postponed harvesting. Due to the greater number of positive fecal samples in Pennsylvania over the course of the study, that site expressed a greater rate than the other two sites (Table 6), but the *E. coli* prevalence rate was within the range reported by Reinstein *et al.* (2009).

Discussion

Feed/forage pathogen prevalence

The greatest number of positive *E. coli* and *Salmonella* spp. feed/forage samples was obtained in the warmer months of June, July and August in Pennsylvania and Minnesota, which were the months frequently associated with higher pathogen prevalence rates in the United States, Finland and Australia (Barkocy-Gallagher *et al.*, 2003; Lahti *et al.*, 2003; Hussein, 2007; Williams *et al.*, 2015). *Salmonella* spp. was detected in 1.89% of the 53 samples in this study, which was lower than that determined by Krytenburg *et al.* (1998), who detected *Salmonella* spp. in 9.8% of feed samples consisting of several feed components, obtained between July and November, from dairy farms and feedlots. Davis *et al.* (2003) isolated *S. enterica* in 0.8% of feed samples, obtained from conventional dairy farms in Washington and northwest Idaho, consisting of apple waste, fish meal, hay, grains, cotton seed, rye pellets, beet pulp

Table 2. Prevalence of *Salmonella* spp. in feed, fecal and hide samples of cattle in the 'Integrated Organic Crops and Livestock' project

| | Feed | | | | | | Fecal | | | | | | | | Hide | | | | | |
| | All locations | | MN | | PA | | All locations | | IA | | MN | | PA | | All locations | | IA | | MN | |
Month	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Jan	0	2	–	–	0	2	0	19	0	4	–	–	0	15	–	–	–	–	–	–
Feb	–[a]	–	–	–	–	–	0	4	–	–	–	–	0	4	–	–	–	–	–	–
Mar	–	–	–	–	–	–	0	2	–	–	–	–	0	2	–	–	–	–	–	–
May	0	2	–	–	0	2	0	10	–	–	–	–	0	10	–	–	–	–	–	–
June	0	6	0	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
July	0	3	0	3	–	–	0	14	–	–	0	14	–	–	0	14	–	–	0	14
Aug	1	14	0	3	1	7	4	33	4	18	0	10	–	–	8	21	4	11	4	10
Sept	0	7	–	–	–	–	0	7	0	7	–	–	–	–	–	–	–	–	–	–
Nov	0	18	0	4	0	8	0	27	0	8	0	8	0	11	–	–	–	–	–	–

[a]Sample not taken in this month.

Table 3. Prevalence of *E. coli* O157:H7 in feed, fecal and hide samples of cattle in the 'Integrated Organic Crops and Livestock' project

| | Feed | | | | | | Fecal | | | | | | | | Hide | | | | | |
| | All locations | | MN | | PA | | All locations | | IA | | MN | | PA | | All locations | | IA | | MN | |
Month	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Jan	0	2	–	–	0	2	8	11	0	4	–	–	8	7	–	–	–	–	–	–
Feb	–[a]	–	–	–	–	–	0	4	–	–	–	–	0	4	–	–	–	–	–	–
Mar	–	–	–	–	–	–	0	2	–	–	–	–	0	2	–	–	–	–	–	–
May	0	2	–	–	0	2	0	10	–	–	–	–	0	10	–	–	–	–	–	–
June	2	4	2	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
July	1	2	1	2	–	–	0	14	–	–	0	14	–	–	0	14	–	–	0	14
Aug	2	13	0	3	2	6	1	36	0	18	1	9	–	–	0	29	0	15	0	14
Sept	0	7	–	–	–	–	0	7	0	7	–	–	–	–	–	–	–	–	–	–
Nov	0	18	0	4	0	8	0	27	0	8	0	8	0	11	–	–	–	–	–	–

[a]Sample not taken in this month.

Table 4. Prevalence of *E. coli* O157:H7 and *Salmonella* spp. by sample type across the three study sites (MN, IA and PA) in the 'Integrated Organic Crops and Livestock' project

Sample type	*E. coli* O157:H7 (% positive samples)	*Salmonella* spp. (% positive samples)
Fecal	7.50a[1]	3.33b
Feed	9.43a	1.89b
Hide	0.00a	18.60a
Meat	0.00a	0.00b
P value	0.0961	0.0003

[1]Means followed by the same letter down the column are not significantly different at $P \leq 0.05$.

Table 5. Prevalence of *E. coli* O157:H7 and *Salmonella* spp. by month across all sample types (feed, fecal and hide) from the three study sites (MN, IA, PA) in the 'Integrated Organic Crops and Livestock' project

Month	*E. coli* O157:H7 (% positive samples)			*Salmonella* spp. (% positive samples)	
	Feed	Fecal	Feed	Fecal	Hide
Jan	0.00a[1]	42.11b	0.00a	0.00a	–
Feb	–[2]	0.00a	–	0.00a	–
Mar	–	0.00a	–	0.00a	–
Apr	–	–	–	–	–
May	0.00a	0.00a	0.00a	0.00a	–
Jun	33.33a	–	0.00a	–	–
July	33.33a	0.00a	0.00a	0.00a	0.00a
Aug	13.33a	2.70a	6.67a	13.51a	27.59b
Sep	0.00a	0.00a	0.00a	0.00a	–
Oct	–	–	–	–	–
Nov	0.00a	0.00a	0.00a	0.00a	–
Dec	–	–	–	–	–
P value	0.1453	<0.001	0.8800	0.0862	0.0295

[1]Means followed by the same letter down the column are not significantly different at $P \leq 0.05$.
[2]Sample not taken in this month.

and pea flour. Over an 8-yr period (2002–2009), Li *et al.* (2012) reported a 12.5% *Salmonella* contamination rate in feeds. *E. coli* O157:H7 was found in three pasture samples from Minnesota and two feed samples consisting of a total mixed ration in Pennsylvania. Overall, the 9.43% *E. coli* O157:H7 prevalence rate was greater than that found in the previous study by Davis *et al.* (2003), but less than the 30.1% rate reported by Lynn *et al.* (1998) in dairy cattle feed samples. The *E. coli* prevalence rate observed in this study, however, was similar to the 14.9% prevalence rate observed by Sargeant *et al.* (2004) in feed bins from feedlots.

Fecal pathogen prevalence

Of the 124 fecal samples over the course of the study, *E. coli* O157: H7 was isolated from eight samples from Pennsylvania in January and one from Minnesota in August. This prevalence rate of 7.26%

was similar to the average rate of 9.3%, and within the range of 0–24.4%, found by Reinstein *et al.* (2009) from fecal samples of organically raised cattle. Jacob *et al.* (2009) found a lower prevalence rate of *E. coli* O157:H7 (5.1%) in an experimental setting where cows were fed dried distillers grains or dry rolled corn. Cho *et al.* (2006) found an equivalent *E. coli* O157:H7 rate of 7.4% at an organic dairy farm in Minnesota. A study of grass-fed cattle in Australia demonstrated a prevalence rate of 10% (Fegan *et al.*, 2004*a*). These studies, however, used molecular techniques for enrichment which may have provided a more sensitive evaluation than typically evaluated (Eriksson and Aspan 2007).

Salmonella spp. prevalence rates were similar to previous studies of organic and conventional production systems. The overall *Salmonella* spp. prevalence rate of 3.26% was similar to reports from Minnesota and Wisconsin where *Salmonella* spp. was detected in 5.2% of fecal samples collected from organic dairy farms (Fossler *et al.*, 2005). Similarly, in a study conducted with feedlot cattle in Nebraska, *Salmonella* spp. prevalence was determined to be 5.4% (Schmidt *et al.*, 2015). Feces of grass-fed beef cattle in Australia were found to have a *Salmonella* spp. prevalence rate of 4.5% (Fegan *et al.*, 2004*b*). Herd size has been found to be a significant factor on farms with higher prevalence rates (Fossler *et al.*, 2005; Habing *et al.*, 2012). Thus, smaller herds dispersed across a larger grazing area may lead to lower *Salmonella* spp. prevalence rates compared with feedlot conditions.

Hide pathogen prevalence

Of the 43 hide samples from cattle in Iowa and Minnesota collected before harvest, no samples tested positive for *E. coli* O157:H7. However, four hide samples from Iowa and four from Minnesota tested positive for the presence of *Salmonella* spp. The overall prevalence rate for *Salmonella* spp. on hides was 18.6%. Most studies isolated higher rates of *E. coli* O157:H7 (30–60%) from hides during harvest (Barkocy-Gallagher *et al.*, 2003; Arthur *et al.*, 2007; Brichta-Harhay *et al.*, 2008). Barkocy-Gallagher *et al.* (2003) determined positive *E. coli* O157:H7 samples on 60.6% of hides in a commercial beef processing plant that sourced cattle from the Midwest, while Brichta-Harhay *et al.* (2008) examined hides of cattle from processing facilities across the United States and obtained 33.3% positive hide samples. The current results are in agreement with a study conducted on feedlot cattle in Texas, where a decrease in *E. coli* O157:H7 was observed on hides after transport and before harvest (Barham *et al.*, 2002).

Salmonella spp. prevalence was higher in hide samples (18.6%) taken before harvest than in fecal samples (3.33%), a pattern observed by both Barham *et al.* (2002) and Barkocy-Gallagher *et al.* (2003), who reported an 89% rate (hide) *vs* 46% (fecal) (Barham *et al.*, 2002) and 71% (hide) *vs* 4.4% (fecal) (Barkocy-Gallagher *et al.*, 2003). Greater variation in *Salmonella* spp. prevalence has been observed within seasons (Brichta-Harhay *et al.*, 2008) and before/after transport (Barham *et al.*, 2002). The *Salmonella* spp. prevalence rate on hides observed in our study was much lower than other studies, which reported rates from 6 to 93.8% (Barham *et al.*, 2002; Barkocy-Gallagher *et al.*, 2003; Arthur *et al.*, 2007; Brichta-Harhay *et al.*, 2008) and were highly variable. An Australian study on *Salmonella* spp. prevalence found similar results, reporting that 2% of hides and 7% of fecal samples from organically raised cows were positive for *Salmonella* spp. (Fegan *et al.*, 2005). The *Salmonella* spp.

Table 6. Prevalence of *E. coli* O157:H7 and *Salmonella* spp. in fecal, feed, hide and meat samples by study sites (MN, IA and PA) in the 'Integrated Organic Crops and Livestock' project

Study sites	*E. coli* O157:H7 (% positive samples)				*Salmonella* spp. (% positive samples)			
	Fecal	Feed	Hide	Meat	Fecal	Feed	Hide	Meat
Iowa	0.00b[1]	0.00a	0.00	0.00	0.00	0.00	26.67a	0.00
Minnesota	3.13b	18.75a	0.00	0.00	0.00	0.00	14.29a	0.00
Pennsylvania	14.55a	10.00a	–[2]	0.00	0.00	5.00	–	0.00
P value	0.017	0.190	–	–	–	0.447	0.332	–

[1]Means followed by the same letter down the column are not significantly different at $P \leq 0.05$.
[2]Sample not taken at this location.

prevalence rate, although low in comparison with most studies in organic and conventionally raised cattle, followed the reported trend of higher rates on hides than in fecal samples. It should be noted that a herbal fly repellent containing plant essential oils was used on cattle at all locations. Plant essential oils have been show to kill both *E. coli* O157:H7 and *Salmonella* species (Burt, 2004; Turgis *et al.*, 2008; Bajpai *et al.*, 2012; Boskovic *et al.*, 2015; Khaleque *et al.*, 2016) and may have reduced pathogen load, but this result would need to be substantiated in future studies.

Meat pathogen prevalence

All meat swabs were negative for *E. coli* O157:H7 and *Salmonella* spp. However, all samples were taken post-chilling, which could considerably reduce the prevalence of both pathogens. Hauge *et al.* (2015) saw a significant reduction (1–11%) post-chilling in total *E. coli* populations in their study of two Norwegian abattoirs, although there was no indication of whether the cattle were organically or conventionally raised. Similar to the 0% prevalence in this study, however, Fegan *et al.* (2005) also did not isolate any *Salmonella* spp. post-chilling in organically raised Australian cattle. The low pathogen prevalence rate from earlier sampling points in this study, and the interventions applied, could have led to a further decrease in the number of pathogens by the time of slaughter.

Conclusions

Of critical concern to consumers is the avoidance of human diseases caused by pathogens, such as *E. coli* O157:H7 and *Salmonella* spp., that could be vectored by livestock in integrated systems. The integrated crop–livestock system in this study demonstrated a high probability of meeting food safety goals for limiting *E. coli* O157:H7 and *Salmonella* spp. contamination in the forage, feed, feces, hide and meat of grass-based organic cattle. Overall, the study found a low prevalence rate of *E. coli* O157:H7 in feed and fecal samples that was similar to previous studies conducted with organic and conventional cattle. The *E. coli* O157:H7 rate of 9.43% in feed in this study was comparable with previous studies of feed bins in feedlots, but higher than previously reported for feed sampled directly from feed mills, suggesting that on-farm management strategies, such as ensuring feed bag and bin closing/coverage after each use, could reduce pathogen load. *Salmonella* spp. prevalence, however, at 1.89% of samples, was similar to feed samples from dairy farms in Washington and northwest Idaho, and lower than other studies. Feed from the months of June, July and August had a higher number of

positive *E. coli* O157:H7 samples than other months, as warmer periods may be particularly vulnerable entry points for these pathogens into the food chain, thereby warranting strict food safety protocols.

Isolation of *E. coli* O157:H7 from 7.26% of fecal samples from study cattle was similar to that previously reported for organic dairy cows in Minnesota. The fecal *Salmonella* spp. prevalence rate of 3.26% was much lower than previous studies conducted in different production systems and may be associated with the smaller sized, more dispersed and grass-based herd compared with other studies. No *E. coli* O157:H7 was detected in hide samples taken prior to harvest, where previous studies indicated a higher prevalence rate on hides. The use of an essential-oil-based organic fly repellent may have served a dual purpose of insect and bacterial reduction, but further testing should occur before recommending for this use. *Salmonella* spp. prevalence on hides, with 18.6% testing positive, matched previous studies conducted at abattoirs. *E. coli* O157:H7 and *Salmonella* spp. were not isolated from any meat samples, which was also observed in a similar sized herd of Australian organic cattle. The previously documented pathogen reduction from chilling operations is likely associated with the lack of pathogens on any meat sample in this study. Further studies to identify exact sources of potential bacterial contamination along the production chain, from field to slaughter, will help increase the implementation of safe, integrated crop–livestock systems.

References

Arthur TM, Bosilevac JM, Brichta-Harhay DM, Kalchayanand N, Shackelford SD, Wheeler TL and Koohmaraie M (2007) Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *Journal of Food Protection* **70**, 1076–1079.

Bajpai VK, Baek K-HH and Kang SC (2012) Control of *Salmonella* in foods by using essential oils: a review. *Food Research International* **45**, 722–734.

Barański M, Średnicka-Tober D, Volakakis N, Seal C, Sanderson R, Stewart G, Benbrook C, Biavati B, Markellou E, Giotis C, Gromadzka-Ostrowska J, Rembiałkowska E, Skwarło-Sońta K, Tahvonen R, Janovská D, Niggli U, Nicot P and Leifert C (2014) Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *British Journal of Nutrition* **112**, 794–811.

Barham AR, Barham BL, Johnson AK, Allen DM, Blanton JR and Miller AMF (2002) Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *Journal of Food Protection* **65**, 280–283.

Barkocy-Gallagher GA, Arthur TM, Rivera-Betancourt M, Nou X, Shackelford SD, Wheeler TL, Koohmaraie M and Hruska RL (2003)

Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of Food Protection* 66, 1978–1986.

Becker B, Stoll D, Schlz P, Kulling S and Huch M (2019) Microbial contamination of organically and conventionally produced fresh vegetable salads and herbs from retail markets in southwest Germany. *Foodborne Pathogens and Disease* 16, 269–275.

Boskovic M, Zdravkovic N, Ivanovic J, Janjic J, Djordjevic J, Starcevic M and Baltic MZ (2015) Antimicrobial activity of thyme (*Tymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms. *Procedia Food Science* 5, 18–21.

Brichta-Harhay DM, Guerini MN, Arthur TM, Bosilevac JM, Kalchayanand N, Shackelford SD, Wheeler TL and Koohmaraie M (2008) *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology* 74, 6289–6297.

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology* 94, 223–253.

Cho S, Bender JB, Diez-Gonzalez F, Fossler CP, Hedberg CW, Kaneene JB, Ruegg PL, Warnick LD and Wells SJ (2006) Prevalence and characterization of *Escherichia coli* O157 isolates from Minnesota dairy farms and county fairs. *Journal of Food Protection* 69, 252–259.

Clark GM, Kaufman A, Gangarosa EJ and Thompson M (1973) Epidemiology of an international outbreak of *Salmonella* serovar Agona. *The Lancet* 2, 490–493.

Crowe SJ, Mahon BE, Vieira AR and Gould LH (2015) Vital signs: multistate food-borne outbreaks-United States, 2010–2014. *Morbidity and Mortality Weekly Report* 64, 1215–1220.

Dargatz DA, Strohmeyer RA, Morley PS, Hyatt DR and Salman MD (2005) Characterization of *Escherichia coli* and *Salmonella enterica* from cattle feed ingredients. *Food-Borne Pathogens and Disease* 2, 341–347.

Davies RH and Wales AD (2013) *Salmonella* contamination of cereal ingredients for animal feeds. *Veterinary Microbiology* 166, 543–549.

Davis MA, Hancock DD, Rice DH, Call DR, Digiacomo R, Samadpour M and Besser TE (2003) Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica*. *Veterinary Microbiology* 95, 199–210.

Eriksson E and Aspan A (2007) Comparison of culture, ELISA and PCR techniques for *Salmonella* detection in faecal samples for cattle, pig and poultry. *BMC Veterinary Research* 3, 21.

Fegan N, Vanderlinde P, Higgs G and Desmarchelier P (2004a) The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *Journal of Applied Microbiology* 97, 362–370.

Fegan N, Vanderlinde P, Higgs G and Desmarchelier P (2004b) Quantification and prevalence of *Salmonella* in beef cattle presenting at slaughter. *Journal of Applied Microbiology* 97, 892–898.

Fegan N, Vanderlinde P, Higgs G and Desmarchelier P (2005) A study of the prevalence and enumeration of *Salmonella enterica* in cattle and on carcasses during processing. *Journal of Food Protection* 68, 1147–1153.

Fossler CP, Wells SJ, Kaneene JB, Ruegg PL, Warnick LD, Eberly LE, Godden SM, Halbert LW, Campbell AM, Bolin CA and Zwald AMG (2005) Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Preventive Veterinary Medicine* 67, 39–53.

Franz E, Van Diepeningen AD, De Vos OJ and Van Bruggen AHC (2005) Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in manure, manure-amended soil, and lettuce. *Applied and Environmental Microbiology* 71, 6165–6174.

Gad A, Abo-Shama U, Harclerode K and Fakhr M (2018) Prevalence, serotyping, molecular typing, and antimicrobial resistance of *Salmonella* isolated from conventional and organic retail ground poultry. *Frontiers in Microbiology* 9, 2653.

Habing GG, Lombard JE, Kopral CA, Dargatz DA and Kaneene JB (2012) Farm-level associations with the shedding of *Salmonella* and antimicrobial-resistant *Salmonella* in U.S. dairy cattle. *Food-Borne Pathogens and Disease* 9, 815–821.

Harvey RR, Zakhour CM and Gould LH (2016) Food-borne disease outbreaks associated with organic foods in the United States. *Journal of Food Protection* 11, 1953–1958.

Hauge SJ, Nesbakken T, Moen B, Røtterud OJ, Dommersnes S, Nesteng O, Østensvik Ø and Alvseike O (2015) The significance of clean and dirty animals for bacterial dynamics along the beef chain. *International Journal of Food Microbiology* 214, 70–76.

Holley R, Walkty J, Blank K, Tenuta M, Ominski K, Krause D and Ng L-K (2007) Examination of *Salmonella* and *Escherichia coli* translocation from hog manure to forage, soil, and cattle grazed on the hog manure-treated pasture. *Journal of Environmental Quality* 37, 2083–2092.

Hsi DJ, Ebel ED, Williams MS, Golden NJ and Schlosser WD (2015) Comparing food-borne illness risks among meat commodities in the United States. *Food Control* 54, 353–359.

Hussein HS (2007) Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *Journal of Animal Science* 85, E63–E72.

Islam M, Morgan J, Doyle MP, Phatak SC, Millner P and Jiang X (2004) Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Food-Borne Pathogens and Disease* 1, 27–35.

Jacob ME, Fox JT, Drouillard JS, Renter DG and Nagaraja TG (2009) Evaluation of feeding dried distiller's grains with solubles and dry-rolled corn on the fecal prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in cattle. *Food-Borne Pathogens and Disease* 6, 145–153.

Jacobsen CS and Bech TB (2012) Soil survival of *Salmonella* and transfer to freshwater and fresh produce. *Food Research International* 45, 557–566.

Khaleque MA, Keya CA, Hasan KN, Hoque MM, Inatsu Y and Bari ML (2016) Use of cloves and cinnamon essential oils to inactivate *Listeria monocytogenes* in ground beef at freezing and refrigeration temperatures. *LWT—Food Science and Technology* 74, 219–223.

Krytenburg DS, Hancock DD, Rice DH, Besser TE, Gay CC and Gay JM (1998) A pilot survey of *Salmonella enterica* contamination of cattle feeds in the Pacific northwestern USA. *Animal Feed Science and Technology* 75, 75–79.

Kudva IT, Blanch K and Hovde CJ (1998) Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64, 3166–3174.

Lahti E, Ruoho O, Rantala L, Hänninen ML and Honkanen-Buzalski T (2003) Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. *Applied and Environmental Microbiology* 69, 554–561.

Li X, Bethune LA, Jia Y, Lovell RA, Proescholdt TA, Benz SA, Schell TC, Kaplan G and McChesney DG (2012) Surveillance of *Salmonella* prevalence in animal feeds and characterization of the *Salmonella* isolates by serotyping and antimicrobial susceptibility. *Foodborne Pathogens and Disease* 9, 692–698.

Lynn TV, Hancock DD, Besser TE, Harrison JH, Rice DH, Stewart NT and Rowan LL (1998) The occurrence and replication of *Escherichia coli* in cattle feeds. *Journal of Dairy Science* 81, 1102–1108.

Merlini V, Pena F, da Cunha D, de Oliveira J, Rostagno M and Antunes A (2018) Microbiological quality of organic and conventional leafy vegetables. *Journal of Food Quality* 1–7. https://doi.org/10.1155/2018/4908316.

Muirhead RW (2009) Soil and fecal material reservoirs of *Escherichia coli* in a grazed pasture. *New Zealand Journal of Agricultural Research* 521, 1–8.

Patterson L, Navarro-Gonzalez N, Jay-Russell MT, Aminabadi P, Antaki-Zukoski E and Pires AFA (2018) Persistence of *Escherichia coli* in the soil of an organic mixed crop-livestock farm that integrates sheep grazing within vegetable fields. *Zoonoses and Public Health* 65, 887–896.

Phillips HN, Heins BJ, Delate K and Turnbull R (2017) Impact of grazing dairy steers on winter rye (*Secale cereale*) versus winter wheat (*Triticum aestivum*) and effects on meat quality, fatty acid and amino acid profiles, and consumer acceptability of organic beef. *PLoS ONE* 12, e0187686.

Reinstein S, Fox JT, Shi X, Alam MJ, Renter DG and Nagaraja TG (2009) Prevalence of *Escherichia coli* O157:H7 in organically and naturally raised beef cattle. *Applied and Environmental Microbiology* 75, 5421–5423.

Ribas-Agusti A, Diaz I, Sarraga C, Garcia-Regueiro JA and Castellari M (2019) Nutritional properties of organic and conventional beef meat at retail. *Journal of the Science of Food and Agriculture* **99**, 4218–4225.

Sargeant JM, Sanderson MW, Smith RA and Griffin DD (2004) Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the Midwestern USA. *Preventive Veterinary Medicine* **66**, 207–237.

Scaglia G (2017) Forage-Fed Beef Production: An Overview and Perspective. Available at http://www.lsuagcenter.com/portals/communications/publications/agmag/archive/2014/fall/foragefedbeefproduction-anoverviewandperspective (Accessed 26 December 2018).

Scharff RL (2012) Economic burden from health losses due to food-borne illness in the United States. *Journal of Food Protection* **75**, 123–131.

Schmidt JW, Agga GE, Bosilevac JM, Brichta-Harhay DM, Shackelford SD, Wang R, Wheeler TL and Arthur TM (2015) Occurrence of antimicrobial-resistant *Escherichia coli* and *Salmonella enterica* in the beef cattle production and processing continuum. *Applied and Environment Microbiology* **81**, 713–725.

Sofos JN (2008) Challenges to meat safety in the 21st century. *Meat Science* **78**, 3–13.

Średnicka-Tober D, Barański M, Seal C, Sanderson R, Benbrook C, Steinshamn H, Gromadzka-Ostrowska J, Rembiałkowska E, Skwarło-Sońta K, Eyre M, Cozzi G, Krogh-Larsen M, Jordon T, Niggli U, Sakowski T, Calder P, Burdge G, Sotiraki S, Stefanakis A, Yolcu H, Stergiadis S, Chatzidimitriou E, Butler G, Stewart G and Leifert C (2016) Composition differences between organic and conventional meat: a systematic literature review and meta-analysis. *British Journal of Nutrition* **115**, 994–1011.

Sustainable Food News (2016) U.S. organic meat and poultry market examined. Sustainable Food News. Available at https://www.sustainablefoodnews.com/printstory.php?news_id=24661 (Accessed 26 December 2108).

Turgis M, Borsa J, Millette M, Salmieri S and Lacroix M (2008) Effect of selected plant essential oils or their constituents and modified atmosphere packaging on the radiosensitivity of *Escherichia coli* O157:H7 and *Salmonella typhi* in ground beef. *Journal of Food Protection* **71**, 516–521.

Umberger WJ, Boxall PC and Lacy RC (2009) Role of credence and health information in determining US consumers' willingness-to-pay for grass-finished beef. *Australian Journal of Agricultural and Resource Economics* **53**, 603–623.

[USDA-AMS-NOP] U.S. Department of Agriculture-Agriculture Marketing Service-National Organic Program (2018) Final Rule: 7 CFR Part 205, USDA-AMS-NOP, Washington, DC. Available at http://www.ams.usda.gov/nop (Accessed 26 December 2018).

[USDA-FDA] U.S. Department of Agriculture-Food and Drug Administration (2017) Bacteriological Analytical Manual (BAM). Available at https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm.

[USDA-FDA] U.S. Department of Agriculture-Food and Drug Administration (2018) Food Safety Modernization Act-Final Rule on Produce Safety. Available at https://www.fda.gov/downloads/food/guidanceregulation/fsma/ucm472887.pdf.

Wales AD, Allen VM and Davies RH (2010) Chemical treatment of animal feed and water for the control of *Salmonella*. *Foodborne Pathogens and Disease* **7**, 3–15.

Williams KJ, Ward MP, Dhungyel OP and Hall EJS (2015) Risk factors for *Escherichia coli* O157 shedding and super-shedding by dairy heifers at pasture. *Epidemiology and Infection* **143**, 1004–1015.