RESEARCH REPORT
PORK SAFETY

Title: Understanding ecology and distribution of Shiga toxin-producing *Escherichia coli* O157 and Non- O157 in US swine feed mills - **NPB 18-070**

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Industry Summary

Ensuring the safety of pork is essential for producers in order to maintain animal and human health, and also to continue serving export market. Although few *Escherichia coli* outbreaks have been associate with contaminated pork products, the characteristics and epidemiology of *E. coli* carried by on-farm pigs remain unknown Few epidemiological studies have elucidated the prevalence of *Enterobacteriacea*, and in particular *E. coli* as carriage in healthy pigs and pork products, and moreover their role in public health. There is therefore the need to further investigate entry route and spread pattern in the pork industry "from feed to fork".
The overall goal of this study was to give to the pork industry a better understanding of the ecology and distribution of *E. coli* and *Salmonella*, and collect valuable data for the development of effective intervention strategies both at pre and post-harvest level. The specific objectives are as follow:

1) determine the presence and distribution of *E. coli* and *Salmonella* population in commercial feed mills manufacturing feed in relation to sampling location and production risk factors;
2) compare *E. coli* and *Salmonella* entry routes and spread pattern within US swine feed mill;
3) characterize isolates by whole-genome sequencing analysis.

A total 405 samples were collected and tested. Of those, 19 (4.7%) were positive for *Salmonella* and 57 (14.1%) for *E. coli*. All mills had at least one sampling site positive for either *Salmonella* or *E. coli*. Sites with higher percentages of positive samples were the receiving, manufacturing, and control area floors. The survey responses show that the age of the mill might be a risk factor for bacterial contamination: the older the facility, the higher the number of positive samples. Other risk factors evaluated, such as the production capacity, did not appear to relate to bacterial prevalence.

Salmonella confirmed isolates were grouped in 10 main serotypes, matching with environmental, feed, pet food, swine and pork products isolates. None of the confirmed

E. coli isolates carried virulent genes. The strains were typed into 12 *E. coli* O groups (O8, O18, O25, O34, O36, O54, O71, O104, O133, O156, O159, O160) and 10 H groups. Some of these serogroups were observed in other studies investigating *E. coli* prevalence through the pork chain.

Overall the data gathered in this study shows the potential role of feed and feed mill environment as entry routes for *E. coli* and *Salmonella* into human food chain. While a sample size of six feed mill facilities, over three sampling seasons might not be enough to detail the impact of mill characteristics and production practices on microbial occurrence, some preliminary trends could be observed. Our observations highlight the relatively frequent occurrence of *Salmonella* and *E. coli* in multiple feed mill environments. Although samples were not quantified in this study, the data add valuable information on biological hazard hot spots and transfer mechanisms within establishments and should be included in future surveys. These observations can contribute to the implementation of biosecurity plans and other preventative strategies in feed mills and to understand microbial ecological niche in the animal feed processing environment.

Keywords: Feed mills, entry route, *Enterobacteriaceae,* pork.

Scientific Abstract:

This study aimed to evaluate the patterns and potential risk factors associated with the occurrence of *Salmonella* spp. and *E. coli* in selected United States swine feed mills. A total of 405 samples were collected during fall 2018, spring and summer 2019 from selected sites including floors, equipment, shoes and feed in six feed mills in the US Midwest region. Each sample was analyzed for the presence of *Salmonella* and *Escherichia coli* with culture methods and confirmed by PCR and Whole Genome Sequencing (WGS). A survey regarding production volumes, hygiene practices and microbial testing capabilities was conducted in each facility All mills had at least one sampling site positive for either *Salmonella* or *E. coli*. Of the 405 samples, 4.7%, and 14.1% were positive for *Salmonella* spp., and *E. coli,* respectively. Sites with higher percentages of positive samples were the receiving, manufacturing, and control area floors. The survey responses indicated that the age of the mill might be a risk factor for bacterial contamination: the older the facility, the higher the number of positive samples. Other risk factors evaluated, such as the production capacity, did not appear to relate to bacterial prevalence. The data documents the presence of *E. coli* and *Salmonella* in selected US swine feed mills, and an association between *E. coli* occurrence and number of ingredient suppliers to feed mill. This information could be used to understand risk factors affecting the occurrence of *Salmonella* spp. and *E. coli* in feed mills and help implement monitoring and mitigation strategies for public health.

Introduction:

Pathogenic and non-pathogenic enteric bacteria of the family *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica* have been detected in animal feeds (Li et al., 2012) as well as in facilities where feeds are produced (Magossi et al., 2019a). Limited data on bacterial occurrence is available to characterize dynamics and assess potential downstream impacts on farm environments, animals, and meat. Contamination can occur at several stages during the feed-to-fork chain. Pre-harvest safety is an ongoing area of interest for the reduction of foodborne illness. Feed and feed mills could potentially act as an entry route or reservoir of contamination in the animal production chain and agricultural environments. Risk factors associated with microbial presence in swine have been extensively investigated outside the US (van der Wolf et al., 2001; Rajić et al., 2007; Dang-Xuan et al., 2017). Feed type, production flow, animal health and, poor hygiene has been generally recognized as important factors associated with the high prevalence of microbial infections (Baer et al., 2013; Hsieh et al., 2016). Nevertheless, the assessment on intervention strategies might be only applicable where the studies were conducted (e.g. Europe and Canada), due to differences in production, industry structure and regulatory framework (Funk and Gebreyes, 2004). It is recognized that feed can harbor ~~enteric~~ bacteria coming from ingredients, or due to cross-contamination within the environment (Jones and Richardson, 2004). However, the relative importance of different entry routes for potential contamination of the human food chain is not well established. Nevertheless, several human outbreaks of *Salmonella* have been attributed to contaminated feed (Österberg et al., 2006; Molla et al., 2010) . Furthermore, while there is some evidence of the importance of mill environmental conditions (Cochrane et al., 2016), the association between facility characteristics, processing practices and bacterial

occurrence has not been extensively explored. Therefore, understanding the influence of management practices and facilities characteristics on the presence of *Salmonella* spp. and *E. coli* in US swine feed mills might assist the swine industry for implementing strategies for pathogen contamination prevention and reduction. The manufacturing process within US feed mills usually includes receiving, processing (pelleting or mashing), storage-packaging, loading and delivery of the finished product (Cochrane et al., 2016). Facilities can vary greatly in size, feed ingredients, finished products, and feed handling and storage practices. For example, some mills produce feed for only one species, whereas others have a variety of product lines. Facilities also vary in terms of origin of ingredients, destination of finished products, and degree of vertical integration with other animal production steps. However, information on facility characteristics and operations have not been typically accounted for in microbial assessment surveys.

In 2015, the FDA released a rule regarding the Current Good Manufacturing Practices (CGMP), hazard analysis, and risk-based preventive controls for food for animals, as a result of the changes taken after the Food Safety Modernization Act (FSMA) implementation. This new regulation makes facilities responsible for the safe "manufacturing, processing, packing, and holding of food for animals" and promotes hazard analysis and risk-based preventive controls (Federal Register, 2015). However, the lack of information on root causes of contamination, association between pathogenic organisms and natural microflora in feed and the role of mill production practices limits the development of best practice documents or interventions.

Objectives:

The overall goal of this study was to evaluate potential risk factors affecting the presence of *Salmonella* spp. and *E. coli* in feed mills. Specific objectives were to: (1) assess occurrence of *Salmonella* spp. and *E. coli* in selected US swine feed mills by culture methods, PCR and WGS; and (2) investigate possible association of bacterial presence with sampling sites, processing steps, facility characteristics and practices.

Materials & Methods:

Sample collection and feed mill characteristics

Six commercial feed milling facilities participating in this study served as a convenience sample from the Midwest region. They were selected based on location, feed produced (swine) and willing to allow sampling. The mills were located in four states, Iowa, Missouri, Kansas and Oklahoma, which are part of the main swine production area of the United States. All mills produce swine feed, four of them exclusively and the remaining two produced feed for other animal species also. In order to maintain anonymity, each mill was assigned an identification number (1-6). Samples were collected using sterile swab sponge sticks, pre-soaked with 10 mL of buffered peptone water (BPW, BD Difco, Sparks, MD; for *Salmonella*) or pre-soaked with 10 mL of neutralizing buffer (NB; for *E. coli*; 3M, St Paul, MN). Each mill was sampled three different times: fall (October of 2018), late winter/early spring (March of 2019), and summer (June of 2019). Sampling sites were selected from areas of high people traffic, ingredients processing and dust accumulation, following production flow, as previously described (Magossi et al., 2019b). Floors were swabbed on 3 different areas to increase test sensitivity, while the other sites were swabbed only

once. All samples were collected by one trained researcher. Each sampling site was swabbed twice, once for *Salmonella* and once for *E. coli*. After the site was swabbed, the sponge was transferred back in its original bag, the plastic handle removed to avoid cross contamination and the bag sealed. Sponge bags were transported to the laboratory at refrigeration conditions and analyzed within 24 hours.

Mill facility survey

A questionnaire was submitted, by email, to the plant manager of each mill, to understand facility characteristics and practices. The list of questions is shown in Table 1. Mills were scored based on the number of suppliers of feed ingredients: small 5-10, medium 10-20, or large 30-40. Answers were aggregated semi-quantitatively or in categories and used to derive summary statistics for the facilities included in this study.

Table 1. Feed mill survey questions submitted to the plant manager of each mill, to understand facility characteristics and practices.

	Question[1]
1	**What is the total production volume in a year (tons)?**
2	How many employees work in the plant?
3	**Which year was the facility built? Did you make any major renovation? YES/NO when?**
4	Which ingredients or raw materials are most commonly used in your plant, and what are the amounts processed per year, in recent years?
5	**What is the approximate number of suppliers for the key ingredients identified above? e.g., between small 5-10, medium 10-20, or large 30-40?**
6	**Where are your ingredients coming from? (e.g. % within the state, % from other US states, % from abroad)**
7	**Are you processing feed only for swine, or for other animal species as well?**
8	Do you carry out microbial testing for incoming ingredients or finished products? If yes, please specify which microbial testing you do, on which products or surfaces, and approximate frequency
9	Do you use any electronic data management? If YES, which one.

[1] Questions in bold were used for univariate analyses.

Salmonella culture- and molecular-based analysis

As per the protocol for the identification of *Salmonella* from environmental samples (USDA-FSIS, 2014), 50mL of BPW (BD Difco, Sparks, MD) was added to the bags containing sponges pre-soaked with BPW and then incubated at 37 ºC for 24 h. Next, 0.5 µl of the pre-enriched sample was added to 10 mL of Tetrathionate broth (TT; BD Difco) and 0.1 µl to 10 mL of Rappaport-Vassiliadis broth (RV; BD Difco). Both enrichment media were incubated at 42 ºC for 24 h. After selective enrichment, a loopful of TT and RV broths was streaked for isolation on both Xylose Lysine Tergitol 4 (XLT4; BD Difco) and Brilliant Green Sulfa (BGS; BD Difco) agar plates and incubated at 37 ºC for 24-48 h. Presumptive *Salmonella* colonies (red with or without black center on XLT4 and pink opaque on BGS) were picked and streaked onto a Tryptic Soy Agar (TSA; BD Difco) plate and incubated at 37 ºC for 24 h for agglutination test. Isolates that tested positive for agglutination were subjected to species confirmation by a real-time PCR assay targeting the *invA* gene (Bai et al., 2018). Isolates with a Ct value 0< and <38 were considered as *Salmonella enterica*.

E. coli identification

The procedure described by Paddock et al. (2012) was followed. Briefly, 50 mL of *E. coli* broth (EC, BD Difco) was added to NB pre-soaked sponge bags and incubated at 40 ºC for 6 h. One mL of the enriched sample was then transferred into a sterile 5 mL tube, boiled for 15 min and then cooled on ice before the next step. The Gene Clean Turbo kit (MP Biomedicals, Santa Anda, CA) was used to purify DNA from the boiled lysate. A PCR assay was carried out in a CFX2000 thermocycler (Bio-Rad, Hercules, CA) targeting the *ybb*W, *uid*A, and *clp*A genes (Walker et al., 2017) (Walker et al., 2017). PCR products were analyzed by capillary electrophoresis on a QIAxcel Advanced

Systems (Qiagen, Germantown, MD) and samples showing amplicons of 449, 454 and/or 447 bp were considered positive for *E. coli* spp.

DNA preparation

Extraction of genomic DNA from bacterial isolates was completed using the Qiagen DNeasy Blood & tissue kit (Qiagen, Hilden, Germany). Genomic DNA concentrations were then measured with a Qubit Fluorometer 3.0. using the dsDNA HS assay kit, according to manufacturer's instructions (Thermo Fisher, Waltham, MA). DNA extract was stored at - 20°C until WGS analysis.

Whole genome sequencing

The Nextera XT Library Preparation kit was used for preparing paired-end libraries and WGS was carried on either a MiSeq or NextSeq sequencer, using a 500-cycle MiSeq reagent V2 kit or a 300-cycle NextSeq 500/550 high-output V2 kit, respectively (Illumina, San Diego, CA). Trimming and de-novo assemblies were obtained with Shovill version 0.9 (https://github.com/tseemann/shovill), available in the GalaxyTrakr pipeline (https://www.galaxytrakr.org) (Afgan et al., 2018) or with CLC Genomics Workbench 11, unless otherwise noted, default parameters were used in all analyses (Zhang et al., 2015). The NCBI Prokaryotic Genomes Automatic Annotation pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) was used to annotate draft genomes of each isolate.

***In-silico* molecular analysis**

The serotype of each isolate was determined *in-silico* by prediction from draft genomes using SeqSero 1.0 (http://www.denglab.info/SeqSero) (Zhang et al., 2015)and

Serotype Finder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/)(Joensen et al., 2015). Strain characterization was performed using an *in-silico* Multilocus Sequence Typing (MLST) approach both for *E. coli* and *Salmonella* comparing isolate genome sequences to seven housekeeping gene fragments and core genome MLST analysis using EnteroBase v1.1.2 (https://enterobase.warwick.ac.uk/).

Statistical Analysis

Phenotypic tests and PCR assays generated binary outcomes for the detection of *E. coli*, and *Salmonella enterica* ("+": presence; "-": absence). Each one binary outcome variable was analyzed separately using a generalized linear mixed model (GLMM) with a logit link at the 5% significance level (α=0.05). The fixed effects included sampling season (fall, spring, and summer) and mill sampling sites run in separate models. Mill was included as random effect (each individual mill received a unique number ID from 1-6). There was no evidence of interaction between sampling season and sampling site on either model. Based on the outcome of the GLMM model, the magnitude of the association between occurrence and season and site respectively was evaluated with the type III test of fixed effects. Fisher's exact test was used to test for the significance of the association between *Salmonella* and *E. coli* prevalence, aggregated by establishment (feed mills) over all sampling seasons. The magnitude of these associations was assessed using the Phi coefficient of association for 2x2 contingency tables. The effect of mill characteristics, obtained from the mill facility survey questionnaire, was evaluated by including each variable into the GLMM model as fixed factor, in addition to sampling sites and season. Mill was once more considered as random factor. Statistical analysis was performed using PROC GLIMMIX (for GLMM)

and FREQ (for exact inference and correlation analysis) in SAS 9.4 (SAS Institute, Inc., Cary, NC).

Results:

Bacterial occurrence and associations

A total 405 samples were collected and tested. Of those, 19 (4.7%) were positive for *Salmonella enterica* and 57 (14.1%) for *E. coli* (Table 2). No significant difference was observed among facilities (p value = 0.874), sampling sites (p value = 0.095) or seasons (p value = 0.061) for *Salmonella* isolates. The number of *E. coli* positive samples did not significantly differ across feed mills (p value = 0.357) or sampling sites (p value = 0.25). Nevertheless, a significant difference was observed across season, with fall having the highest positive samples for *E. coli* (p value <0.001). The overall association between *E. coli* and *Salmonella* was not significant (p value =0.165), therefore these factors were considered independent from each other.

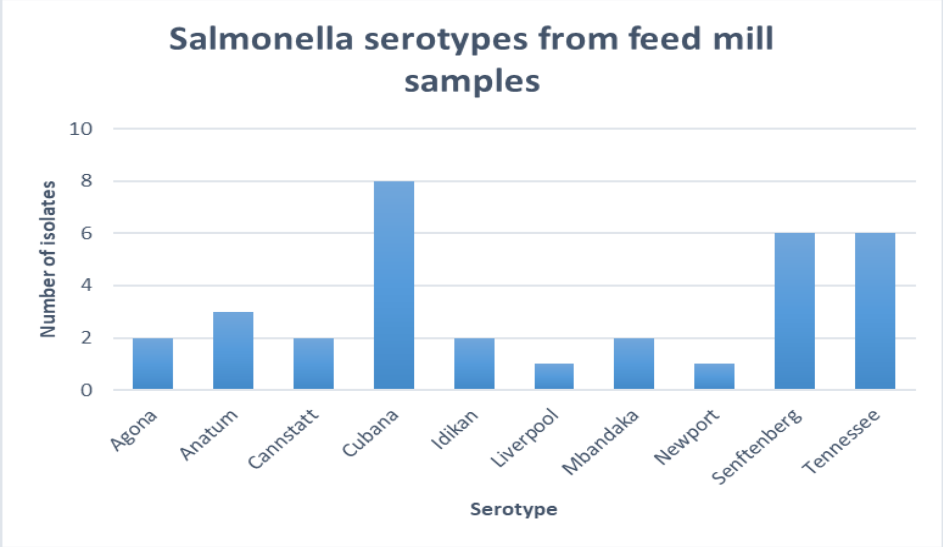
Table 2. Number of *Salmonella* and *E. coli* positive samples (culture tests and PCR assays) across seasons, mills, and sampling sites.

		E. coli	*Salmonella*
Season	Fall	23.0% (31/135)	8.1% (11/135)
	Spring	5.1% (7/136)	3.7% (5/136)
	Summer	14.2% (19/134)	2.2% (3/134)
Mill ID	1	17.1% (12/70)	2.9% (2/70)
	2	7.4% (5/68)	7.4% (5/68)
	3	13.6% (9/66)	3.0% (2/66)
	4	16.9% (11/65)	6.2% (4/65)
	5	20.9% (14/67)	1.5% (1/67)
	6	8.7% (6/69)	7.2% (5/69)
Sampling site	Receiving ingredients pit grate*	20.4% (11/54)	1.9$ (1/54)
	Fat intake inlet	0.0% (0/18)	0.0% (0/18)
	Pellet mill	16.7% (2/12)	0.0% (0/12)
	Discharge bin boot	5.6% (1/18)	0.0% (0/18)
	Load-out auger	6.7% (1/15)	0.0% (0/15)
	Finished feed	26.3% (5/19)	10.5% (2/19)
	Control room floor*	11.1% (6/54)	1.9% (1/54)
	Receiving area floor*	22.2% (12/54)	16.7% (9/54)
	Manufacturing area floor*	16.7% (9/54)	5.6% (3/54)
	Warehouse area floor*	3.8% (2/53)	0.0% (0/53)
	Worker shoes	13.9% (5/36)	5.6% (2/36)
	Broom	16.7% (3/18)	5.6% (1/18)
	Total	14.1% (57/405)	4.7% (19/405)

* Floors were swabbed on 3 different areas, while other sites once.

Ten different *Salmonella* serotypes were identified among the *Salmonella* enterica sequenced isolates by WGS (Figure 2), *Salmonella* Cubana was the most prevalent, with a total of 8 isolates (24.2%), followed by *Salmonella* Senftenberg and *Salmonella* Tennessee (n=6, 18.2%), Anatum (n=3, 9.1%), Agona, Cannstatt, Idikan, and Mbandaka (n=2, 6.1%), Liverpool and Newport (n=1, 3.05).

Figure 2. *Salmonella* serotypes frequency from feed mill samples



The *E. coli* isolates belonged to 22 O groups (O5, O8, O9, O18, O25, O26, O32, O34, O36, O54, O59, O62, O71, O91, O104, O112, O120, O133, O156, O159, O160, and O185) and 19 H groups. *E. coli* serotypes O8, O156, O159, and O160 were isolated previously also from swine feces, O8, O9, and O91 have been previously isolated from samples collected from pigs and human samples. Serotype O104 has been linked to pigs and pork products in recent years and it was responsible for a major outbreak in Germany, in 2011, linked to sprouts, leading to many reported cases of HUS among the patients.

Survey of facility characteristics

Most of the sampled facilities included in this study produced exclusively swine feed (four out of six) while the remaining two mills produce feed for swine and other animal species: cattle, ovine, equine, and poultry. The mills were built between 1956 to late 1990s (hence 25 to 63 years old) with only two of them having undergone major renovations in the last 10 years. Facility size varied: mills had between 3 and 43 employees, with four mills having ten or fewer employees. Overall feed production volume ranged between 14,000 and 450,000 tons. The mix and relative amounts of ingredients processed in the mill were relatively homogeneous. For five mills, corn was the main ingredient (approximately 60-80% of the total ingredients), followed by soybean meal (~10-25%), and distillers' grains (~15-25%). Only in one mill was wheat middling the main ingredient. Minor ingredients included animal fat, vegetable oil, salt, calcium carbonate or limestone, amino acids, vitamins, mineral supplements and antibiotics. The number of suppliers for each mill varied between 5 and 40 in alignment with production volume. Five mills had more than 95% of ingredients sourced within the US. The proportion of ingredients sourced in-state vs. out of state varied widely: four mills received 20-30% of ingredients from in-state sources, and two mills 80-90%. No relationship with production volume were observed. None of the mills sampled in this study had internal capability for microbial analysis.

Association between bacterial occurrence and facility characteristics

The correlation analysis between bacterial occurrence *Salmonella*, and *E. coli* positives) and facility characteristics (based on the survey) is shown in Table 3. The age of the facility, production volume, and the type of feed produced (only swine vs. multiple species) was not significantly correlated with microbial occurrence. The analysis

demonstrated that, at a significance level of α=0.05 (5%), the number of suppliers and the occurrence of *E. coli* were negatively associated (*p* value = 0.044). The odds ratio proposes that a facility with, for example 20 suppliers, is 3.6 times more likely to have a sample test positive for *E. coli* than a facility with 10 fewer suppliers.

The log of production volume was marginally significant at a 10% confidence level for the presence of *E. coli* (*p* value = 0.105). The odds ratio of 0.61 and 0.57 were < 1, indicating a negative correlation between production volume and contamination. The odds of having a sample test positive for *E. coli* resulted to be inversely proportional to the log of production volume.

Table 3. Odd ratio associations between mill characteristics *E. coli* and *Salmonella* occurrence in environmental and feed samples using a generalized linear mixed model.

	E. coli		*Salmonella*	
Mill Characteristics	Odds ratio	*p* value[1]	Odds ratio	*p* value[1]
Number of suppliers	0.966	0.044	1.028	0.241
Log of production volume	0.566	0.105	1.553	0.414
Swine feed only	0.540	0.07	1.465	0.537
Proportion of in state suppliers	1.401	0.661	0.785	0.813
Mill age	1.000	0.991	0.994	0.752

[1]Significance level α < 0.05

Discussion:

This study presents a preliminary evaluation of potential risk factors associated with feed mills establishment characteristics, highlighting the occurrence of *Salmonella* spp. and *E. coli* in different locations and processing steps within facilities and advancing the characterization of potential critical points for bacteria entry and persistence in feed mill environments. In our study, *E. coli* was detected at a higher frequency than *Salmonella*, across mills and sampling sites. Our findings agree with previous research where a relatively high detection frequency of *Salmonella*, and *E. coli* in animal feed and feed mill environments was observed (Jones and Richardson, 2004; Molla et al., 2010J; ones, 2011; Li et al., 2012; Magossi et al., 2019a). Notwithstanding, in the present study *Salmonella* prevalence was not significantly associated with season, in contrast to previous observation by Magossi et al. (2019a). Our research encompassed a smaller number of facilities and geographical coverage. Season is a complex factor that may include a wide range of variables, including environmental temperature and humidity, ingredient origin, quality, storage conditions, volume or rate of production, and possibly workforce composition and handling practices. It was outside the scope of this survey to test these variables in detail. However, sampling season seems to be a significant factor for *E. coli* presence, as reported in other studies documenting seasonal occurrence in *E. coli* with a higher prevalence in the fall. Other studies have reported significant associations between microbial contamination and weather-related variables (Rasmussen and Casey, 2001; Jahne et al., 2015; Fink et al., 2018). Human pathogens often present seasonal variation, with a higher presence during warm months and lower in cold months (Pangloli et al., 2008; Ravel et al., 2010). This phenomenon is probably influenced by

human activities: during warmer months walk around facilities more often, leaving doors and windows open, thus facilitating microbial spread.

Multiple sites and production steps were identified in our sampling plan as possible bacterial contamination points. Some preliminary trends could be observed (Table 2). *Salmonella* presence was high in receiving area floor. The prevalence of *E. coli* was observed mostly in receiving ingredient pit grate, receiving of ingredients floor and manufacturing area floor. In our previous study (Magossi et al., 2019) we also observed a high number of positive samples in the receiving ingredient pit gratin and receiving floor. Similar findings were reported by Binter et al. (2011), indicating the role of raw grain ingredients and transporting trucks as microbial entry routes into mills. Further, in our study worker shoes showed bacterial prevalence that raise concerns on the role of people traffic around different areas of the mill on contamination spread. Amass, et al. (2000) and Otake, et al. (2002) demonstrated that porcine reproductive virus and respiratory syndrome virus could be carry inside mill facilities through workers' shoes. All these observations highlight the importance of biological hazard analysis and consequential mitigation strategies to control and prevent microbial entrance and contamination in feed mill facilities as outlined by (Cochrane et al., 2016) and (Jones et al., 2019). Potentially, the spread of contaminated dust accumulation, or dust particles, throughout the manufacturing areas within the mill can result in contaminated feed (Huss et al., 2015; Schumacher et al., 2016; Huss et al., 2017). Bacterial prevalence differed across sampling sites within mills, suggesting higher contamination levels in areas where ingredients were received. *Salmonella* was detected at a lower frequency than what was observed previously (Magossi et al., 2019a) across mills and sites and the presence of *E. coli* was significantly associated with seasons. These observations potentially suggest that

Salmonella and *E. coli* have different mechanisms of entry, transfer, and persistence within-mill, therefore different monitoring and risk management practices may be needed. To our knowledge, this is the first study that aims to correlate mill facility characteristics and production practices to microbial contamination in feed mill environment. From our observations, the number of suppliers was positively associated with bacterial presence, suggesting a potentially higher likelihood of contamination entering the mill through ingredients or transport equipment such as trucks. Similar observation were concluded by Larson et al. (2008) when conducting a survey investigating the presence of stored-product insects in feed mills. Whyte et al. (2003) and Davies and Wales (2010) investigated the prevalence of bacterial species in commercial poultry feed mills and observed that contamination was bacterially diverse and reflected ingredient sources and the environment conditions from mills but no association with practices or facilities characteristics were considered.

Summary of the results

The data gathered in this study shows the potential role of feed and feed mill environment as entry routes for *Enterobacteriaceae (e.g. Salmonella enterica* and *Escherichia coli)* into human food chain. While a sample size of six feed mill facilities, over three sampling seasons might not be enough to detail the impact of mill characteristics and production practices on microbial occurrence, some preliminary trends could be observed. Our observations highlight the relatively frequent occurrence of *Salmonella* and *E. coli* in multiple feed mill environments. Although samples were not quantified in this study, the data add valuable information on biological hazard hot spots and transfer mechanisms within establishments and

should be included in future surveys. These observations can contribute to the implementation of biosecurity plans and other preventative strategies in feed mills and to understand microbial ecological niche in the animal feed processing environment.

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