RESEARCH REPORT
PORK SAFETY

Title From feed to meat: investigation of the prevalence and distribution of *Salmonella enterica* serotype I 4, [5], 12:1:- a pathogen of interest in pork- **NPB # 16-192**

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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 markets. One barrier to this is the rising occurrence of *Salmonella* contamination in pork. In order to best prevent *Salmonella* in post-harvest pork, the pathogen must first be prevented from entering the farm-to-fork supply chain. Recently *Salmonella enterica* serotype I 4,[5], 12:i:- have been linked to swine feed and pork products. The magnitude of its presence in the U.S. and its pathogenicity are currently unknown. Therefore, the overall objective of this study was to give to the pork industry a better understanding of the ecology and distribution of *Salmonella enterica* and in particular of the serotype I 4,[5], 12:i:-; and collect valuable data for the development of effective intervention strategies both at pre and post-harvest level. In order to achieve these overall objectives, the specific deliverables were as follow:

- determine the presence and distribution of *Salmonella enterica* population and in particular the prevalence of serotype I 4,[5], 12:i:- in commercial feed mills manufacturing feed;
- characterize the distribution of *Salmonell*a positive isolates in relation to sampling location and establishment-production associated risk factors.

For objective 1, results indicated that 9 of the total 11 feed mills had at least one *Salmonella* positive site. From the total isolates, 49 (12.8%) were confirmed *Salmonella*: two isolates were identified in feed, while the other 47 on equipment and/or surfaces. A higher pathogen prevalence was observed in fall and summer season. A total of two ST and three STM were identified during the visits. These isolates showed a similar seasonal presence being found most commonly during fall and summer.

For the second objective mill ID was the significant factor associated with the presence of *Salmonella*. Differences in management, geographical location, hygiene practices, quality of incoming raw ingredients, volume of feed produced, number of workers, and time the facility has been operational were all important variables for pathogen presence. No differences between mash and pelleted feed were observed, while production flow and plant design were identified as important in preventing microbial introduction and recontamination of finished feed. Worker shoes also represented an important vehicle for pathogen spread around the mill: control room and manufacturing floor area (zones with the most human flow) had the highest percentage of *Salmonella* samples.

Overall the data gathered in this study shows the potential role of feed and feed mill environment as entry routes for *Salmonella spp,* ST and STM into human food chain. Hygiene, management, production flow, and cross-contamination within facility were all significant factors linked with pathogen contamination in mills. We found that both the mill and the season were significantly associated with the presence of *Salmonella* in the production facilities. These results contribute both to the implementation of biosecurity plans and other preventative strategies in feed mills and to understand *Salmonella* behavior at pre-harvest level.

Keywords: Feed mills, seasons, entry route, *Salmonella* Typhimurium, *Salmonella* I 4,[5],12:i:- , pork.

Scientific Abstract:

Salmonella is a pathogen of public health concern. Each year, *Salmonella* infections cost to the food industry approximately $2.3 billion and 33% of the reported cases are associated with beef, poultry or pork. Pathogen presence in feed mills can represent one of the many potential routes for entry and transmission into the food production chain. Nevertheless, little is known about *Salmonella* incidence and association with these type of environments. The objective of this research was to investigate presence, distribution and seasonal prevalence of *Salmonella* spp., *Salmonella enterica* serovar Typhimurium (ST) and its monophasic variant 4,[5],12:i:- (STM) in different feed mills across the United States. Eleven facilities were selected in 8 states and 12 sites were sampled within each feed mill. Visits were conducted during fall 2016, early spring 2017 and summer 2017. Samples were analyzed following the FSIS guidelines for isolation and identification of *Salmonella*. Culture positive isolates were further examined by a PCR analysis targeting the *invA* gene to differentiate for *Salmonella enterica*. A multiplex real-time PCR was used to differentiate ST and STM from other serotypes. Associations between season, mill and sample site with *Salmonella* presence were investigated using generalized linear mixed effects models. Both season ($P < 0.007$) and mill ($P < 0.005$) were significantly associated with *Salmonella* spp. presence. Fall months were associated with a higher *Salmonella* prevalence (13.2%) compared to early spring and summer. A total of 5 isolates, among the 383 samples, were serotyped as ST and STM. These two serotypes showed a similar seasonal presence throughout the study, being mostly found during fall and summer seasons. These findings demonstrated the seasonal presence of *Salmonella* spp. in feed mills and the role of these environments as potential pathogen entry route into the human food chain.

Introduction:

Salmonella is a resilient microorganism that can live in low water activity conditions and adapt to different temperatures. This pathogen can survive outside the animal host and in the environment for long periods of time (Baer et al., 2013). The Centers for Disease Control and Prevention (CDC) estimated that *Salmonella* is responsible for

approximately one million foodborne illnesses, 19,000 hospitalizations and 380 deaths in the United States of America (USA) each year (CDC, 2014). The yearly impact for the food industry is around $2.3 billion. From 2006 to 2015, the number of cases of *Salmonella* linked to pork products has increased (CDC, 2014). Although pork has the lowest association with human foodborne illness, when compared to beef and chicken, it is the most consumed meat in the world (Delgado et al., 2001). Therefore, *Salmonella* has become a food safety concern also for the American swine industry: ensuring the safety of pork is essential for producers to maintain animal and human health, and to continue serving export markets (Baer et al., 2013*)*. Recent studies have demonstrated the role of pigs as *Salmonella* reservoir (De Knegt et al., 2015). For example, pigs can ingest or inhale the pathogen from the environment or through contaminated feed (Fedorka-Cray and Hogg, 1997) and carry it during transfer from the farrowing farm to the finishing farm or to the slaughter house (Kranker et al., 2003). During these stressful events, pigs can shed *Salmonella*, leading to pork contamination via feces during processing (Arguello et al., 2013; Rostagno et al., 2009). Moreover, *Salmonella* can persist for long periods of time, adapt to suitable host and be a transient member of the animal gastrointestinal population. Some strains can live in animals without causing illnesses until consumed by humans (Rostagno and Callaway, 2012). Furthermore, also animal feed can represent a potential source of *Salmonella* infections for animals (Carter et al., 2003; Davis et al., 2003; Kidd et al., 2002). Several studies have estimated the presence of *Salmonella* in feed as generally low, and historically no evidence of direct link to animal or human illness has been demonstrated in US (Burns et al., 2015; Cochrane et al., 2015; Davies et al., 2004; Molla et al., 2010). Nevertheless, the importance of feed as pathogen contamination source in pigs, the potential risk of transmission and survival in slaughter houses and the possible infection for consumers has been highlighted as significant and potentially high in several risk assessment models *(*Rönnqvist et al., 2017; Österberg et al., 2006). Surveillance programs for *Salmonella* in animal products and feed have been already implemented in USA (AFSS – Animal Feed Safety System, Feed Contaminants Program from 2002-2006, and the *Salmonella* Assignment from 2007-2009) and in Europe (Swedish National *Salmonella* Control Programme) (Abrahantes et

al., 2009; Österberg et al., 2006). Moreover, a surveillance study conducted in USA from 2002 to 2009 reported that 12.5% of feed and feed ingredient samples collected from manufacturing facilities were contaminated with *Salmonella* (Li et al., 2012). These results support the importance to investigate pathogen presence and possible infection sources from feed to fork. The risk of salmonellosis from feed is difficult to quantify due to inconsistent data, sampling constrains and lack of epidemiological information (Crump and Griffin, 2002; Jones, 2011). Limited practices have been implemented for animal feed environments, even if these facilities have been recognized as potential source of infections in different occasions (Podolack et al., 2010; Rostagno et al., 2012). While *Salmonella* spp. contamination in livestock feed is low (Li et al., 2012), it is important to understand locations of entry into the animal feed value chain.

Among the emerging *Salmonella* serotypes linked to pork product, a monophasic variant of *Salmonella* Typhiumurium (ST), *Salmonella enterica* 4, [5], 12:i:- (STM) has recently caused a large recall from whole roaster hogs (CDC, 2015). Investigations traced the source of contamination to a pork slaughter establishment in Graham, WA, and the potential sources of contamination were identified as the raw pork meat, the inadequate employee handwashing practices, and the poor cleaning conditions of the surfaces and utensils used (CDC, 2015). This serotype is a particular concern because of its known resistance to many common antimicrobials, including netilmicin, tetracycline, chloramphenicol, gentamicin, kanamycin, ampicillin, cephalothin, sulfonamides, sulfamethoxazole-trimethoprim, amoxicillin-clavulanic acid, streptomycin, amikacin, and nalidixic acid (Moreno Switt et al., 2009). Before the mid-1990s, STM had rarely been identified in clinical samples. One of the first isolates was obtained from chicken carcasses in Portugal around 1986/87 (Machado and Bernardo, 1990). Since 1995, the reported cases of STM have increased in the United States (Moreno Switt et al., 2009), and within recent years STM has been progressively implicated in human disease worldwide. Since *Salmonella* spp. has been identified as a potential biological hazard in many livestock feeds (Cochrane, 2016; Crump et al., 2002), understanding this pathogen's ecological niche and potential pre-harvest entry routes into the human food chain is critical for producers and consumers.

Objectives:

The overall objective of this study is to give to the pork industry a better understanding of the ecology and distribution of *Salmonella enterica* and in particular of the serotype I 4,[5], 12:i:-; and collect valuable data for the development of effective intervention strategies both at pre and post-harvest level. In order to achieve these overall objectives, the specific deliverables are as follow:

- determine the presence and distribution of *Salmonella enterica* population and in particular the prevalence of serotype I 4,[5], 12:i:- in commercial feed mills manufacturing feed;
- characterize the distribution of *Salmonell*a positive isolates in relation to sampling location and establishment- production associated risk factors.

Materials & Methods:

Swabbing method and sites

A diverse geographical pool of 11 feed manufacturing facilities was selected for this study representative of the main swine production areas within the US (Figure 1). Each of the chosen mills supply feed to swine operations. Six mills produced only mash feed, while the other five facilities produced both mash and pelleted feed with average conditioning temperatures of 71°C for 45 sec. Within each feed manufacturing facility, twelve sampling sites were selected, taking into consideration production flow, people traffic, and dust accumulation (Table 1). Samples were collected with a sterile sponge-stick pre-soaked in 10 mL of Buffered Peptone Water (3M, St Paul, MN) using a 10 cm × 10 cm sterile template. Surfaces in receiving ingredient pit grating, floors in receiving area, manufacturing area, warehouse and control/brake room were sampled in triplicates. Single samples were collected from fat intake inlet, exterior of pellet mill, finished product bin boot/product discharge, load-out auger and broom. Worker shoes samples were collected from both left and right shoe. Finished feed was obtained from fresh feed manufactured the same day of sample collection, usually after conditioning. Only for feed samples the method described in Chapter 5 of the Bacteriological Analytical Manual was followed and a 50g feed portion was used for further testing

(BAM, 2011). All samples were kept under refrigeration conditions and transported to the laboratory. Processing and testing of samples was conducted within 48 hours of sampling. Samples were collected over three seasons: fall (October and November 2016), early spring (February and March 2017) and summer (June and July 2017).

Culture-based analysis

The USDA-FSIS laboratory guidebook for the isolation and identification of *Salmonella* from meat, poultry, pasteurized egg and catfish products and carcass and environmental sponges was followed for culture-based analysis (USDA/FSIS, 2014). Positive colonies were selected and one colony per plate was picked. In addition, culture-based positive isolates were analyzed with a combination of biochemical assays: Lysine Iron Agar test (BD Difco, Sparks, MD) and Triple Sugar Iron Agar test (BD Difco, Sparks, MD). Positive colonies were further investigated in a slide agglutination assay using a *Salmonella* O antiserum polyvalent test for groups A through G + iv following manufacturer's instructions (BD Difco *Salmonella* O Antisera, Sparks, MD).

Molecular-based analysis

Positive culture- based samples were further analyzed by real-time PCR. One colony from each agar plate was transferred directly and without any treatment to the PCR mixture. A protocol developed in our laboratory, that targets the invasion gene *inv*A present in all *Salmonella enterica* was followed (Bai et al., 2018). For every experiment, a non-template control, a non-*Salmonella* control (*Escherichia coli* O157:H7 ATCC 43888) and four positive controls (*Salmonella* Newport from ATCC 6962, *Salmonella* Typhimurium from ATCC BAA-215, *Salmonella* Typhimurium monophasic variant 4, [5], 12:i:- CA RM 17 305 obtained from USDA ARS Albany CA and *Salmonella* Typhimurium monophasic variant 4, [5],12:i:- NY FSL5-580 obtained from the Department of Food Science at Cornell University) were added. A sample was considered PCR positive when the Ct value was lower than 40.

Multiplex PCR

PCR+ isolates were further analyzed by a multiplex PCR assay to differentiate ST and STM from other serotypes. The protocol described by Prendergast et al. (2013) was

followed with minor modifications. A pick from a colony was transferred directly from an agar plate, with a pipet tip, to the PCR mixture without any treatment. The reaction was carried out in a final total volume of 25µl, containing 1µl of primer mix (0.4µM of each primer) (Bioresearch Technologies, Petaluma, CA), 0.5µl (0.2µM) of each probe (Bioresearch Technologies, Petaluma, CA), 12.5µl of 2X IQ Multiplex Power mix (Bio-Rad, Hercules, CA), and 10µl of nuclease-free molecular biology grade water (Integrated DNA Technologies, Coralville, IA). Three sets of primers and probes were used in the assay and the targeted genes were fliC (present in ST and STM), fljB 1,2 (present in ST), and fliB/IS200 (present in ST and STM) (Prendergast et al., 2013). The PCR was carried out in a CFX96 thermocycler (Bio-Rad, Hercules, CA), with initial denaturation step of 94°C for 2 min, followed by 40 cycles of 95°C for 20 seconds and 60°C for 90 seconds. Reaction was considered positive when Ct values ≤ 40. Samples were characterized as ST if expressing all three genes (fliC, fljB 1,2, and fliB/IS200) and STM if expressing both fliC and fliB/IS200 genes.

Statistical analysis

Generalized linear mixed models were fitted in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using the glimmix procedure. Binary distribution, logit link, Laplace approximation, and ridge-stabilized Newton-Raphson algorithm were used. The outcome consisted of the presence of *Salmonella* spp. in environmental samples as determined by the PCR test (dichotomous: positive vs negative). Independent variables included: season (fall, spring, and summer), mill ID (each individual mill received an ID consisting of a number from 1 to 11), mill type (divided into mills producing mash or pelleted feed), and sample site (numbered from 1 to 12, representing the sites on table 1). When at least one of the subsamples of floor and worker shoes tested positive (PCR +) sample sites were considered positive. An initial univariable screen for the fixed effects of season, mill ID, mill type, and sample site was followed by a multivariable model if more than one fixed effect was significant ($P < 0.05$) in the univariable screen. Random effects considered for the univariable models were season, state, mill ID, mill type, month, and season. Mean probabilities and their 95% confidence intervals were computed (Table 1).

Figure 1. Map of the main swine production areas across the United States (highlighted), where feed mills were selected for this study.

Adapted from USDA (2015): Overview of the United States Hog Industry.

Results:

1) A total of 383 environmental and feed samples were collected from eleven feed mills during three season. From the total isolates, 49 (12.8%) were *Salmonella* PCR +: two isolates (5.1%) were identified in feed, while the other 47 (13.7%) on equipment and/or surfaces. Nine of the total 11 feed mills had at least one *Salmonella* spp. PCR+ sample and the mean prevalence varied from 1.9 % to 37.5% across mills. Facility 4 and 7 had the highest mean prevalence with 28.5% and 37.5%, respectively, whereas mills 9 and 11 had no test positive samples (Table 1). A multiplex PCR was designed to identify ST

and STM among the 49 PCR+ *Salmonella* isolates. A total of two ST and three STM were identified by the multiplex PCR. Both ST isolates originated from mill 5, one STM isolate came from mill 1 and the other two STM isolates were found in mill 10.

2) Based on the univariable models, season ($P < 0.007$) and mill ID ($P < 0.005$) were significantly associated with the presence of *Salmonella* spp., while mill type ($P > 0.952$) and sample site ($P > 0.170$) were not (Table 1). Samples collected during fall months had a significantly higher mean prevalence (13.2%) of *Salmonella* compared to samples collected during early spring (3.6%) or summer (6.7%) (Table 1). As shown in Table 1, a higher mean prevalence of *Salmonella* spp. was observed in sites corresponding to receiving area floor (20.9%), manufacturing area floor and receiving ingredients pit gratin (14.7%), followed by control room floor and worker shoes (11.9%). Interaction between significant fixed effects (mill ID* season) were tested (P=0.999) and the random effects month and state were considered confounded with season and mill ID, respectively. When an effect was considered fixed, it was removed from the list of random effects and the factors were analyzed independently. Therefore, Mill ID ($P < 0.003$) and season ($P < 0.005$) were significantly associated with prevalence of *Salmonella* in our multivariable model. ST isolates were recovered from the receiving area floor during summer in mill 5. One STM isolate came from mill 1 and was identified in the control room floor during summer. The other two STM isolates were found in mill 10 during fall from the receiving ingredients pit gratin and receiving area floor. These results show that the ST and STM isolates followed the same seasonal pattern as PCR + *Salmonella*, with higher prevalence during fall and summer. Additionally, the sample sites where ST and STM were recovered matched the highest percentage of PCR + samples.

Table 1. Presence of *Salmonella* PCR + samples in feed mills by season, mill id, mill typ and sampling site.

Variable	n[1]	Model-adjusted[2]		
		Mean prevalence (%)	95% CI (%)	*P* - value
Season				0.006
Fall 2016	25	13.2	5.1-29.7	
Spring 2017	9	3.6	1.1-11.0	
Summer 2017	15	6.7	2.3-17.9	
Mill ID				0.005
1	5	10.8	3.9-26.8	
2	1	1.9	0.2-13.6	
3	3	6.2	1.7-19.9	
4	11	28.4	14.0-49.1	
5	6	13.9	5.4-31.5	
6	2	3.9	0.9-16.1	
7	13	37.4	19.9-58.8	
8	3	6.7	1.8-21.4	
9	0	0.0	0.0-100	
10	5	11.9	4.1-29.6	
11	0	0.0	0.0-0.0	
Mill ID*season				0.999
Mill type				0.952
Mash	27	6.8	1.7-23.5	
Pelleted	22	7.3	1.8-25.6	
Sample site				0.170
1. Receiving ingredients pit gratin	7	14.7	4.0-41.5	
2. Fat intake inlet	1	1.6	0.2-14.7	
3. Pellet mill	2	7.5	1.1-36.1	
4. Discharge bin boot	3	5.0	1.0-21.9	
5. Load-out auger	0	0.0	0.0-100	
6. Finished feed	2	3.0	0.5-16.6	
7. Control room floor	6	11.9	3.1-36.7	
8. Receiving area floor	9	20.9	6.3-50.7	
9. Manufacturing area floor	7	14.7	4.0-41.5	
10. Warehouse area floor	3	4.7	1.0-21.9	
11. Worker shoes	6	11.9	3.1-36.7	
12. Broom	3	5.0	1.0-21.9	
Total	49			

[1] Number of *Salmonella* positive (PCR+) samples per variable considered in this study.

[2]Model-adjusted prevalence estimates from univariable models evaluating the association between each variable with the presence of *Salmonella* spp

Discussion:

Salmonella have been proven to exhibit seasonal variation: higher prevalence in warmer months and lower in colder months (D'Souza et al., 2004; Pangloli et al., 2008;

Ravel et al., 2010). Also in our study, we observed a higher PCR + samples prevalence in fall and summer seasons (Table 1), which is consistent with the data reported from other research (Ravel et al., 2010; Jahne et al., 2015). During warmer months' people tend to walk around the facility more often, go outside, and keep doors and windows open for air circulation. This behavior leaves the mill more susceptible to the entrance and spread of microorganisms. Conversely, during colder months' people have the tendency of remaining inside and keep doors and windows closed. Other factors contributing to the seasonality of bacterial contamination are the airborne transmission of *Salmonella* from high air particulate matter created by harvesting crops and the use of swine manure as fertilizer during the fall. That combination increases the availability and transmission of airborne pathogens into the mills. (Jahne et al., 2015). We also observed a significant association between the feed mill ID and the prevalence of *Salmonella*. Differences in management, geographical location, hygiene practices, quality of incoming raw ingredients, volume of feed produced, number of workers, and time the facility has been operational are all important variables for pathogen presence as described by (Cochrane, 2016). Two different type of mills were included in the research: one producing mash and the other pelleted feed. Facilities were structurally different: extra equipment was present for the pelleting process (conditioner, extruder, pellet mill and cooler). However, no significant differences were observed between these two mill types, probably due to same amount of dust accumulation and human flow as vehicles of microbial spread around the facility. Production flow and plant design might also play a role in preventing microbial introduction and recontamination of finished feeds (Whyte et al., 2003). Research studies showed that raw grain ingredients and transporting trucks were the vehicle of contamination into the mill facilities (Binter et al., 2011; Fedorka-Cray and Hogg, 1997). As in our study, a high number of PCR+ samples were found in the receiving ingredient pit gratin and receiving area floor. Additionally, birds and bird feces were found in some facilities, highlighting the vulnerability of these production environments to pests, wildlife, weather condition, and human/vehicle traffic (Torres et al., 2011; Whyte et al., 2003). Because *Salmonella* can survive for long periods of time in dry and hostile environments, in our analysis we considered worker shoes as a

potential microorganism reservoir (Table 1). Amass, et al. (2000) and Otake, et al. (2002) proved that shoes can carry biological hazards, like porcine reproductive virus and respiratory syndrome virus. Therefore, the worker shoes can also represent a vehicle for pathogen spread around the mill. Not surprisingly, in our study, the control room and manufacturing floor area with the highest human flow) showed a high percentage of PCR+ samples. Since our intent was to understand if high environmental contamination could lead also to final product contamination as highlighted by Jones and Richardson (2004), finished feed was collected during each visit. Among feed samples only two (5.1%) were PCR+ and came from mills 4 and 7. Both facilities had the highest mean prevalence of *Salmonella*, 28.4 and 37.4 % respectively. These results are consistent with the FDA surveillance findings from 2007-2009, where 5.6% of finished feed was contaminated with *Salmonella* (Li et al., 2012). The high bacterial prevalence in feed mill environments can be easily connected with a greater risk of cross-contamination of finished feed.

ST and STM showed overall a similar seasonal presence throughout the study, as compared to S*almonella* spp., being found most commonly during fall and summer. STM, along with ST, is one of the most commonly found serotype in humans, swine and pork products in recent decades (Hauser et al., 2010; Moreno Switt et al., 2009).

Summary of the results

The data gathered in this study shows the potential role of feed and feed mill environment as entry routes for *Salmonella spp,* ST and STM into human food chain. A seasonal pattern was observed with higher pathogen prevalence in fall and summer. A total of 5 ST and STM isolates were found. Hygiene, management, production flow, and cross-contamination within facility were all important factors linked with pathogen contamination in mills. We found that both the mill and the season were significantly associated with *Salmonella* prevalence. These observations might contribute to the implementation of biosecurity plans and other preventative strategies in feed mills and to understand *Salmonella* ecological niche in the animal feed processing environment.

References

Abrahantes, J. C., K. Bollaerts, M. Aerts, V. Ogunsanya, and Y. Van der Stede. 2009. S*almonella* serosurveillance: Different statistical methods to categorise pig herds based on serological data. *Preventive Veterinary Medicine*. 89:59-66.

Amass S, Stevenson G, Anderson C, Grote LA, Dowell C, Vyverberg DV, Kanitz C, Ragland D. Investigation of people as mechanical vectors for porcine reproductive and respiratory syndrome virus. *Swine Health Prod*. 2000; 8:161–166

Arguello, H., Álvarez-Ordoñez, A., Carvajal, A., Rubio, P., Prieto, M. Role of Slaughtering in *Salmonella* Spreading and Control in Pork Production. Journal of Food Protection 2013; 76:899–911.

Bacterial Analytical Manual. Chapter 5: *Salmonella.* 2011. Available at: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm, accessed April 3, 2016.

Baer, A. A., M. J. Miller, and A. C. Dilger. 2013. Pathogens of interest to the pork industry: A review of research on interventions to assure food safety. *Comprehensive Reviews in Food Science and Food Safety*. 12:183-217.

Bai, J., V. Trinetta, X. Shi, G. Magossi, E. Porter, N. Cernicchiaro, D. G. Renter, and T. G. Nagaraja. 2018. A Multiplex Real-Time PCR Assay, Based on i*nvA* and p*agC* Genes, for the Detection and Quantification of *Salmonella* Species in Cattle Feces, Lymph nodes and Environmental Samples. *Journal of Microbiological Methods.* https://doi.org/10.1016/j.mimet.2018.03.019

Binter, C., Straver, J.M., Häggblom, P., Bruggeman, G., Lindqvist, P.-A., Zentek, J., Andersson, M.G. Transmission and control of *Salmonella* in the pig feed chain: A

conceptual model. International Journal of Food Microbiology 2011; 145:S7–S17. https://doi.org/10.1016/j.ijfoodmicro.2010.09.001

Burns, A. M., P. G. Lawlor, G. E. Gardiner, E. M. McCabe, D. Walsh, M. Mohammed, J. Grant, and G. Duffy. 2015. *Salmonella* occurrence and *Enterobacteriaceae* counts in pig feed ingredients and compound feed from feed mills in Ireland. *Preventive Veterinary Medicine*. 121:231-239.

Carter, P.J., P. Cullen, A. Glenn, S. McDermott, J. Paige, and D. Wagner. Prevalence and antibiotic susceptibility profiles of *Salmonella* and *Escherichia coli* recovered from rendered animal feed commodities—results of a national survey. Presented at the American Society of Microbiology, 2003, Washington, DC.

[CDC] Centers for Disease Control and Prevention. 2014. National Enteric Disease Surveillance: *Salmonella* Surveillance Overview. Available at: https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html, accessed June 7, 2018.

Cochrane, R. A., S. S. Dritz, J. C. Woodworth, C. R. Stark, A. R. Huss, J. P. Cano, R. W. Thompson, A. C. Fahrenholz, and C. K. Jones. 2015. Feed mill biosecurity plans: A systematic approach to prevent biological pathogens in swine feed. *Journal of Swine Health and Production*. 24 (3), 154-164.

Crump, J. A., and P. M. Griffin. 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. *Clinical infectious diseases*. 35(7): 859-865.

Davies, P. R., S. Hurd, J. A. Funk, P. J. Fedorka-Cray, and F. T. Jones. 2004. The role of contaminated feed in the epidemiology and control of *Salmonella enterica* in pork production. *Foodborne pathogens and disease*. 1(4):201-215.

Davies, R.H., Wales, A.D. Investigations into *Salmonella* contamination in poultry feed mills in the United Kingdom: *Salmonella* in poultry feed mills. Journal of Applied Microbiology 2010; 109:1430–1440. https://doi.org/10.1111/j.1365-2672.2010.04767

Davis, M.A., Hancock, D.D., Rice, D.H., Call, D.R., DiGiacomo, R., Samadpour, M., Besser, T.E. Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella* enterica. Vet Microbiol 2003; 95:199–210. https://doi.org/10.1016/S0378-1135(03)00159-7

Delgado, C., M. Rosegrant, H. Steinfeld, S. Ehui, and C. Courbois. 2001. Livestock to 2020: The Next Food Revolution. *Outlook on Agriculture*. 30:27-29.

De Knegt, L.V., Pires, S.M. and Hald, T. Attributing foodborne salmonellosis in humans to animal reservoirs in the European Union using a multi-country stochastic model. *Epidemiology & Infection* 2015; *143*(6):1175-1186.

D'Souza, R.M., Becker, N.G., Hall, G., Moodie, K.B.A. Does Ambient Temperature Affect Foodborne Disease Epidemiology 2004; 15:86–92. https://doi.org/10.1097/01.ede.0000101021.03453.3e

Fedorka-Cray, P. J., Hogg, A., Gray, J. T., Lorenzen, K., Velasquez, J., & Von Behren, P. Feed and feed trucks as sources of *Salmonella* contamination in swine. *Journal of Swine Health and Production* 1997; *5*:189-193.

Food and Drug Administration (FDA). Compliance Policy Guide Section 690.800 *Salmonella* in Food for Animals 2013. Available at http://www.fda.gov/downloads/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/UCM361105.pdf., accessed 13 March 2018.

Hauser, E., Tietze, E., Helmuth, R., Junker, E., Blank, K., Prager, R., Rabsch, W., Appel, B., Fruth, A., Malorny, B. Pork Contaminated with *Salmonella* enterica Serovar 4,[5],12:i:-, an Emerging Health Risk for Humans. Applied and Environmental Microbiology 2010; 76:4601–4610. https://doi.org/10.1128/AEM.02991-09

Jahne, M.A., Rogers, S.W., Holsen, T.M. et al. Aerobiologia 2015; 31:73. https://doi-org.er.lib.k-state.edu/10.1007/s10453-014-9348-0

Jones, F. T. 2011. A review of practical *Salmonella* control measures in animal feed. *Applied Poultry Research*. 20:102-113.

Jones, F.T., Richardson, K.E. *Salmonella* in Commercially Manufactured Feeds. Poultry Science 2004; 83:384–391. https://doi.org/10.1093/ps/83.3.384

Kidd, R.S., Rossignol, A.M., Gamroth, M.J. *Salmonella* and other Enterobacteriaceae in dairy-cow feed ingredients: antimicrobial resistance in western Oregon. J Environ Health 2002; 64:9–16.

Kranker, S., Alban, L., Boes, J., Dahl, J. Longitudinal Study of *Salmonella enterica* Serotype Typhimurium Infection in Three Danish Farrow-to-Finish Swine Herds. J. Clin. Microbiol 2003; 41:2282–2288. https://doi.org/10.1128/JCM.41.6.2282-2288.2003

Li, X., L. A. Bethune, Y. Jia, R. A. Lovell, T. A. Proescholdt, S. A. Benz, T. C. Schell, G. Kaplan, and D. G. McChesney. 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.

Machado, J., Bernardo, F. Prevalence of *Salmonella* in chicken carcasses in Portugal. J. Appl. Bacteriol 1990; 69:477–480.

Moreno Switt, A.I., Soyer, Y., Warnick, L.D., Wiedmann, M. Emergence, Distribution, and Molecular and Phenotypic Characteristics of *Salmonella enterica* Serotype 4,5,12:i:–. Foodborne Pathogens and Disease 2009; 6:407–415. https://doi.org/10.1089/fpd.2008.0213

Molla, B., A. Sterman, J. Mathews, V. Artuso-Ponte, M. Abley, W. Farmer, P. Rajala-Schultz, W. E. Morgan Morrow, and W. A. Gebreyes. 2010. *Salmonella enterica* in Commercial Swine Feed and Subsequent Isolation of Phenotypically and Genotypically Related Strains from Fecal Samples. *Applied and Environmental Microbiology*. 76:7188-7193.

Otake S, Dee S, Rossow K, Deen J, Joo H, Molitor T, Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod.* 2002; 10:59–65.

Österberg, J., I. Vågsholm, S. Boqvist, and S. Sternberg Lewerin. 2006. Feed-borne Outbreak of Salmonella Cubana in Swedish Pig Farms: Risk factors and Factors affecting the restriction period in infected farms. *Acta Veterinaria Scandinavica*. 47:13-22.

Pangloli, P., Dje, Y., Ahmed, O., Doane, C.A., Oliver, S.P., Draughon, F.A. Seasonal Incidence and Molecular Characterization of *Salmonella* from Dairy Cows, Calves, and Farm Environment. Foodborne Pathogens and Disease 2008; 5:87–96. https://doi.org/10.1089/fpd.2007.0048

Prendergast, D.M., Hand, D., Ní Ghallchóir, E., McCabe, E., Fanning, S., Griffin, M., Egan, J., Gutierrez, M. A multiplex real-time PCR assay for the identification and differentiation of *Salmonella* enterica serovar Typhimurium and monophasic serovar

4,[5],12:i:–. International Journal of Food Microbiology 2013; 166:48–53. https://doi.org/10.1016/j.ijfoodmicro.2013.05.031

Podolack, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of Salmonella in Low-Moisture Foods. *Journal of Food Protection*. 73:1919-1936.

Ravel, A., Smolina, E., Sargeant, J.M., Cook, A., Marshall, B., Fleury, M.D., Pollari, F. Seasonality in Human Salmonellosis: Assessment of Human Activities and Chicken Contamination as Driving Factors. Foodborne Pathogens and Disease 2010; 7:785–794. https://doi.org/10.1089/fpd.2009.0460

Torres, G.J., Piquer, F.J., Algarra, L., de Frutos, C., Sobrino, O.J. The prevalence of *Salmonella* enterica in Spanish feed mills and potential feed-related risk factors for contamination. Preventive Veterinary Medicine 2011; 98:81–87. https://doi.org/10.1016/j.prevetmed.2010.11.009

USDA Food Safety and Inspection Service. Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg, and catfish products and carcass and environmental sponges 2014. Available at https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES, accessed September 13, 2016.

Whyte, P., McGill, K., Collins, J.D. A survey of the prevalence of and other enteric pathogens in a commercial poultry feed mill. Journal of Food Safety 2003; 23:13–24. https://doi.org/10.1111/j.1745-4565.2003.tb00348.x