Longitudinal study of *Salmonella* shedding in naturally infected finishing pigs

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SUMMARY

A 3-year longitudinal study was conducted on a multi-site farrow-to-finish production system. For each of 18 cohorts at three finishing sites, 50 pigs were randomly selected. Faecal samples were collected every 2 weeks for 16 weeks. *Salmonella* was cultured from 453 (6·6%) of 6836 faecal samples. The pig-level incidence of *Salmonella* was 20·8% (187/899 pigs). *Salmonella* prevalence varied between cohorts and within pigs. The adjusted *Salmonella* prevalence decreased over the finishing period from 6·4% to 0·8%. Intermittent detection of *Salmonella* was found in more than 50% of pigs that were positive at more than one collection. The finding that the majority of pigs shed intermittently has implications for surveillance and research study design when determining *Salmonella* status. The variability in shedding over time, as well as between and within cohorts and pigs suggests that there may be time-variant risk factors for *Salmonella* shedding in swine.

Key words: Longitudinal, *Salmonella*, swine.

INTRODUCTION

It has been well documented that *Salmonella* spp. are one of the major causes of foodborne diseases in the USA and worldwide [1–3]. In the USA alone, it is estimated that 1027 million non-typhoidal *Salmonella* human infections result in 19336 hospitalizations and 378 deaths annually [3], costing US$365 billion in direct medical expenditure per year [2]. Swine are a potential reservoir for human salmonellosis. The most common serotypes isolated in swine (*S.* Typhimurium, *S.* Heidelberg, *S.* Agona, *S.* Infantis) are common to those found in human cases [4, 5]. It has been suggested that reduction of *Salmonella* contamination of pork requires interventions at three levels: pre-harvest (farm), harvest (slaughter) and post-harvest (distribution systems and consumer handling) [6, 7]. In order to put in place on-farm control and inter-vention measures it is crucial to understand the dynamics of *Salmonella* infection in swine.

A large number of epidemiological studies have been conducted to determine prevalence and risk factors for *Salmonella* infection in swine. Most of these studies have used a cross-sectional study design. A limited number have assessed the faecal prevalence over time, with longitudinal studies showing high

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variability in *Salmonella* shedding at the farm, cohort and individual animal level [8–15]. Longitudinal studies at the pig level during the finishing phase have reported time variability of faecal shedding associated with cohort (or batch) of pigs [11–14]. Intermittent faecal shedding is also commonly reported in epidemiological studies of swine [12–14].

Therefore, longitudinal studies at the individual level based on bacteriological culture should be performed in order to investigate the dynamics of *Salmonella* infection in swine. The objective of this study was to describe the shedding pattern of *Salmonella* in faeces of naturally infected finishing pigs.

METHODS

A longitudinal study was conducted on a multi-site farrow-to-finish production system located in the Midwestern USA. The presence of *Salmonella* had been confirmed by culture of pooled faecal samples prior to initiation of the study. Selection criteria for the production system were willingness to cooperate in a long-term research project and to share health management and production records. The production system had three-site management, meaning that overall production was separated into three stages of production, breeding and farrowing, nursery (weaning until age ∼10 weeks) and finishing (10 weeks to slaughter, 24–26 weeks), with each stage housed at a separate site. The production system had all-in/all-out management in nursery and finishing sites. This system consisted of two farrowing sites (F1 and F2), two nursery sites (N1 and N2), and 12 finishing sites. The farrowing sites had a total inventory of 3700 sows (F1 = 1300, F2 = 2400), the average one-time inventory of the finishing sites was 25 000 (75 000 finishing pigs/year marketed). During the study period the system transitioned from two farrowing sites to one farrowing site of 3000 sows. The number of nursery and finisher sites remained unchanged. Three finishing sites (A, B, C) were conveniently selected, based on building design and willingness to participate in the study. At each finishing site one barn was selected for study inclusion. Site A had four barns in separate buildings. Pigs were allocated into 40 pens (20–25 pigs per pen). Sites B and C had identical building structures. Each site had four barns grouped in two buildings (two barns/rooms with one shared wall). Each barn housed about 1000 pigs. Pigs were housed in 12 pens; ten pens were initially stocked with pigs at placement (eight pens with a range of 100–125 pigs

and two pens with a range of 40–50 pigs). The remaining two pens were used for sick pigs or pigs deemed to be at risk for illness. Sites A and B were finishing sites (age 10–26 weeks). Site C transitioned to a weaning-to-finishing site after the second cohort of pigs. For the weaning-to-finishing cohorts at site C, piglets were placed in the barn at age 3 weeks and remained until marketing. Finishing site A received pigs from nursery N1 in all cohorts; site B received pigs from nursery N2 in four cohorts and from N1 and N2 in the last two cohorts of pigs. The first cohort for site C was supplied from nursery N2, for all other cohorts piglets were placed directly from the farrowing sites due to the transition to a weaning-to-finishing site.

Sample collection

Nursery sampling

In order to evaluate the *Salmonella* status of the cohort of pigs prior to sampling during the finishing phase, ten pools were collected from the nursery rooms ∼1 week prior to movement to the finishing barn. A pool consisted of a minimum of 5 g fresh faecal material collected from five different locations on the same pen floor (25 g/pool). In the weaning-to-finishing site (site C), ten pools were collected from ten random pens when pigs were aged ∼9 weeks.

Environmental sampling

Finisher barns were cleaned and disinfected between batches of pigs. The disinfectants (Synergize, Preserve International, USA and VirkonS, Antec International, UK) were alternated following the standard operating procedure of the production system. In order to assess contamination, culture of environmental samples was performed after cleaning and disinfection and before placement of pigs in the barn. Drag swabs and environmental swab samples were obtained from cleaned and disinfected floors, walls, gates and feeders/drinkers following previously described methods [16]. Briefly, swabs were moistened with 10 ml of sterile buffered peptone water (BPW, Acumedia, Neogen Corporation, USA) before collection. To sample floors, one drag swab was used for four pens in site A. In sites B and C, one drag swab was used per 1–2 pens depending on pen size. To sample other environmental surfaces, a single 4 × 4 gauze moistened with BPW was used to sample each surface. Ten, five, three and two swabs were collected

from floors, walls, gates and feeders/waterers, respectively in each barn prior to every cohort.

Individual faecal sampling

At the beginning of each cohort, 50 pigs (aged 10 ± 2 weeks) were randomly selected and individually identified with ear tags. Random number generation was conducted in Microsoft Excel 2007 (Microsoft, USA). In site A, a simple random sample was generated to select one pig from every pen ($n = 40$). Another ten pens were randomly selected to identify and select a second pig using a simple random sample (additional ten pigs for a total of $n = 50$/barn; 30 pens with one study pig, ten pens with two study pigs). A random proportional sampling scheme based on the number of pigs in each pen was conducted in sites B and C within each cohort. A range of 1–7 pigs per pen was selected. In sites B and C no pigs were selected for study inclusion from the pens identified for sick or at-risk pigs.

Individual faecal samples were collected from the rectum with gloved hand, and placed in sterile containers (Specimen cups, VWR International LLC, USA). Gloves for collecting the faecal samples were changed between pigs. After collection, samples were stored at ambient temperature for transport to the laboratory. Individual pig faecal samples were collected every 2 weeks for 16 weeks (eight total sampling periods per pig). A total of 400 individual samples (50 pigs × 8 sample periods) per cohort and 7200 faecal samples overall (400 samples × 18 cohorts) were planned for collection.

Bacteriological culture

Faecal samples

Bacteriological culture for *Salmonella* was performed by the Diagnostic Center for Population and Animal Health, Michigan State University. Faecal samples were transported to the laboratory the day of collection or stored for 48 h at $2 \cdot 8$ °C.

Faecal samples were cultured using standard enrichment methods from Davies *et al.* [17]. Briefly, for pooled samples from the nursery, 25 g of pooled faecal samples were diluted in 225 ml tetrathionate broth (TTB) (Becton Dickinson, USA) and incubated at 37 °C for 48 h. For individual pig faecal samples 10 g of the individual faecal samples were inoculated into 90 ml TTB and incubated at 37 °C for 48 h. After incubation, an aliquot (100 μl) of the faecal-TTB

solution was inoculated into $9 \cdot 9$ ml Rappaport–Vassiliadis broth (RV, Becton Dickinson) and incubated at 42 °C for 24 h. The RV broth was plated onto xylose lysine tergoitol 4 agar (XLT4, Remel, Thermo Fisher Scientific, USA) selective agar plates and incubated at 37 °C, overnight. Suspect *Salmonella* colonies from microbiological culture were screened using *Salmonella* poly O antisera antiglutination (Becton Dickinson, USA).

Environmental samples

Environmental samples were cultured following the same protocol [17] using a volume of TTB sufficient to submerge the swabs ($\sim$60 ml).

Data analysis

Bacteriological culture data were entered into an Excel 2007 spreadsheet using appropriate coding and subsequently verified for accuracy by checking each entry with the original hard copy result. The spreadsheets were transferred to a relational database (Access 2007, Microsoft Corporation). Data were retrieved from the database and imported into SAS 9.3 (SAS Institute Inc., USA) for data management and statistical analysis.

Descriptive statistics of demographic data (number of pigs sampled, gender), loss to follow-up and morbidity were presented in proportions.

Descriptive statistics of bacteriological culture were described for the nursery, barn environment, site, cohort, pig, and faecal sample (observation). *Salmonella* apparent prevalence (proportion of positive samples/ tested) and respective 95% confidence intervals (CI) were estimated at each unit of observation: cohort (e.g. all collections combined within cohort), site (e.g. all cohorts combined), pig age (by collection period) and individual sample. Logistic models (Proc Glimmix, SAS 9.3, SAS Institute Inc.) accounting for clustering within pig, cohort and site, were applied to compare prevalence at each unit of investigation. Logistic models accounting for clustering within cohort and site were used to estimate adjusted prevalence for cohort and site, to compare the proportion of *Salmonella*-positive samples in sites, and to estimate the association between the proportion of *Salmonella*-positive samples by collection period and proportion of *Salmonella*-positive samples in nursery and environmental samples. Model-adjusted apparent prevalences are presented for cohorts, age and site. Pearson χ^2 analysis with Bonferroni adjustment

was used to compare the proportion of *Salmonella*-positive samples in the cohorts classified based on nursery and barn environmental *Salmonella* status (positive or negative). A significance level of 0·05 was used for all comparisons.

Patterns and duration of shedding were estimated for those pigs which met the following inclusion criteria: (1) survival until marketing (excluded $n=3$ dead and $n=5$ early shipment); (2) no more than one period from which a sample was not collected (excluded $n=1$); (3) had no more than two negative cultures between two positive culture results (excluded $n=10$).

In order to estimate the duration of shedding of individual pigs, we assumed that the shedding began 7 days prior the first detected positive culture and lasted until 7 days after the last isolation. The 7-day interval was selected taking into account data from experimental studies indicating that pigs start to shed *Salmonella* as early as 2–7 days post-exposure [18] and as late as 7–14 days [19, 20], after exposure to a *Salmonella*-contaminated environment or when commingled with pigs shedding *Salmonella*. This interval (7 days) is also the midpoint between two consecutive sampling periods.

RESULTS

Demographic results

A total of 900 pigs were selected for inclusion in the study. Forty-six percent (410/900) were barrows or castrated males and 54% (490/900) were females.

The total loss to follow-up for faecal sample collection was 5·1% (364/7200). Causes for loss to follow-up were: death, unable to collect a specimen (e.g. empty rectum, sick animal), or shipped to market prior to final collection. A total of 17 (17/900, 1·9%) pigs died during the study. The majority of the pigs were sampled eight (71·4%, 643/900) or seven (23·1%, 208/900) times.

At the observation level (individual pig × number of sample periods pig observed) diarrhoea was described in 2·4% (164/6836) of the observations. At the pig level, 15·1% (136/900) were observed to have diarrhoea at least once.

Nursery and barn environment

The total proportion of positive samples and respective 95% CI of nursery, barn environment samples and sites, stratified by cohort are summarized in Table 1. Pooled faecal samples from the source nursery were collected and cultured in 17/18 cohorts. *Salmonella* was detected in at least one nursery pool in 76·5% (13/17) of the cohorts. A total of 36·5% (62/170) of the pooled nursery samples were *Salmonella* positive. The proportion of positive nursery samples ranged from 0% to 100% in the cohorts (Table 1).

Environmental samples were collected for all cohorts. *Salmonella* was detected in at least one environmental swab in 61·1% (11/18) of the cohorts. The total number of positive swabs was 40 (40/360, 11·1%). The proportion of positive barn environmental swabs ranged from 0% to 85% in the cohorts (Table 1).

Site, cohort and age apparent prevalence

Salmonella was isolated from at least one sample type (nursery, environmental or individual faecal) sample at all three sites. In 17/18 cohorts at least one individual faecal sample was positive. *Salmonella* was cultured from 6·6% (453/6836, 95% CI 6·0–7·2) of individual faecal samples. The proportion of positive faecal samples within a cohort (eight collection periods combined per cohort) ranged from 0% to 44·1%. Within site, the adjusted *Salmonella* apparent prevalence per cohort (six cohorts/site) ranged from 1·9% (95% CI 0·7–5·4) to 12·1% (95% CI 7·9–18·3) in site A, 1·1% (95% CI 0·3–4·0) to 43·1% (95% CI 35·7–50·9) in site B and 0% to 5·7% (95% CI 3·2–10·1) in site C (Table 1).

For the 17 cohorts with both nursery and environmental swab collections, there were nine cohorts with at least one positive sample in both sample types that also had at least one individual faecal sample positive. Three cohorts were *Salmonella* positive in the nursery but *Salmonella* negative for environmental swabs. One cohort was negative in the nursery and had at least one environmental swab positive. Three cohorts were negative for both sample types. One cohort had at least one positive sample for both nursery and environmental samples but was negative for individual faecal samples. The proportion of *Salmonella*-positive samples was significantly greater in those cohorts in which both the nursery and the barn environment were *Salmonella* positive ($P<0.05$) (Table 2). No significant difference was found in cohorts negative for both types of samples and nursery positive and environment negative or nursery negative and

Table 1. *Proportion of samples positive for* Salmonella *spp. by site and cohort (samples represent individual faecal samples, pooled faecal samples from the source nursery and barn environmental swabs) and respective 95% confidence intervals (CI)*

Site/cohort	Environment*		Nursery†		Total positive faecal samples tested, n/N (%)	Adjusted Salmonella apparent prevalence‡	
	%	(95% CI)	%	(95% CI)		%	(95% CI)
Site A							
1	5·0	(0·1–24·9)	60·0	(26·2–87·9)	30/388 (7·7)	7·6	(4·5–12·5)
2	25·0	(8·7–49·1)	80·0	(44·4–97·5)	42/396 (10·6)	10·4	(6·7–15·7)
3	0·0	n.a.	n.s.	n.s.	7/390 (1·8)	1·9	(0·7–5·4)
4	85·0	(62·1–96·8)	60·0	(26·2–87·8)	46/382 (12·0)	12·1	(7·9–18·3)
5	5·0	(0·1–24·9)	20·0	(2·5–55·6)	16/386 (4·1)	4·7	(2·5–8·6)
6	5·0	(0·1–24·9)	10·0	(2·5–44·5)	6/396 (1·5)	1·9	(0·7–5·0)
Site B							
1	10·0	(1·2–31·7)	10·0	(0·25–44·5)	12/383 (3·1)	3·3	(1·5–7·1)
2	10·0	(1·2–31·7)	100·0	(69·2–100)	156/354 (44·1)	43·1	(35·7–50·9)
3	0·0	n.a.	20·0	(2·5–55·6)	4/379 (1·1)	1·5	(0·50–4·5)
4	30·0	(11·9–54·3)	100·0	69·2–100	57/339 (16·8)	16·4	(11·3–23·0)
5	5·0	(0·1–24·9)	0·0	n.a.	2/362 (0·6)	1·1	(0·3–4·0)
6	0·0	n.a.	0·0	n.a.	16/386 (4·1)	4·2	(2·1–8·3)
Site C							
1	0·0	n.a.	0·0	n.a.	1/387 (0·3)	0·6	(0·1–3·0)
2	0·0	n.a.	80·0	(44·4–97·5)	24/393 (6·1)	5·7	(3·2–10·1)
3	0·0	n.a.	0·0	n.a.	6/390 (1·5)	1·6	(0·6–4·6)
4	15·0	(3·2–37·9)	60·0	(26·2–87·8)	18/371 (4·9)	4·5	(2·4–8·8)
5	5·0	(0·1–24·9)	10·0	(2·5–55·6)	0/376 (0·0)	0·0	n.a.
6	0·0	n.a.	10·0	(2·5–44·5)	10/378 (2·6)	2·5	(1·0–6·1)

n.s., Not sampled; n.a., not applicable.
* Total of 20 environmental samples per cohort.
† Total of 10 pooled samples per cohort.
‡ Adjusted apparent prevalence accounting for clustering.

Table 2. *Distribution of cohorts and proportion of samples positive for* Salmonella *spp. by the* Salmonella *status of nursery and environmental swabs**

| Nursery and environment status	No. of positive cohorts	No. of negative cohorts	Positive faecal samples tested (n/N)	Proportion of positive faecal samples (%)†	95% CI
Nursery + environment +	9	1	383/3771	10·2[a]	9·2–11·2
Nursery + environment–	3	0	38/1150	3·3[bd]	2·4–4·5
Nursery – environment +	1	0	2/362	0·6[ce]	0–2·0
Nursery – environment–	3	0	23/1163	2[de]	1·3–3·0

CI, Confidence interval.
* Seventeen cohorts are represented, one cohort was excluded as no nursery samples were collected.
† Different superscript letters indicate a significant difference ($P < 0.05$) of proportion of positive faecal samples.

environment positive ($P > 0.05$) (Table 2). There was a positive association between the proportion of positive samples at each collection and the proportion of positive pooled nursery samples ($P < 0.0001$).

There was also a trend of a positive association between the proportion of positive barn environmental swabs ($P = 0.07$) and the proportion of positive faecal samples at each collection.
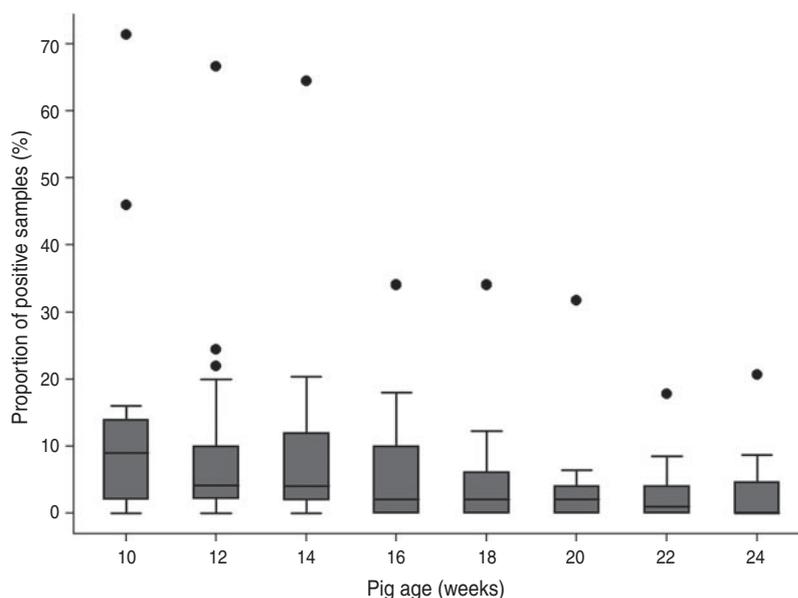
Fig. 1. Box plot representing the distribution of *Salmonella*-positive faecal samples within each cohort by pig age.

Although there was a numerical difference of the overall proportion of positive samples in the sites [site A: 6·3% (147/2338); site B: 11·2% (247/2203); site C (2·6% (59/2295)], no significant difference in the adjusted *Salmonella* prevalence ($P > 0·05$) between sites was found (site A: 5·1%, 95% CI 1·7–13·8; site B: 5·5%, 95% CI 1·9–15·1; site C: 1·8%, 95% CI 0·6–5·5).

For all 18 cohorts, the proportions of positive samples per cohort were plotted by age (Fig. 1). The overall median was 2·0%; 25%, 75% and 95% quartiles were 0%, 7·4% and 25·5%. The adjusted *Salmonella* apparent prevalences were 6·4% (95% CI 3·2–12·6), 4·3% (95% CI 2·1–8·8) and 3·7% (95% CI 1·8–7·8) at ages 10, 12 and 14 weeks, respectively, and were significantly different ($P < 0·0001$) from the end of finishing period (at 24 weeks: 0·8%, 95% CI 0·3–2·0). There were no significant differences ($P > 0·05$) in adjusted *Salmonella* apparent prevalence in the last four collections (18 weeks: 1·2%, 95% CI 0·6–2·7; 20 weeks: 0·9%, 95% CI 0·6–1·5; 22 weeks: 0·6%, 95% CI 0·3–1·5; 24 weeks: 0·3%, 95% CI 0·3–2·0).

Pig apparent prevalence and duration of shedding

Most pigs were detected as *Salmonella* positive for the first time at the first collection period (age 10 weeks, 61·5%, 115/187). This was followed by collections 2 and 3 (ages 12 and 14 weeks, 14·4%, 27/187), collection 4 (age 16 weeks, 4·3%, 8/187), collection 6 (age 20 weeks, 3·7%, 7/187) and collections 5, 7 and 8 (ages 18, 22 and 24 weeks, 0·5%, 1/187).

Overall incidence of *Salmonella* was 20·8% (187/899 pigs, 95% CI 18·2–23·6). Of the positive pigs, 87 were culture positive once (46·5%) and 27 (14·4%), 31 (16·6%), 17 (9·1%), 10 (5·4%), six (3·2%), and seven (3·7%) pigs were positive, two, three, four, five, six, and seven times, respectively. Only two pigs were *Salmonella* positive in all eight collection periods. The duration of shedding was clustered within site and cohort. The majority of the pigs with two or more positive samples belonged to site B (61/100 pigs), with sites A and C having 30 and nine pigs detected as culture positive for *Salmonella* at two or more collection periods, respectively. In site B, two cohorts had the majority (53/61) of pigs with two or more positive samplings (40 and 13 pigs, in cohorts 2 and 4, respectively). In site A, two cohorts had the majority of pigs with two or more positive samples (19/30; nine and 10 pigs in cohorts 2 and 4, respectively).

There were 95 pigs detected positive on more than two sampling occasions that had consecutive sampling collections. Of these, 46·3% (44/95) had consecutive positive culture samplings, 23·2% (22/95) had one culture-negative faecal sample between positive culture samples and 30·5% (29/95) were culture negative on two or more occasions between the first and last culture-positive sample collection period for each pig.

A total of 168 pigs met the inclusion criteria for estimation of shedding period. The median time of shedding was 14 days (S.D. = 32·5, range 14–112 days). Eighty-five (50·6%) pigs shed for 14 days, 15 (8·9%)

pigs shed for 28 days, 18 (10·7%) pigs shed for 42 days, 11 (6·6%) pigs shed for 56 days and 39 (23·2%) pigs shed for between 70 and 112 days.

DISCUSSION

Estimates of *Salmonella* prevalence in finishing pigs in the USA range from 3·4% to 48% [10, 11, 15, 21–26]. The observed proportion of *Salmonella*-positive samples and cohort prevalence were within the range of these reports. The overall incidence of positive pigs was 20·8%, which is, to the best of our knowledge, the first estimate of incidence in naturally infected swine in one large swine production system in the USA.

Several longitudinal studies have been conducted at the farm [8, 9, 15, 27, 28] and cohort/pig group [10, 29, 30] level. A limited number of studies have repeatedly sampled individual pigs [11–14]. Similar to these previous studies, we report variability of prevalence by cohort and within pig. This may suggest there are risk factors at the cohort and pig level that might be associated with *Salmonella* prevalence. This variability reinforces that point estimates of prevalence might misclassify farm and pig status and that prospective studies are needed to assess time-dependent risk factors for *Salmonella* in swine with consideration for risk factors that may be distributed at different levels of organization (farm, cohort, pig) [31].

The majority of the pigs were detected as *Salmonella* positive at the beginning of the finishing period (age 10 weeks). Although individual sampling during the nursery phase was not performed in this study, *Salmonella* was isolated in nursery pool samples from a majority of the cohorts and there was a positive association between the nursery pool prevalence and the proportion of positive individual samples by collection period. This suggests that pigs were exposed to *Salmonella* in the nursery and may have been shedding at arrival to the finishing barn. *Salmonella* shedding during the nursery phase has been reported [13], in some cases representing the peak prevalence during the nursery period [12]. Several authors have reported increased prevalence when pigs were moved to finishing units [13, 30], which may be a result of multiple potential factors: stress caused by transportation, comingling with new pigs, changes in feed type and exposure to residual contamination [13, 30].

Contaminated facilities are a source of *Salmonella* [10, 11, 32, 33] and may in part explain the high prevalence of *Salmonella* at the first collection period. In agreement with other authors we observed that cleaning and disinfection did not eliminate *Salmonella* in the barn environment. The elimination of *Salmonella* from the barn environment is difficult and residual contamination might be responsible for new infections [11, 14, 32, 33]. The trend of a positive association between the proportion of positive barn environmental swabs and the proportion of positive individual samples suggests that the contaminated environment may have contributed to *Salmonella* infections in the finishing phase.

There was a significant difference in *Salmonella* prevalence between age groups and shedding decreased as pig age increased. Other authors have reported a decrease in prevalence during the finishing period [12, 13, 30]. It is unclear whether this association represents the natural history of *Salmonella* in swine, with young animals being more susceptible and ultimately clearing the infection over time, or if other factors are involved. Further research to understand whether control of *Salmonella* in young pigs ultimately would decrease the risk of shedding at the time of harvest is warranted.

More than 50% of the *Salmonella*-positive pigs were detected at two sampling periods. Other studies that have followed pigs over time have reported a lower percentage of pigs that were detected on more than two occasions. Beloeil *et al.* [14] reported that a majority of pigs shed only once in weekly samplings. In other studies the comparison is not as direct, since in this study the sampling period was more frequent than other reports [11, 12]. In this study, pigs identified as *Salmonella* positive on more than two occasions were clustered within site and cohort. This is in agreement with Kranker *et al.* [12] who reported characteristic patterns (shorter or longer periods of shedding) by cohort. This might suggest that there are cohort-level effects that are related to duration of shedding or transmission dynamics.

The median and range of shedding duration in this study is similar to that described by Kranker *et al.* [12] who reported a mean duration of shedding of 18 or 26 days (range 7–101 days). Although our estimates are limited by an imperfect diagnostic test, the sampling frequency and the assumption of no new infections, these data present critical information regarding the duration of shedding in naturally infected swine. Further research to understand risk factors for duration of *Salmonella* shedding in swine are warranted.

There was intermittent detection of shedding in more than 50% of the pigs with multiple culture-positive collections. *Salmonella* carriers can shed intermittently and for long periods [11, 12, 34]. It is difficult to separate intermittent shedding of *Salmonella* from intermittent detection or new infections. Despite being an imperfect diagnostic test, faecal culture is considered the 'gold standard' for *Salmonella* isolation. Estimates of the relative sensitivity of faecal culture range from 6·5% to 95%, depending on culture method and parallel estimation of the sensitivity [17, 22, 35–38]. Although a relative short sampling interval (2 weeks) was conducted in this study, new infections could occur between sampling occasions. Therefore, the intermittent shedding could be intermittent detection of an ongoing infection or a new infection after clearance of a previous infection.

These data represent one production company in one region of the USA. Although this may limit external validity, we believe that this limitation is minimal. This farm is typical of many US swine production systems in size and production practices. Furthermore, there are many similarities between the results in this study compared to studies both in the USA and other countries. A further limitation for interpretation is in regard to the univariate analyses reported in this paper. Statistical inferences regarding these associations should be interpreted with caution, as univariate analyses may bias the results reported. Further risk-factor analyses using multivariate analysis were not the aim of this paper and are currently in process. Despite this limitation, the findings presented in this paper are consistent with what has been previously reported in the literature [11–14, 30, 32, 33].

These descriptive data regarding the incidence, duration and pattern of shedding in swine provide critical data for understanding risk factors for *Salmonella* in finishing swine. The variability and clustering of *Salmonella* shedding by site, cohort and pig not only suggest a need to evaluate time-variant risk factors, but also guide the design of future epidemiological studies for identification of potential risk factors at different levels of clustering (site, cohort, pig). Future research of the epidemiology of *Salmonella* in swine should focus on longitudinal study designs focused on multilevel and time-variant risk factors. This study also reinforces that point-source estimates of prevalence might misclassify herd or pig *Salmonella* status.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Greig JD, Ravel A.** Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology* 2009; **130**: 77–87.
2. **Center for Disease Control and Prevention.** Vital signs: incidence and trends of infection with pathogens transmitted commonly through food: foodborne diseases active surveillance network, 10 U. S. Sites, 1996–2010. *Morbidity and Mortality Weekly Report* 2011; **60**: 749–755.
3. **Scallan E, et al.** Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* 2011; **17**: 7–15.
4. **Foley SL, Lynne AM, Nayak R.** *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science* 2008; **86**: E149–162.
5. **Center for Disease Control and Prevention.** Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, 2009. *Morbidity and Mortality Weekly Report* 2010; **59**: 418–422.
6. **Boyen F, et al.** Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control. *Veterinary Microbiology* 2008; **130**: 1–19.
7. **Lo Fo Wong DMA, et al.** Epidemiology and control measures for *Salmonella* in pigs and pork. *Livestock Production Science* 2002; **76**: 215–222.
8. **Rajic A, et al.** Longitudinal study of *Salmonella* species in 90 Alberta swine finishing farms. *Veterinary Microbiology* 2005; **105**: 47–56.
9. **Farzan A, et al.** A longitudinal study of the *Salmonella* status on Ontario swine farms within the time period 2001–2006. *Foodborne Pathogens and Disease* 2008; **5**: 579–588.
10. **Dorr PM, et al.** Longitudinal study of *Salmonella* dispersion and the role of environmental contamination in commercial swine production systems. *Applied and Environmental Microbiology* 2009; **75**: 1478–1486.
11. **Funk JA, Davies PR, Nichols MA.** Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems. *Veterinary Microbiology* 2001; **83**: 45–60.

12. **Kranker S, et al.** Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds. *Journal of Clinical Microbiology* 2003; **41**: 2282–2288.

13. **Nollet N, et al.** Distribution of *Salmonella* strains in farrow-to-finish pig herds: a longitudinal study. *Journal of Food Protection* 2005; **68**: 2012–2021.

14. **Beloeil PA, et al.** Longitudinal serological responses to *Salmonella enterica* of growing pigs in a subclinically infected herd. *Preventive Veterinary Medicine* 2003; **60**: 207–226.

15. **Rostagno MH, Hurd HS, McKean JD.** Variation of bacteriologic and serologic *Salmonella enterica* prevalence between cohorts within finishing swine production farms. *Food Research International* 2012; **45**: 867–870.

16. **Kingston DJ.** A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. *Avian Diseases* 1981; **25**: 513–516.

17. **Davies PR, et al.** Comparison of methods for isolating *Salmonella* bacteria from faeces of naturally infected pigs. *Journal of Applied Microbiology* 2000; **89**: 169–177.

18. **Fedorka-Cray PJ, et al.** Transmission of *Salmonella* Typhimurium to swine. *Veterinary Microbiology* 1994; **41**: 333–344.

19. **van Winsen RL, et al.** Monitoring of transmission of *Salmonella enterica* serovars in pigs using bacteriological and serological detection methods. *Veterinary Microbiology* 2001; **80**: 267–274.

20. **Osterberg J, Lewerin SS, Wallgren P.** Direct and indirect transmission of four *Salmonella enterica* serotypes in pigs. *Acta Veterinaria Scandinavica* 2010; **52**: 1–7.

21. **Davies PR, et al.** Prevalence of *Salmonella* in finishing swine raised in different production systems in North Carolina, USA. *Epidemiology and Infection* 1997; **119**: 237–244.

22. **Hurd HS, et al.** Estimation of the *Salmonella enterica* prevalence in finishing swine. *Epidemiology and Infection* 2004; **132**: 127–135.

23. **Bahnson PB, et al.** Herd-level risk factors for *Salmonella enterica* subsp. enterica in U. S. market pigs. *Preventive Veterinary Medicine* 2006; **76**: 249–262.

24. **Gebreyes WA, Thakur S, Morrow WEM.** Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant *Salmonella* in antimicrobial-free and conventional pig production. *Journal of Food Protection* 2006; **69**: 743–748.

25. **Wang B, et al.** Sub-iliac lymph nodes at slaughter lack ability to predict *Salmonella enterica* prevalence for swine farms. *Foodborne Pathogens and Disease* 2010; **7**: 795–800.

26. **USDA-APHIS.** *Salmonella* on U. S. swine sites – prevalence and antimicrobial susceptibility. (http://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_is_salmonella.pdf). Accessed 23 February 2011.

27. **van der Wolf PJ, et al.** A longitudinal study of *Salmonella enterica* infections in high-and low-sero-prevalence finishing swine herds in The Netherlands. *Veterinary Quarterly* 2001; **23**: 116–121.

28. **Rajic A, et al.** *Salmonella* infections in ninety Alberta swine finishing farms: serological prevalence, correlation between culture and serology, and risk factors for infection. *Foodborne Pathogens and Disease* 2007; **4**: 169–177.

29. **Merialdi G, et al.** Longitudinal study of *Salmonella* infection in Italian farrow-to-finish swine herds. *Zoonoses and Public Health* 2008; **55**: 222–226.

30. **Vigo GB, et al.** *Salmonella enterica* subclinical infection: bacteriological, serological, pulsed-field gel electrophoresis, and antimicrobial resistance profiles-longitudinal study in a three-site farrow-to-finish farm. *Foodborne Pathogens and Disease* 2009; **6**: 965–972.

31. **Dohoo IR, Martin W, Stryhn H.** *Veterinary Epidemiologic Research*, 2nd edn. Charlottetown, Prince Edward Island, Canada: VER Inc., 2010, pp. 173–174.

32. **Mannion C, et al.** Efficacy of cleaning and disinfection on pig farms in Ireland. *Veterinary Record* 2007; **161**: 371–375.

33. **Zewde BM, et al.** Comparison of swiffer wipes and conventional drag swab methods for the recovery of *Salmonella* in swine production systems. *Journal of Food Protection* 2009; **72**: 142–146.

34. **Scherer K, et al.** Time course of infection with *Salmonella* Typhimurium and its influence on faecal shedding, distribution in inner organs, and antibody response in fattening pigs. *Journal of Food Protection* 2008; **71**: 699–705.

35. **Funk JA, Davies PR, Nichols MA.** The effect of faecal sample weight on detection of *Salmonella enterica* in swine feces. *Journal of Veterinary Diagnostic Investigation* 2000; **12**: 412–418.

36. **Rostagno MH, et al.** Culture methods differ on the isolation of *Salmonella enterica* serotypes from naturally contaminated swine faecal samples. *Journal of Veterinary Diagnostic Investigation* 2005; **17**: 80–83.

37. **Love BC, Rostagno MH.** Comparison of five culture methods for *Salmonella* isolation from swine faecal samples of known infection status. *Journal of Veterinary Diagnostic Investigation* 2008; **20**: 620–624.

38. **Funk J.** Pre-harvest food safety diagnostics for *Salmonella* serovars. Part 1: Microbiological culture. *Journal of Swine Health and Production* 2003; **11**: 87–90.