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Prevalence of salmonella in finishing swine raised in different production systems in North Carolina, USA

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SUMMARY

We compared the prevalence of salmonella in faecal samples from finishing pigs and in feed samples from swine herds in North Carolina, USA. Farms were either finishing sites using all-in/all-out management of buildings in multiple-site systems (14 farms) or farrow-to-finish systems using continuous flow management of finishing barns (15 farms). The two groups of herds differed with respect to several management variables. Salmonella were isolated from 565 of 2288 (24·6%) faecal samples and from at least 1 faecal sample on 24 of 29 (83%) farms. Predominant serotypes were *S. derby*, *S. typhimurium* (including *copenhagen*), *S. heidelberg*, *S. worthington* and *S. mbandaka*. Fewer farrow-to-finish farms were detected as positive compared with all-in/all-out farms. Prevalence was lower for pigs raised on slotted floors compared with all other floor types, and was highest for pigs raised on dirt lots. Modern methods of raising pigs in multiple-site production systems, using all-in/all-out management of finishing pigs, appear to have no benefit in reducing the prevalence of salmonella compared with conventional farrow-to-finish systems.

INTRODUCTION

In recent years, outbreaks of foodborne diseases associated with the consumption of animal products have received much attention from the media in the USA, leading to increased consumer concern about the safety of the food supply. Outbreaks of human illness linked to *Salmonella enteritidis* in eggs [1], verotoxigenic *Escherichia coli* O157:H7 in ground beef [2], and *Listeria monocytogenes* in soft cheeses [3], have prompted discussion of the adequacy of conventional organoleptic methods of food inspection and brought suggestions that reduction of foodborne pathogens in animal production systems is necessary to ensure safe food [4].

In 1995, the Food Safety Inspection Service and Animal and Plant Health Inspection Service of the United States Department of Agriculture reviewed foodborne disease in the USA linked to red meat and poultry products and ranked salmonellosis to be the most important disease [5]. A general conclusion of this review was that there is insufficient epidemiologic knowledge about most foodborne agents in animal production systems to enable reliable and cost-effective control measures to be implemented on farms. In support of this conclusion, there is little published literature describing the epidemiology of salmonella infection in modern swine production systems.

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Over the last 10 years, the structure of the US swine industry has changed radically with the emergence of large integrated production systems, most notably in North Carolina, outside the traditional swine producing region of the US mid-west. Features of these large systems are the use of multiple-site production (different phases of production raised on separate sites) and all-in/all-out (AIAO) management (all animals are removed from a location before introducing a new group) of both nursery and finisher phases of production. These methods for spatial and temporal separation of populations of pigs of different ages appear to be effective in controlling some infectious diseases of swine [6], but no study of the prevalence of foodborne agents in these systems has been reported. Here we report a study of the prevalence of salmonella in faecal samples from finishing pigs raised in multiple-site, AIAO systems or in more traditional systems. A concurrent study of the prevalence of antibodies to *Toxoplasma gondii* and *Trichinella spiralis* in pigs on the same farms is reported elsewhere [7].

MATERIALS AND METHODS

Selection of herds

The study design included 28 farms of which 14 used AIAO management of finishing barns within multiple-site systems (group A), and 14 used continuous flow management of finishing barns within single-site, farrow-to-finish systems (group B). Within the 14 AIAO farms, 7 had fully slotted floors (group A-SF) and 7 had solid floors draining into open flush gutters which were flushed with recycled lagoon effluent (group A-FG). Among the continuous flow farms, 7 kept all finishing pigs in total confinement on fully-slotted concrete floors (group B-TC), and 7 (group B-OA) gave finishing pigs some access to outside (3 with concrete pens, 3 with dirt lots, 1 with 200 finishing pigs on 7 acres of pasture). In addition, one University herd (continuous flow, total confinement, group B-TC) was included in the study. Herds were selected purposefully according to the criteria above and willingness of the producers to participate. Details on some management procedures including feed source and delivery systems, water source, pest (flies and rodents) control measures and presence of cats on farms were obtained by a questionnaire administered to farm staff on the day samples were collected or by telephone. Samples were collected between November 1994 and August 1995. To avoid potential confounding due to seasonal effects, visits were scheduled in blocks of four farms that included one of each farm type (A-SF, A-FG, B-TC, B-OA).

Sampling

On all farms, producers indicated which pigs they considered to be within 1 month of slaughter age. Where multiple barns of pigs of similar age occurred, the barn with the heaviest pigs was sampled. Target sample sizes for each herd were calculated to enable estimation of prevalence within 10% at the 95% confidence level, assuming a prevalence of 50% [8]. In most cases, these sample sizes were adequate to be 99% confident of detecting one positive animal at a prevalence of 5%. On each farm, all pens containing eligible pigs were sampled, and we sampled an equal number of pigs per pen (some adjustments were made if large variation in the number of pigs per pen was evident). Fresh individual faecal samples (32–92 per farm) were collected into Whirl-pak bags with plastic spoons (one per sample) and transported to the laboratory to be processed on the same day. Samples were collected as pigs defaecated, or from the pen floor after the pigs were observed defaecating. On one farm (herd 3) selected as a continuous flow, outdoor barn (B-OA), some finishing pigs were also housed in a continuous flow barn with open flush gutters. Both populations were sampled, but only data from the outdoor pigs are included here. The comparative data are published in a separate paper [9].

On 26 of the 29 farms, samples of feed (approximately 100 g) were collected and transported to the National Animal Disease Center, Ames, Iowa for culture for salmonella as part of a study of salmonella in swine feed [10]. Generally, feed was collected from the sites where feed entered the feeders, which was usually inaccessible to pigs. On one farm with outdoor pigs, it was not possible to collect from within the feeder and samples were collected from sites where the pigs were eating.

Bacteriology

To detect salmonella organisms, approximately 25 g from each faecal sample was diluted 1:9 by weight with 2% buffered peptone water (BPW, Difco) and incubated at 37 °C static for 16–18 h. A 100 μl aliquot was transferred to 9·9 ml of Rappaport-Vassiliadis R10 (RV) broth (Difco) and incubated in a water bath at 42 °C static for 24 h [11]. A loopful of broth was streaked on XLT4 agar (Difco) and Modified Brilliant

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Table 1. *Numbers of farms employing certain management procedures among multiple-site systems with all-in/all-out management of finishing barns (MSAIAO) or farrow-to-finish farms with continuous flow finishing barns (FTFCF)*

Management procedure	MSAIAO ($n = 14$)	FTFCF ($n = 15$)	P*
All buildings bird-proofed	13	6	$< 0{\cdot}01$
Boots and coveralls used	13	9	$< 0{\cdot}05$
Cats on farm	1	10 (of 14)	$< 0{\cdot}001$
Rodent control programme	13	14	n.s.
Footbaths	7	3	n.s. (0·13)
Perimeter fence	1	4	n.s.
Showering necessary to enter	1	1	n.s.
Feed prepared on farm	1	9	$< 0{\cdot}01$
Pelleted feed for finishers	12	6	0·02

* Two-tailed probability from Fisher's exact test.

Green agar (Oxoid) plates which were incubated overnight at 37 °C. Suspect salmonella colonies were transferred to triple-sugar-iron (Difco) and urea agar (Difco) slopes.

Samples of feed were cultured for salmonella at the National Animal Disease Center, Ames, IA. Samples were processed for qualitative bacteriology as previously described [10]. Approximately 10 g of feed was placed into 100 ml of BPW and allowed to incubate overnight at 37 °C under static conditions. The following morning, approximately 100 μl of the BPW culture was transferred into 10 ml each of GN-Hajna (GN) broth (Difco) and tetrathionate (Tet) broth (Accumedia). Both GN and Tet broths were incubated at 37 °C static. After 18–24 h for GN and 48 h for Tet, approximately 100 μl of culture was transferred into RV medium. All RV cultures were incubated at 37 °C for 18 h, then streaked on brilliant green agar (BGS) with sulfadiazine (Accumedia) plates. The BGS plates were incubated for 24 h at 37 °C. Colonies having the appearance typical of salmonella were inoculated into triple-sugar-iron and lysine-iron agar slopes. All isolates from faeces or feed that were presumptively identified as salmonella were forwarded to the National Veterinary Services Laboratories, Ames, IA, for serotyping.

Analysis

The proportions of herds in groups A and B that were positive for salmonella were compared using Fisher's exact test. Owing to the non-normal distribution of prevalence of positive faecal cultures among farms, comparisons of prevalence among groups were made using the Mann–Whitney U-test or Kruksal–Wallis one-way ANOVA. In addition to comparisons between groups A and B, comparisons between farms, ignoring group, were made according to individual factors (e.g. slotted floors). Analyses were performed using a commercial software package (Statistix 4.0, Analytical Software).

RESULTS

Mean herd size for farrow-to-finish farms was 283 sows (range 20–1000), selling an average of 5014 pigs per year (range 400–19000). The mean number of pigs sold per year out of AIAO finishing farms (group A) was 10910 (range 1200–20000). Among farrow-to-finish farms (group B), mean herd size was 223 sows for 8 farms raising finishing pigs in total confinement, 667 sows for 3 farms raising finishing pigs outdoors in concrete pens, and 117 sows for 4 farms raising finishing pigs on dirt or pasture. The two groups of farms (multiple site *vs.* farrow-to-finish) differed with respect to the use of several management procedures (Table 1). All but two farms had rodent control programmes (baits) and only one farm required visitors to shower before entering the facilities. Finishing sites in multiple-site systems (group A) were more likely to be bird-proofed and to require workers to use boots and coveralls, but were less likely to keep cats on the farms. Pigs on all farms were fed diets based on corn and soya bean meal. Farrow-to-finish farms (group B) were more likely to mix feed on the farm, and to feed meal rather than pelleted rations to finishing pigs.

Salmonella were isolated from 565 of 2288 (24·6%)

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Table 2. *Numbers of farms and samples from which serotypes of* salmonella *were isolated from faecal samples*

Serotype	No. of farms	Positive (%)	No. of samples	Positive (%)
derby	8	28	144	6·29
worthington	7	24	43	1·88
typhimurium	7	24	40	1·75
typhimurium (*copenhagen*)	6	21	91	3·98
heidelberg	6	21	72	3·15
mbandaka	6	21	26	1·14
schwarzengrund	4	14	85	3·72
agona	3	10	8 (34)*	0·35
new brunswick	2	7	9	0·39
cholerae-suis	2	7	6	0·26
Untypable†	4	14	5	0·22
kentucky	2	7	4	0·17
infantis	1	3	4	0·17
litchfield	1	3	4	0·17
muenster	1	3	4	0·17
johannesburg	1	3	3	0·13
anatum	1	3	2	0·09
arkansas	1	3	1	0·04
binza	1	3	1	0·04
montevideo	1	3	1	0·04
Multiple serotypes	—	—	4	—
Not done‡	—	—	8	—

* Includes isolates from confined pigs in herd 3.
† 4, 12: poorly motile (2); rough 'O':Z10-e,n,z15; rough O:E,H1-6; rough 'O':i-1,2.
‡ 1 contaminated; 3 broken in transit; 3 non-viable, 1 not done.

individual faecal samples and from at least one faecal sample on 24 of 29 (83%) farms. More than one serotype was isolated from faecal samples from 16 farms, and 4 or 5 serotypes were isolated from faecal samples on 8 farms. Among positive herds, prevalence ranged from 2–84% of faecal samples (mean 31%, S.D. 25·5). The serotypes isolated on a farm and sample basis are shown in Table 2. Of 591 samples (including 26 samples from confined pigs in herd 3) from which salmonella were isolated, no typical salmonella colonies were present on XLT4 plates for 13 (2·2%) samples and no typical colonies were present on MBG plates for 24 (4·1%) samples. None of six isolates of *S. cholerae suis* grew on XLT4 agar, and none of four isolates of *S. litchfield* grew on MBG agar.

The proportion of herds which were positive for salmonella differed ($P = 0{\cdot}04$) between groups A and B, with all five negative herds in the farrow-to-finish, continuous flow group (group B). Four of these were total confinement herds, and the other herd comprised pigs raised on pasture. All herds in group A (all-in/all-out management of finishing barns) were positive for salmonella. Salmonella were isolated from at least one faecal sample from all 11 farms on which farmers recalled a previous diagnosis of clinical salmonellosis, and from 13 of 18 farms with no apparent history of salmonellosis ($P = 0{\cdot}12$).

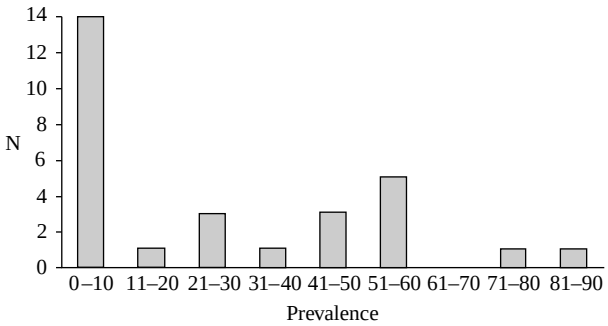

Fig. 1. Frequency distribution of farms by prevalence of faecal samples positive for *Salmonella*.

A histogram of the prevalence of positive samples among farms suggested a bimodal distribution (Fig. 1). On 14 farms, prevalence was less than 10% with

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Table 3. *Prevalence of samples positive for* salmonella *among farms within farm types* (*in ascending order of prevalence*)

Farm type	*n*	Prevalence among farms
A-SF		
All-in/all-out, multiple site, slotted floor	7	2, 7, 28, 30, 31, 41, 47
A-FG		
All-in/all-out, multiple site, open flush gutters	7	4, 4, 17, 51, 58, 59, 60
B-TC		
Continuous flow, farrow to finish, total confinement	8	0, 0, 0, 0, 3, 6, 6, 58
B-OA		
Continuous flow, farrow to finish, outside access	7	
Outdoor pens with concrete floors	3	3, 9, 21
Dirt lots	3	46, 72, 84
Pasture	1	0

Table 4. *Numbers of samples and farms from which serotypes of* salmonella *were isolated from feed samples, and numbers of farms where the same serotype was isolated from faecal samples*

Serotype	No. of samples ($n = 1044$)	No. of farms ($n = 26$)	No. of farms with same serotype from pigs and feed
worthington	12	3	2
derby	4	1	1
kentucky	2	1	1
johannesburg	1	1	1
typhimurium (*copenhagen*)	1	1	1
orion	2	1	—
mbandaka	1	1	—
tennessee	1	1	—
orianenburg	1	1	—
thomasville	1	1	—

the mean ((S.D.) prevalence being 3·1% (2·9%). For the remaining 15 herds, prevalence ranged from 17–84%, with a mean (S.D.) of 46·6 (19·0%). Wide variability in prevalence of positive samples among farms was found within all groups (Table 3), and differences in mean prevalence between groups A (31%) and B (20%) were not statistically significant ($P = 0{\cdot}08$). However, mean prevalence tended to be lower ($P = 0{\cdot}06$) for herds where pigs were housed on slotted floors (16·5%) compared with herds raising pigs on other surfaces (36·7%). The mean prevalence for three herds raising pigs outdoors on concrete was only 11%, but this small number of herds precludes meaningful interpretation of this observation. With the exception of one herd (prevalence 58%), salmonella were isolated at low prevalence (< 10%) from samples from continuous flow, total confinement herds, all of which had fully slotted floors. Mean herd prevalence tended to be highest in herds where animals were raised on dirt lots (mean = 67·3%) or in barns with open flush gutters (39%), and these farms combined had higher prevalence than other farms ($P < 0{\cdot}01$). When analysed by feed type, prevalence was higher ($P = 0{\cdot}001$) in farms feeding pelleted rations (38·1%) compared with farms feeding meal rations (5·7%). On most farms it was evident that positive faecal samples did not occur randomly in barns, but were clustered in individual pens. For example, on a farm where 87 samples were collected from 12 pens, 12 of 16 samples from 2 pens were positive compared with 6 of 71 samples from all other pens.

Ten salmonella serotypes were identified among 26 (2·5%) isolates from 1044 feed samples, and at least

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one feed sample was positive on 9 (35%) of 26 farms (Table 4). A high prevalence (36%) of positive feed samples was found on one farm where contamination was possible because feed could only be collected from the site of the feeders to which pigs had access. The prevalence of positive faecal samples did not differ ($P = 0{\cdot}98$) between farms according to the detection of salmonella in feed samples. On 6 farms, the same serotypes were recovered from feed and faecal samples, and on 5 farms a serotype was isolated from the feed but not from faecal samples. On one farm, *S. worthington* was isolated from a feed sample, but none of 75 faecal samples was culture positive.

DISCUSSION

The primary objective of this study and a concurrent study [7], was to compare the prevalence of muscle-borne (toxoplasma, trichinella) and faecal-borne (salmonella) foodborne pathogens in pigs produced in markedly different production systems in North Carolina. To control foodborne pathogens in animal production systems it is necessary to identify sources of infection of herds and risk factors for transmission of agents among pigs within farms. Owing to the heterogeneity of traditional swine farming systems, and likely confounding of potential risk factors, considerable resources are required to conduct herd-level studies of risk factors for the prevalence of any agent in randomly selected samples of herds.

In recent years, expansion of large swine producing enterprises in North Carolina has led to greater homogeneity of production systems with respect to factors such as facility design, genotype, nutrition and feed management, manure management, etc. A recent comparison of management practices in corporate and independent swine operations in Quebec concluded that the two populations of production systems should be considered distinct [12]. This conclusion is supported by our data which show group A and group B farms differed in several respects regarding management. Key features of corporate swine enterprises in North Carolina are the adoption of all-in/all-out principles in all phases of production and rearing of different age-groups at different locations (multi-site production). These methods were designed to reduce the impact of common infectious diseases of swine on production [6]. For our initial studies of foodborne pathogens in swine populations in North Carolina, we believed that evaluating these modern systems in relation to more traditional farming systems would be a more efficient approach than attempting a large study on a random sample of herds. Consequently, the data presented cannot be considered representative of the overall industry in North Carolina. However, we suggest that the data from the multiple-site farms may be representative of a large proportion of pigs produced in North Carolina under very similar conditions.

In contrast to our findings for muscle-borne parasites [7], multiple-site production systems using all-in/all-out management of finishing pigs appear to have no benefit in reducing the prevalence of salmonella compared with conventional farrow-to-finish systems. In fact, our data indicate that salmonella may be more common in finishing pigs produced in these systems. However, the large variability in prevalence of salmonella observed among herds within all farm types implies either the existence of important risk factors affecting prevalence within each system or large random error in estimating prevalence. While we believe that the sample sizes chosen in this study were adequate to achieve reasonable accuracy in estimating the prevalence of faecal shedding of salmonella at the time of sampling, there have been no published longitudinal studies designed to evaluate the temporal variability of prevalence of salmonella in populations of finishing swine and, therefore, the repeatability of point estimates of prevalence. In addition, the clustering of positive samples in individual pens suggests that pen-level risk factors are important. It is probable that some, if not all, of the five farms on which salmonella were not isolated did in fact harbour some infected pigs. This is supported by the finding of a positive feed sample on one of these farms. Based on the apparent bimodal distribution of prevalence among farms, we propose that on some farms there is limited transmission among finishing-age pigs, while on other farms transmission among finishing pigs is a frequent event. Given that it is extremely difficult to maintain animal populations completely free from salmonella in the long term [13], defining risk factors for horizontal transmission and duration of shedding of salmonella in finishing-pig populations should be a high priority for research.

Several of the most prevalent serotypes (*S. typhimurium*, *S. typhimurium* (*copenhagen*), *S. heidelberg* and *S. agona*) recovered in this study are among the most common isolates from cases of clinical disease in humans in the USA [14]. The predominance of *S.*

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derby is not unusual, as this serotype is a common isolate from pigs in many countries [15–17]. *S. derby* is the second most common serotype isolated from clinical cases in swine in the USA [15], but is not commonly isolated from other animal species or human sources [14, 15]. *S. cholerae suis*, the most common isolate from clinical cases in swine in the USA [15], was isolated from only 6 samples on 2 farms. The absence of *S. cholerae suis* from a large number of samples is not surprising given that clinical disease was inapparent on the farms. However, the high prevalence of *S. derby* in apparently healthy pigs in this study raises the question of the clinical significance of isolation of this serotype from cases of diarrhoea in the absence of histological evidence of enteritis. There is no published information regarding the pathogenicity of *S. derby* in swine.

Although prevalence of faecal shedding of salmonella tended to be higher in barns managed all-in/all-out than in continuous flow barns, it is unlikely that all-in/all-out management *per se* is detrimental to salmonella control. Firstly, all-in/all-out management of barns with open-flush gutters flushed with recycled effluent should not be expected to be highly effective with respect to control of enteric organisms, assuming survival of pathogens in the recycled effluent [18]. Although exposure to recycled effluent may not directly lead to a high prevalence of infection, it is a likely source of infection for a group and open-flush gutters may facilitate transmission within and among pens [9, 19]. However, prevalence did tend to be higher in AIAO barns with slotted floors compared with continuous flow barns with slotted floors. Stocking density or number of pigs per pen was identified as a risk factor for shedding of salmonella by pigs housed in pens [20]. However, although not determined accurately, space allowance per finishing pig did not vary greatly among confinement operations in this study and was therefore unlikely to be an important source of variation among herds. Because the AIAO barns were, by selection, part of multiple-site systems studied, pigs were transported between sites in vehicles, while movement of pigs between phases of production was minimal in continuous flow systems (all phases on one site). There is research implicating transport as a factor leading to increased faecal shedding of salmonella by swine and other species at slaughter [21, 22], and transport of growing pigs between sites may facilitate transmission.

The role of feed as a potential source of salmonella is well established [23], however its role as a risk factor for prevalence among populations is not well established. Some 2·5% of the feed samples, which were approximately 10 g, were positive for salmonella, indicating a high probability of exposure for pigs expected to consume 200–300 kg of feed during the finishing phase. The difference we found between predominant serotypes in isolates from feed and isolates from pigs also was consistent with previous studies [17, 24], and implies that sources other than feed are important on many farms. In addition to contamination of feedstuffs, effects of feed formulation and processing may be important. Field studies in Denmark found a lower prevalence of salmonella on farms mixing their own feed and feeding liquid feed, suggesting that dietary factors might influence salmonella prevalence [25]. A study of 40 fattening farms in Holland found salmonella from 19·4% of samples from farms using whey compared with 64·4% of farms using water [26]. There is some recent evidence, based on serological data, that fineness of grind of the feed can affect the prevalence of salmonella, possibly via effects on intestinal flora or organic acid distribution [27]. This is consistent with the difference we observed between pigs fed pelleted or meal rations, but we are cautious in making this association owing to the high level of confounding of management factors in our study. Earlier studies have shown pelleted feed to be superior to meal for controlling salmonella in pigs and poultry [23, 28]. In a recent study, 5·4% of samples of pelleted swine feed were contaminated, compared with 1·8% of ground feed samples [10]. However, feed or feed ingredients could not be singled out as the definitive source of salmonella as they may have been contaminated from the farm environment. It is important to separate the issues of feed contamination from possible effects of diet on the gastro-intestinal flora.

The complexity of the epidemiology of salmonella is such that management practices adopted in modern animal production systems cannot be expected to reliably control the organism in commercial herds. In contrast, adequate preharvest control of toxoplasma and particularly trichinella, where sources of infection are relatively few and potential for pig-to-pig transmission is limited, appears to be achieved by management practices inherent to modern systems in North Carolina [7]. Better understanding of the epidemiology of salmonella in these types of production systems is necessary to evaluate the feasibility of pre-harvest control of salmonella under commercial conditions.

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