Split Marketing as a Risk Factor for *Salmonella enterica* Infection in Swine

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Abstract

On-farm reduction of *Salmonella* carriage prevalence in pigs requires the identification of risk factors to direct interventions development. This study was designed to determine if split marketing of finishing pigs constitutes a risk factor for *Salmonella* infections, by comparing *Salmonella* prevalence in the first group of pigs selected for harvest (“first pull”) versus the prevalence in the last group of pigs selected for harvest (“close out”) from multiple commercial finishing lots. Nine paired samplings were conducted consisting in matched groups of pigs from individual barns as the first pull and the close out with a 4-week interval between groups. From each group, fecal and meat samples were collected, on-farm and at harvest, respectively. Fecal samples were selectively enriched, and analyzed for the presence of *Salmonella*, whereas meat juice samples were analyzed for the presence of antibodies against *Salmonella*. In 7/9 (77.8%) of the studied barns, an increase in *Salmonella* prevalence was observed, based on both bacteriologic and serologic analysis. Overall, there was an increase of 9.2% ($p < 0.05$) in bacteriologic prevalence, and 31.3% ($p < 0.05$) in serologic prevalence from first pull to close out groups. This study demonstrates that a significant increase in *Salmonella* prevalence occurs between the first and the last group of pigs harvested from finishing lots, with close out groups of market pigs posing a higher risk for *Salmonella* contaminations.

Introduction

Subclinical *Salmonella* infections in pigs constitute an important food safety problem, as carrier animals pose a potential risk for contamination of pork products. In the United States, more than 76 million cases of foodborne illness occur annually, and more than 95% of all cases of salmonellosis in humans are attributed to contaminated food (Mead *et al.*, 1999). It has been estimated that between 5% and 30% of all cases of foodborne salmonellosis are caused by contaminated pork (Bryan, 1980, 1988; Baird-Parker, 1994).

Although intervention strategies to assure food safety can be applied at all levels of the pork production chain, emphasis has been placed on the potential for reduction of meat contamination by minimizing contaminants at the preharvest level (i.e., on-farm). In theory, reducing the number of animals infected at the farm and carrying the pathogen into the abattoir can decrease contamination of final products. However, an in-depth understanding of the on-farm ecology and epidemiology of *Salmonella* is fundamental to identify strategic intervention points. A critical step in this endeavor consists in identifying risk factors as a precursor to the development of monitoring and intervention (control) strategies.

Many on-farm studies have been conducted, and considerable data published on *Salmonella* prevalence in pigs. A number of cross-sectional studies have broadly investigated on-farm risk factors for *Salmonella* infections in pigs, and a variety of potentially contributing factors have been found, including livestock other than pigs in the same farm, herd size, previous cases of clinical salmonellosis, bowl-type drinkers, dry feeding, pelleted feed, *Salmonella*-positive breeding herd, solid or partially slatted floors, reduced floor space allowance, persistent floor contamination, coinfections (with *Lawsonia intracellularis* or porcine reproductive and respiratory syndrome virus), lack of hygiene and biosecurity practices, contact between pigs in adjacent pens, continuous flow system, multiple pig suppliers, environmental temperature fluctuation, and *Salmonella*-contaminated feed (Berends *et al.*, 1996; Davies *et al.*, 1997; van der Wolf *et al.*, 1999, 2001; Funk *et al.*, 2001; Kranker *et al.*, 2001; Leontides *et al.*, 2003; Beloel *et al.*, 2004; Lo Fo Wong *et al.*, 2004; Nollet *et al.*, 2004; Bahnson *et al.*, 2006; Farzan *et al.*, 2006; Mejia *et al.*, 2006). The diversity of potential risk factors for...
Salmonella infection reported clearly illustrates the complexity of the on-farm ecology of this foodborne pathogen. This scenario is further complicated by limitations of current diagnostic methods to precisely define determinants (or effectors) in the dynamic Salmonella epidemiology within swine production systems. Therefore, to be able to design adequate intervention strategies, potential risk factors for the occurrence of infections need to be individually investigated. In this study, we focused on a single potential risk factor and evaluated it by applying two prevalence estimate approaches (i.e., bacteriology and serology).

Owing to the variability of individual growth patterns within groups of finishing pigs, it is common practice in swine production operations to remove animals for market over a period of time (USDA, APHIS, 1996). Conventionally, the heaviest pigs would be removed first (“first pull”), thus allowing more time for the lighter pigs to reach a targeted market weight (“close out”). This practice is known as split marketing (i.e., splitting groups to be delivered to harvest into multiple shipments over time). Research has shown that by removing up to 50% of the heaviest pigs from a pen, growth performance of the remaining animals is increased (Bates and Newcomb, 1997; Woodworth et al., 2000; DeDecker et al., 2005). Removing pigs from a pen results in an increase in floor and feeder space for the remaining animals, but also changes the social dynamic of the group (Meese and Ewbank, 1973; Fernandez et al., 1994; Tuchscherer et al., 1998). There is some concern that this marketing strategy may serve as a potential stressor to the remaining animals causing the reactivation of latent infections and/or increased predisposition to new infections.

Therefore, this study was designed to compare the Salmonella enterica prevalence in the first group of pigs selected for harvest (first pull) versus the last group of pigs selected for harvest (close out) to determine if close out groups of finishing pigs pose a higher risk of Salmonella contaminations. A secondary objective of this study was to compare group-matched bacteriologic and serologic Salmonella prevalence estimates, which are commonly applied in monitoring programs.

Materials and Methods

Two finishing farms containing multiple production sites, and components of a large integrated production system were included in this study. Each production farm was visited multiple times (four paired samplings from farm A and five paired samplings from farm B). Each paired sampling consisted of matched groups of pigs from the same barn/lot as the first pull (i.e., the first group of pigs selected to harvest) and the close out (i.e., the last group of pigs selected to harvest). In each sampling, 45 individual fecal samples were collected directly from the rectum (two to three pigs randomly sampled per pen). At the abattoir, the same groups of pigs were followed, and individual meat samples (diaphragm, 40–70 g) were randomly collected (n = 50 samples per group). The time interval between first pull and close out groups was the same for all lots in both farms (4 weeks).

Each fecal sample (10 g) was inoculated into 90 mL of thionate broth, and incubated at 37 °C for 20–24 hours. From the primary enrichment, 0.1 mL was transferred into 10 mL of Rappaport-Vassiliadis broth containing 20 µg/mL of novobiocin (Sigma Chemical, St. Louis, MO), and incubated at 42 °C for 20–24 hours. After incubation, an aliquot (1 mL) of the last enrichment was analyzed for the presence of Salmonella using a antigen-capture ELISA (Assurance® Gold EIA Salmonella, BioControl Systems, Bellevue, WA), previously evaluated in our laboratory (>98.9% sensitivity and 96% agreement with culture method) (Rostagno et al., 2001). All bacteriologic media used during the sample processing were obtained from Becton Dickinson Microbiology Systems ( Sparks, MD).

Individual meat samples were kept frozen (–20 °C) until processed. Samples were then thawed, and the resulting fluid (meat juice) was collected for each sample (1 mL) and analyzed for the presence of anti-Salmonella antibodies using an indirect ELISA (HerdChek® Swine Salmonella; IDEXX Laboratories, Westbrook, ME), based on lipopolysaccharide antigens (Camitz et al., 2001). The cut-off value (S/P ratio) applied was 0.25, according to manufacturer’s recommendation.

Sample size was determined, based on a population of 1000 pigs per lot/barn with an expected prevalence of 10–15%, with a precision of 10%. The number of replicates per farm was determined by power analysis (minimum of four replicates per farm required). Salmonella bacteriologic and serologic prevalence and respective 95% confidence interval (CI) were determined for each group sampled, and overall. Proportions were compared by chi-square test, and the statistical significance level applied for inferences was p < 0.05. Additionally, prevalence data for each matched groups of pigs harvested (first pull and close out; n = 9) were subjected to a hypothesis test to determine if the prevalence in the different groups of pigs (i.e., difference between dependent groups) was statistically equal (p > 0.05) or different (p < 0.05), by applying the nonparametric Wilcoxon’s t-test. Correlation between group-matched fecal prevalence (i.e., bacteriology) and seroprevalence was determined using the nonparametric statistic for correlation Spearman’s rho (JMP 4.0.0; SAS Institute, Cary, NC).

Results

All finishing barns/lots studied were Salmonella positive, based on sampling from first pull and close out, both through bacteriologic and serologic analysis. There was no difference between production farms sampled for the bacteriologic and serologic prevalence estimates (p > 0.05). The mean Salmonella prevalence, based on fecal samples, was 43/405 (10.6%; 95% CI 6.03–15.2%) for first pull groups, and 80/405 (19.8%; 95% CI 11.3–28.2%) for close out groups. Based on meat juice samples (i.e., serology), the Salmonella prevalence for first pull groups was 85/450 (18.9%; 95% CI 12.7–25.1%), and 226/450 (50.2%; 95% CI 26.8–73.6%) for close out groups. The differences between first pull and close out groups in overall bacteriologic (+9.2%) and serologic (+31.3%) prevalence were statistically significant (p < 0.05) in both cases (using chi-square test to compare proportions). Median prevalence for first pull and close out groups was 11.1% and 17.8% (bacteriologic), and 20% and 48% (serologic), respectively.

When considering the individual groups studied, a bacteriologic prevalence increase from first pull to close out occurred in 7/9 (77.8%), whereas in only one group (11.1%) the prevalence decreased, and in one group (11.1%) the prevalence was the same for both groups (Fig. 1). Similarly, a serologic prevalence increase from first pull to close out...
occurred in 7/9 (77.8%), whereas in two groups (22.2%) the prevalence decreased (Fig. 2). The minimum and maximum bacteriologic prevalence in first pull and close out groups was 2.2% and 20%, and 11% and 35.6%, respectively. The minimum and maximum serologic prevalence in first pull and close out groups was 8.1% and 30%, and 30.5% and 96%, respectively.

When considering each sampled lot as the experimental unit \( (n = 9) \), the statistical comparison of the two treatments (i.e., first pull vs. close out) using the \( t \)-test also revealed statistically significant \( (p < 0.05) \) differences between treatments for both bacteriologic and serologic prevalence.

Overall, combining first pull and close out groups resulted in a bacteriologic prevalence of 123/810 (15.2%), and a serologic prevalence of 311/900 (34.6%), which differed statistically (chi-square test; \( p < 0.05 \)). Of the total 18 paired fecal and meat samplings conducted, 14 (77.8%) had higher serologic prevalence, whereas 3 (16.7%) had higher bacteriologic prevalence, and only 1 (5.6%) had equal bacteriologic and serologic prevalence estimates (Fig. 3). The correlation (Spearman’s rho) between fecal bacteriologic culture and meat juice serology prevalence estimates was moderate (0.48; \( p < 0.05 \)).

Discussion

In the present study, split marketing was associated with a significant increase of Salmonella prevalence from first pull to close out groups of finishing pigs harvested. There are two potential explanations for the increase in Salmonella prevalence between first pulls and close outs: (1) the reactivation of latent infections and subsequent increased transmission, due to the stress caused by the social disruption consequent to the removal of the heaviest pigs from the pens (and most likely, the dominant pigs from each group) and (2) mechanical transmission (i.e., dissemination or spread) of the bacteria by the personnel entering the barns to select and remove the heaviest pigs from the pens. Although no definitive evidence exists, it may also be possible that increased concentration of the infected animals in the population may have occurred, if the growth performance of subclinically Salmonella-infected pigs was detrimentally affected by the infection (which remains to be clinically demonstrated). However, based on the serologic prevalence increase observed between groups within the studied farms, it is more likely that new infections occurred due to the transmission of the bacteria between the pigs and/or through the personnel involved in the selection and removal of the heaviest pigs.

According to the USDA/APHIS (1996), delivery of uniform-sized pigs for harvest is very important for pork producers, with most operations (96%) frequently assembling uniform sized pigs for harvest. These data underscore the importance of the results reported here and its potential food safety implications. To our knowledge, this is the only study addressing split marketing as a risk factor for Salmonella infection in finishing pigs. Although a number of studies have been conducted to determine risk factors for Salmonella infection in commercial swine herds (Berends et al., 1996; Davies et al., 1997; van der Wolf et al., 1999, 2001; Funk et al., 2001; Kranker et al., 2001; Leonidades et al., 2003; Beloel et al., 2004; Lo Fo Wong et al., 2004; Nollet et al., 2004; Bahrson et al., 2006; Farzan et al., 2006; Mejia et al., 2006), none has included the variable (or risk factor) investigated in this study. Further, our results serve to illustrate how dynamic the ecology and epidemiology of Salmonella can be in swine populations, and how it can be affected by simple management practices at the farm.

It is critically important to know how results from bacteriologic and serologic diagnostic methods correlate under field conditions, and particularly, how well serologic results reflect the current infection status in herds and groups of pigs.
delivered to abattoirs. The overall prevalence estimates obtained in this study by applying the two diagnostic tools (i.e., bacteriologic and serology) differed markedly, with an overall higher serological prevalence estimate (15.2% vs. 34.6%, respectively). Our results are in agreement with previous on-farm studies reporting discrepancies between bacteriologic and serologic prevalence estimates, with serological estimates being higher than bacteriologic estimates (Stege et al., 2000; Lo Fo Wong et al., 2003; Hurd et al., 2004; Funk et al., 2005).

However, when comparing bacteriologic and serologic prevalence estimates, it has to be kept in mind that a temporal disassociation exists between infection and serologic response. Recent Salmonella infections (i.e., less than 5–7 days) usually cannot be detected by serologic analysis. Therefore, it is possible that pigs infected during the last days of the finishing production stage are not serologically detected, when samples are collected at slaughter. More importantly, it is possible that animals with positive serological status (which has been shown to persist for several weeks; Nielsen et al., 1995) do not continue to shed Salmonella in the feces and, therefore, would not be detected with bacteriologic analysis. This scenario would explain the common finding of higher serologic prevalence estimates, as reported here, and by others (Stege et al., 2000; Lo Fo Wong et al., 2003; Hurd et al., 2004; Funk et al., 2005). Studies with experimentally infected pigs have shown that the onset of serologic response and peak seroprevalence occur at approximately 7 and 30 days post-inoculation, respectively (Wood et al., 1989, 1991; Nielsen et al., 1995; Srinand et al., 1995). Therefore, it is not unexpected that a different pattern of bacteriologic and serologic responses may, and probably do, occur under natural conditions. This observation may occur because pigs within groups are infected at different points in time with variability in both exposure rate and level, and in individual host responses. Moreover, the occurrence of multiple Salmonella enterica serovars within a group of pigs (i.e., lot or barn) will further complicate this scenario, as it has been shown that transmission and serological response are serovar dependent (van Wissen et al., 2001).

The effective identification and evaluation of risk factors and intervention measures, and the development and implementation of reliable preharvest monitoring and control programs depend on the ability to accurately assess the Salmonella status of animal groups or populations. However, the diagnostic tools currently available for preharvest investigations of Salmonella are limited. Despite the known limitations of fecal bacteriologic culture for determination of Salmonella status (Funk et al., 2000; Hurd et al., 2004), it remains the gold standard for on-farm Salmonella investigations. Estimates of its sensitivity are frequently low to moderate. In the other hand, serologic methods have the ability to assay a large number of samples rapidly and at relatively low cost, as is necessary in monitoring and control programs. However, considering that the presence of antibodies reflects previous exposure to Salmonella rather than current infection, the relationship between serologic status and microbial risk at harvest is less evident than with preharvest bacteriologic culture.

Asymptomatic carriage (i.e., subclinical infection) and intermittent shedding of low numbers of Salmonella characterize most Salmonella-infected pig herds. Identification of risk factors that may influence the prevalence of this intestinal carriage is critical for development of effective intervention measures to reduce Salmonella contamination of market pigs.

As our knowledge on the ecology and epidemiology of Salmonella in swine populations evolves, the effect of multifactorial issues becomes evident underlying the complexity of the microbial preharvest food safety risks challenging the pork industry. The permarketing stage of the pork production chain (i.e., when pigs are removed from the production farms and transported to the abattoirs for slaughter and processing) includes a multitude of variable factors that make it difficult to completely control for potential confounding and interactions. Research to investigate the effect of each component of the permarketing complex stage is needed, particularly under more controlled conditions. This study demonstrates for the first time (under commercial conditions) that a significant increase in Salmonella prevalence occurs between the first and the last group of pigs harvested from finishing lots, when a split marketing strategy is practiced. Therefore, we conclude that close out groups of market pigs constitute a higher risk for Salmonella contamination of the abattoir environment, and potentially, of pork products.

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Disclosure Statement

No competing financial interests exist.

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