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1. Introduction

Subclinical Salmonella infections in pigs constitute an important food safety problem as carrier animals pose a risk for pork product contamination. Although intervention strategies to assure food safety can be applied at all levels of the pork production chain, increased emphasis has been placed on the potential reduction of meat contamination by reducing contaminants at the pre-harvest level (i.e., on-farm). Theoretically, reducing the number of animals infected at the farm can decrease contamination of final products. However, before attempting to reduce the number of Salmonella-infected pigs leaving the farm, and consequently, entering the abattoir, it is critical to determine if on-farm intervention and control measures are necessary and effective. This situation has increased the need for a better understanding of the on-farm ecology and epidemiology of Salmonella.

Many studies have been conducted and considerable data published on Salmonella prevalence in pigs, on-farm and at slaughter. However, although a number of longitudinal investigations of Salmonella epidemiology in swine herds have been conducted (Beloeil et al., 2003; Funk, Davies, & Nichols, 2001; Kranker, Alban, Boes, & Dahl, 2003; Lo Fo Wong et al., 2004; Rajic et al., 2005; van der Wolf et al., 2001), the Salmonella transmission dynamics in swine populations is mostly unknown, limiting our knowledge of the epidemiology of this important foodborne pathogen. Therefore, this study was conducted with the objective of evaluating the consistency of Salmonella prevalence (bacteriologic and serologic) in cohorts of finishing pigs, on-farm and at slaughter. The study was conducted with the objective of evaluating the consistency of Salmonella prevalence (bacteriologic and serologic) in cohorts of finishing pigs, on-farm and at slaughter. The study was conducted with the objective of evaluating the consistency of Salmonella prevalence (bacteriologic and serologic) in cohorts of finishing pigs, on-farm and at slaughter. The study was conducted with the objective of evaluating the consistency of Salmonella prevalence (bacteriologic and serologic) in cohorts of finishing pigs, on-farm and at slaughter. The study was conducted with the objective of evaluating the consistency of Salmonella prevalence (bacteriologic and serologic) in cohorts of finishing pigs, on-farm and at slaughter.
within the same production farm occurred every 1–2 weeks. Each sampling consisted of 30 individual fecal samples collected directly from the rectum (2–3 pigs were randomly sampled per pen, allowing the sampling of at least 10 different pens randomly selected within a building group or lot consisting of 32 pens). At the abattoir, the same lots of pigs were followed and individual meat samples (diaphragm, 40–70 g) were collected (N = 50 samples per lot). Fecal samples were transported to the laboratory for immediate processing, whereas meat samples were kept frozen (−20 °C) until processing.

2.2. Bacteriology

Each fecal sample (10 g) was inoculated in 90 mL of Tryptone broth (Becton Dickinson and Company, Sparks, MD), and incubated at 37 °C for 24 h. From the primary enrichment, 0.1 mL was transferred to 10 mL of Rappaport-Vassiliadis broth (Becton Dickinson and Company, Sparks, MD) containing 20 µg/mL of novobiocin (Sigma-Aldrich Co., St. Louis, MO), and incubated at 42 °C for 24 h. An aliquot (1 mL) of the last enrichment was analyzed for the presence of *Salmonella* using a commercially available antigen-capture ELISA (Assurance Gold *Salmonella EIA*, BioControl Systems Inc., Bellevue, WA), previously evaluated in our laboratory (Rostagno, Hurd, Gailey, McKein, & Leite, 2001).

2.3. Serology

Meat samples were kept frozen (−20 °C) and thawed for processing. After thawing, the resulting fluid (“meat juice”) was collected for each sample (1 mL) and analyzed for the presence of anti-*Salmonella* antibodies using a commercially available indirect ELISA (HerdChek Swine *Salmonella*, IDEXX Laboratories Inc., Westbrook, MA), which is based on lipopolysaccharide antigens (Camitz, Holmquist, Ballagi, & Rodgers, 2001). The cut-off value (S/P ratio) applied was 0.25, according to the manufacturer’s instructions.

2.4. Statistical analysis

The number of samples to be collected per sampling was determined through power analysis, based on expected prevalence (10%), population size (800–1000 pigs per lot), precision of the estimate (5–10%), and confidence level (95%). Bacteriologic and serologic *Salmonella* prevalences, and respective 95% confidence intervals, were determined for each production farm and overall. Frequency distributions were analyzed and proportions were compared by Chi-square test. Correlation between fecal (bacteriologic) and “meat juice” (serologic) prevalence was determined using the non-parametric statistic for correlation Spearman’s rho. The statistical significance level applied for inferences was *P* < 0.05.

3. Results

All production farms studied were *Salmonella*-positive in at least two fecal samplings (bacteriologic), and in at least four meat samplings (serologic). The overall bacteriologic prevalence was 12.9% (139/1080; 95% CI 8.0–17.8%), whereas the overall serologic prevalence was 35.4% (637/1800; 95% CI 24.5–46.4%). There was a significant difference (*P* < 0.05) between the overall bacteriologic and serologic *Salmonella* prevalences for all production farms (i.e., all farms combined). However, this difference was not consistent between production farms. In three out of the six production farms studied (A, C, and D) a significant higher serologic prevalence (*P* < 0.05) was found, whereas in the other three production farms (B, E, and F), there was no statistical difference between bacteriologic and serologic prevalence estimates (Table 1).

The comparison of the proportion of *Salmonella*-positive pigs bacteriologically and serologically, based on the individual lots studied (n = 36), resulted in 38.9% (14/36) of the lots with similar bacteriologic and serologic prevalence estimates (*P* > 0.05), and 61.1% (22/36) with higher serologic than bacteriologic prevalence estimates (*P* < 0.05). In only 2/36 (5.6%) lots, *Salmonella* was detected by culture, but not serologically, whereas in 11/36 (30.6%) lots, *Salmonella* was detected serologically, but did not produce positive cultures. Only 1/36 (2.8%) lot was *Salmonella*-negative for both culture and serology.

The comparison of the overall bacteriologic prevalence by production farm revealed no statistical difference (*P* > 0.05) between farms (Table 1). Also, the comparison of the overall serologic prevalence by production farm revealed no difference (*P* > 0.05) between farms (Table 1). A wide prevalence variation (bacteriologic and serologic) was observed in all production farms, which is evidenced by the standard deviations presented in Table 1. The correlation (Spearman’s rho) between fecal bacteriologic culture and “meat juice” serology prevalence estimates was moderate (0.59; *P* < 0.05). A scatter plot of the bacteriologic and serologic prevalences of *Salmonella*, including all finishing lots studied is presented in Fig. 1.

4. Discussion

This study demonstrates the occurrence of wide variations in *Salmonella* prevalence (bacteriologic and serologic) of different finishing pig lots within individual production farms, which otherwise were static. The observed prevalence variation prevented the categorization (or separation) of the production farms as high or low *Salmonella* prevalences. A scatter plot of the bacteriologic and serologic prevalences of *Salmonella enterica* prevalences for 36 lots of finishing pigs from multiple production farms.

### Table 1

<table>
<thead>
<tr>
<th>Production farm</th>
<th>Bacteriologic prevalence 1 (%) ± SD</th>
<th>Serologic prevalence 2 (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.5 ± 12.4 ab</td>
<td>39 ± 37.1 ab</td>
</tr>
<tr>
<td>B</td>
<td>6.7 ± 5.6 ab</td>
<td>11.3 ± 11.5 ab</td>
</tr>
<tr>
<td>C</td>
<td>22.8 ± 15.8 ab</td>
<td>55.7 ± 32.8 ab</td>
</tr>
<tr>
<td>D</td>
<td>9.4 ± 18.7 ab</td>
<td>51.9 ± 27.7 ab</td>
</tr>
<tr>
<td>E</td>
<td>15 ± 15.3 ab</td>
<td>20.4 ± 23.6 ab</td>
</tr>
<tr>
<td>F</td>
<td>13.9 ± 16.2 ab</td>
<td>30 ± 28.9 ab</td>
</tr>
<tr>
<td>Overall</td>
<td>12.9 ± 14.6 a</td>
<td>35.4 ± 28.2 b</td>
</tr>
</tbody>
</table>

Upper case superscript letters: statistical comparison within column (*P* < 0.05).

Lower case superscript letters: statistical comparison within row (*P* < 0.05).

1 Total of 6 lots per farm (30 samples per lot; total of 180 samples per farm).

2 Total of 6 lots per farm (50 samples per lot; total of 300 samples per farm).

![Fig. 1. Scatter plot of bacteriologic (fecal samples) and serologic (meat juice samples) *Salmonella enterica* prevalences for 36 lots of finishing pigs from multiple production farms.](Image)
prevalence farms. Our results complement reported longitudinal studies that observed variation of Salmonella prevalence overtime in the same groups of pigs repeatedly sampled (Beloel et al., 2003; Funk et al., 2001; Kranker et al., 2003; Lo Fo Wong et al., 2004; Rajic et al., 2005; van der Wolf et al., 2001). Additionally, our results are supported by Farzan et al. (2008) and Rajic et al. (2007) that reported significant variability in the proportion of Salmonella-positive farms, based on repeated samplings. Therefore, our results indicate that caution is needed when using point estimates (i.e., based on a single sampling) for on-farm evaluations of intervention and control strategies, as well as for monitoring and surveillance purposes.

Sanchez, Dohoo, Christensen, and Rajic (2007) conducted a systematic review and meta-analysis study to identify variables that could explain the variation in Salmonella prevalence estimates at study-level. According to that study, diagnostic procedure was one of the most important predictors in explaining the differences in Salmonella prevalence between studies. Specifically in the case of the study reported here, in addition to the known low detection sensitivity of fecal sampling (Davies et al., 1997; Funk, Davies, & Nichols, 2000; Hurd, McKean, Griffith, & Rostagno, 2004; Wilkins et al., 2010), possible reasons for the variation in the Salmonella bacteriologic prevalence within production farms over time would be: 1) occurrence of intermittent shedding following exposure, and 2) evolution and resolution of Salmonella epidemics without clinical manifestations.

The intermittent shedding of Salmonella within swine populations has been demonstrated (Osterberg & Wallgren, 2008; Osterberg, Lewerin, & Wallgren, 2010; Scherer et al., 2008; van Winsen et al., 2001; Williams & Newell, 1967), and as a consequence, timing of the sampling is critical for detecting intermittent shedders. Absence of clinical manifestations to indicate shedding limits success of timing single sampling schedules. Multiple samplings may be used to moderate the effects of intermittent shedding on the prevalence estimates.

The pathogenesis of Salmonella infections is complex being affected by several variables, including infectious dose, route of infection, and serotype (Boughton, Egan, Kelly, Markey, & Leonard, 2007; Fedorka-Cray, Kelley, Stabel, Gray, & Laufe, 1995; Fedorka-Cray, Whipp, Isaacson, Nord, & Lager, 1994; Gray, Stabel, & Fedorka-Cray, 1996; Osterberg & Wallgren, 2008; Osterberg, Lewerin, & Wallgren, 2009; van Winsen et al., 2001). Under experimental conditions, pigs infected with moderate to low doses of Salmonella are able to clear the infection in a period of time ranging from days to weeks (Fedorka-Cray et al., 1994, 1995; Gray et al., 1996; Osterberg & Wallgren, 2008; van Winsen et al., 2001). Therefore, we hypothesize that, under natural conditions, multiple infections (possibly, with the same serotype, but also with multiple Salmonella serotypes) may generate a series of infections over the finishing production stage, corresponding to the initiation and resolution of shedding within the finishing lots. As in the case of intermittent shedding previously mentioned, multiple temporally spaced samplings may be used to reduce the impact of a series of infections on prevalence estimates based on fecal sampling (i.e., bacteriologic).

The variation observed in the serologic prevalence, which would not be affected by the previously discussed factors (i.e., low sensitivity of fecal sampling, and occurrence of intermittent shedding), supports the hypothesis of periodic initiation and resolution of infections during the finishing production stage. Fortunately, our knowledge of the longitudinal time-course of the on-farm serologic response to Salmonella infections is limited. Based on studies with experimentally infected pigs, it has been shown that the onset of serologic response and peak seroprevalence occur at approximately 7 and 30 days post-inoculation, respectively (Nielsen, Baggesen, Bager, Haugegaard, & Lind, 1995; Osterberg et al., 2009; Srinand, Robinson, Collins, & Nagaraja, 1995; Wood, Posspichil, & Rose, 1989; Wood, Rose, Coe, & Ferris, 1991). Longer time periods between peak of shedding prevalence, and peak seroprevalence have been reported (Kranker et al., 2003), and according to the authors, the different course of the bacteriologic and serologic responses occurred, because under natural conditions, pigs within groups are infected at different points in time, with variability in both exposure level and host response. Therefore, in conjunction with the previously mentioned studies, our results suggest that Salmonella infections in swine populations are very dynamic under natural conditions, and consequently, still far from being fully understood.

As expected, the overall prevalence estimates obtained by applying the two diagnostic tools (i.e., bacteriology and serology) differed significantly, although the difference was not consistent in all production farms studied with either test system. Nevertheless, a moderate correlation (r=0.59) between bacteriologic and serologic prevalences was observed, in agreement with previous studies (Barron, Sounpasis, Butler, & Duffy, 2009; Funk, Harris, & Davies, 2005; Lo Fo Wong et al., 2003; Rajic et al., 2007; Rostagno, Hurd, & McKean, 2009). However, it should be considered that recent Salmonella infections (~1 week) cannot be detected by serological examination. Therefore, it is possible that pigs get infected during the last days of the finishing production stage and are not detected (serologically), when samples are collected at slaughter. On the other hand, it is also possible that animals with positive serological status do not continue to shed Salmonella in the feces. When comparing prevalence estimates, it has to be kept in mind that a temporal disassociation exists between infection and serological response. Although the number of published studies on the epidemiology of Salmonella in subclinically infected pig herds has tremendously increased over the past decade, additional longitudinal studies following both the bacteriologic and serologic statuses of pigs are necessary to understand these relationships. Moreover, to design effective pre-harvest monitoring and control programs, an understanding of transmission of Salmonella, taking into account a number of variables (e.g., serotype, age of infection, infectious dose, route of infection, diet configuration, and environmental exposures) on farms, is essential.

It is clear that defining a sampling strategy to effectively monitor on-farm Salmonella prevalence and dynamics constitutes a challenging task. Different sampling approaches and statistical methods to categorize pig herds have been investigated (Abrahantes, Bollaerts, Aerts, Ogunsanya, & Van der Stede, 2009; Arnold & Cook, 2009; Davies, Heath, Coxon, & Sayers, 2003; Ekeroth, Alban, & Feld, 2003; Snary, Munday, Arnold, & Cook, 2010). However, a consensus still does not exist as to what would be the best approach. Therefore, further research is still required.

Based on the results of this study, it can be concluded that Salmonella prevalence varies widely between finishing pig lots within production farms under common management. This observation makes the categorization of high and low prevalence farms difficult, especially if based on a single point sampling (or point estimate). This study suggests that risk factors and Salmonella infection dynamics are lot-related, instead of farm-related. As a direct consequence of the results obtained in this study, a critical question arises: Are there high and low Salmonella prevalence farms/herds, or is prevalence determination just a matter of timing?

Acknowledgments

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References
