Salmonella enterica Subclinical Infection: Bacteriological, Serological, Pulsed-Field Gel Electrophoresis, and Antimicrobial Resistance Profiles—Longitudinal Study in a Three-Site Farrow-to-Finish Farm

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Abstract

The aim of this surveillance was to study both Salmonella spp. shedding patterns and the time course of serological response in farrow-to-finish reared pigs from a subclinically infected farm. Antimicrobial resistance profile, molecular subtyping, and the relationship among the isolates were determined by pulsed-field gel electrophoresis (PFGE). A farrow-to-finish farm of 6000 sows, with a history of Salmonella Typhimurium septicemia, was selected. A longitudinal bacteriological and serological study was conducted in 25 sows before farrowing (M1 = S1) and in 50 offspring at 21 (M2 = S2), 35 (M3 = S3), 65 (M4 = S4), 86 (M5 = S5), 128 (M6 = S6), and 165 (M7 = S7) days of age. Serum antibodies were tested using Herdcheck® Swine Salmonella antibody test kit (Idexx Laboratories, ME). Bacteria were isolated from pooled fecal samples. Suspected isolates were confirmed by conventional biochemical assays, and those identified as Salmonella spp. were serotyped. A variation between seropositive percentages and positive fecal samples was observed. Serologically positive pigs decreased from S1 to S4, and subsequently increased from S4 to S7. The percentages of fecal positive culture increased from M1 to M3, and then declined in M4, increased in M5, and were negative in M6 and M7.

In the study three serovars, Salmonella 3,10:e,h:-, Salmonella Muenster, and Salmonella Bovismorbificans, were identified with low pathogenicity for swine. Three multidrug resistance strains (one belonged to Salmonella 3,10:e,h:- and two belonged to Salmonella Muenster) were found. PFGE results showed three different but closely related patterns among the 13 isolates of Salmonella Bovismorbificans, and two patterns for the three Salmonella Muenster and Salmonella 3,10:e,h:- isolates.

This longitudinal study established critical points of Salmonella spp. infection in the farm and the production stages, where appropriate control measures must be taken. PFGE showed clonal relationships in each serovar. Antibiotic resistance profiles should be periodically included due to public health concerns.

Introduction

Salmonella spp. infection is of major concern to the swine industry for several important reasons: (1) contaminated pork distributed in retail stores can present very significant risk to public health, and (2) septicemic salmonellosis, due to host-adapted Salmonella choleratis, or severe enteritis, due to nonhost-adapted Salmonella Typhimurium (Lanza, 1998; Weiss et al., 2002; Griffith et al., 2006), causes significant mortality in piglet and grower pigs, resulting in important economic loss. Salmonella spp. are one of the most important foodborne pathogens transmitted to humans worldwide (Asai et al., 2002; Beloeil et al., 2003; Oliveira et al., 2005). Pork is the

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second most important source of *Salmonella* spp. in some countries, and many human outbreaks reported in the last decade have been traced to consumption of pork meat (Griffith et al., 2006).

It is assumed that the greater the number of *Salmonella* spp. that are carried into the slaughter process, via the pigs’ intestines, the greater the risk of equipment and final product contamination (Hurd et al., 2002; Sauli et al., 2005; Griffith et al., 2006). Therefore, reductions in preslaughter infection rates should result in increased pork safety (Hurd et al., 2002; Oliveira et al., 2005; Sauli et al., 2005).

Infection of swine by one or more serovars is common (Griffith et al., 2006), but nowadays *Salmonella* spp. do not usually cause clinical disease in swine (Kranker et al., 2003). Enteroocolitis due to *Salmonella* Typhimurium occurs more frequently than expected in high health herds (Griffith et al., 2006); however, this serovar can also live on swine intestines without clinical signs (Griffith et al., 2006). *Salmonella* spp. are frequently isolated as a sequel to other enteric or debilitating diseases such as porcine circovirus 2 (Griffith et al., 2006; Silva et al., 2006) and other predisposing factors (Schawrtz, 1997; Vigo et al., 2004; Ha et al., 2005).

Generally, to estimate *Salmonella* spp. prevalence in pig herds, bacteriological examinations are performed on fecal samples or tissues from clinically affected animals (Asai et al., 2002). Culturing fecal samples for *Salmonella* spp. is a useful tool to determine current infections in a pig herd. However, *Salmonella* spp. shedding by pigs is intermittent with a low number of bacteria in the feces of subclinically infected pigs (Oliveira et al., 2005). Therefore, conventional culture methods are labor intensive, time consuming, and expensive, and may not be practical or economically feasible for routine application due to low sensitivity (Ekeroth et al., 2003; Lo Fo Wong et al., 2003). Modern serological techniques, such as enzyme-linked immunosorbent assay tests, have proven to be convenient and cost-effective methods for screening for antibodies against *Salmonella* spp. (Lo Fo Wong et al., 2003). Measuring antibody responses is an indirect but more sensitive method to determine the prevalence of pigs harboring the bacteria in a herd (Ekeroth et al., 2003). Importantly, the presence of serum antibodies reflects previous exposure rather than current infection (Funk et al., 2005). Neither fecal culture nor detection of serum antibodies represents a perfect diagnostic test for *Salmonella* spp. Apart from this, the limitations of both tests should be considered to interpret the results (Nollet et al., 2005b).

To determine the dynamics of *Salmonella* spp. infection in swine herds over time (e.g., age, duration of infection, and disease transmission patterns), longitudinal studies following the bacteriological and serological status of pigs should be performed (Kranker et al., 2003; Oliveira et al., 2005). Further, the distribution of *Salmonella* spp. and its clonal relationships between strain types circulating among different farm facilities provide valuable information to understand the epidemiology of the infection (Zhao et al., 2007).

Approaches to prevent and control salmonellosis in the food animal industry include, among others, improved biosecurity, vaccination, and use of competitive exclusion products, although each of these practices has had limited success. The control and prevention of *Salmonella* spp. needs to be reinforced by the use of antimicrobial chemotherapy (Zhao et al., 2007). Data show increased antimicrobial resistance among several *Salmonella* serovars, and multiple drug resistance (MDR) is an emerging problem (Douris et al., 2008). The antimicrobial resistance makes it more difficult for clinicians to empirically select an appropriate antibiotic (Zhao et al., 2007).

Conventional and molecular epidemiology and antimicrobial susceptibility profiles are important epidemiological tools to determine potential sources of infections and have been widely used to understand the epidemiology of many infectious diseases (Liebana, 2002). The current gold standard method of choice for molecular typing of *Salmonella* spp. as a source identification is pulsed-field gel electrophoresis (PFGE) (Ridley et al., 1998; Liebana, 2002; Best et al., 2007; Vigo et al., 2007).

Currently, there is limited published data relative to the prevalence, antimicrobial susceptibility, and molecular subtypes of *Salmonella* spp. present in swine herds in Argentina (Vigo et al., 2004, 2007). The aim of this survey was to study both the *Salmonella* spp. shedding patterns and the time course of the serological response in farrow-to-finish reared pigs from a subclinically infected farm, and to determine the antimicrobial resistance profile, molecular subtype by PFGE, and the relationship among the isolates.

### Materials and Methods

#### Farm selection and sampling size

A farrow-to-finish farm (6000 sows) was selected. The farm consisted of three operation units and used a batch farrowing system (250 sows/week) with an all-in/all-out hygiene policy at farrowing, postweaning, and fattening sections. The piglets were weaned at 3 weeks and moved to site 2. Site 2, where growers are raised, has nine barns and all of them are loaded with piglets weaned for a full week (3000 piglets). After 8 weeks in site 2, growers are moved to site 3, which has 14 barns and allows raising 1500 finishers in each one of them, until they reach the market weight.

*Salmonella* spp. positive status of the farm was confirmed by a preliminary study carried out in October 2005, when an increased mortality in fattening units was observed, and *Salmonella* Typhimurium was isolated from the spleen of pigs that suffered from porcine dermatitis and nephropathy syndrome (Vigo et al., 2004).

#### Sampling scheme

**Site 1 (farrowing).** At the beginning of this study, 25 sows, within a weekly batch, were randomly selected (Fig. 1). They were bled the day before farrow (S1). The day after farrow, 10–25 g of feces was collected from the rectum of sows with sterile gloves or from the floor using sterile plastic bags (M1). From 10 of the previously selected sows, a total of 50 piglets were randomly selected and tagged. Piglets were bled at weaning age (21 ± 3 days old) (S2). At the same time, fecal samples, 10 pools of 10–25 g, each pool coming from five animals, were collected from the floor of the farrowing crates (M2).

**Site 2 (nursery).** The 50 pigs previously tagged were sampled 2 weeks after their transfer to site 2 (35 ± 3 days old). Individual blood samples (S3) and pooled fecal samples for bacteriological studies were collected (M3). They were taken...
from the floor in five different points of each pen where the piglets were held. The same sample scheme for serological (S4) and bacteriological (M4) studies was carried out 1 week before the pigs left site 2 (65 ± 3 days old).

**Site 3** (fattening). In this stage, three samples for serological and bacteriological studies were taken as described above. The first sample was collected 2 weeks after the pigs reached site 3 (86 ± 3 days old) (S5) (M5), the second sample was collected 8 weeks into the finisher stage (midway through the fattening period, 128 ± 3 days old) (S6) (M6), and the last sample was taken 1 week before the pigs left the farm for slaughter (165 ± 3 days old) (S7) (M7).

**Serology**

Blood was taken from the cava cranialis vein. The samples were centrifuged the same day, and sera were collected and stored at −20°C until study. Serum antibodies were tested using HerdCheck Swine Salmonella antibody test kit (Idexx Laboratories) following the manufacturer’s instructions. Optical densities (ODs) were determined by a spectrophotometer with a 650 nm filter. The test detects the most common serogroups (B, C1, and D) isolated in Europe, Asia, and America, and has a specificity of 99.4% (HerdChek Swine Information Sheet; Idexx Laboratories).

The ODs were calculated as:

\[
OD = \frac{OD\text{-ample} - OD\text{-negative control}}{OD\text{-positive control} - OD\text{-negative control}}
\]

Samples with OD ≥ 0.4 were considered to be positive. Software xChek™ 3.3 (Idexx Laboratories) was used to obtain the results.

**Bacteriology**

From each pooled sample, 10–25 g of feces was diluted 1/10 in buffered peptone water. Each sample was incubated for 24 h at 37°C (preenrichment). Thereafter, 1 mL of broth was inoculated in 10 mL of tetrathionate broth for 24 h at 37°C (enrichment). A loopful of enrichment broth was transferred to enteric Hektoen agar with novobiocin (10 μg/mL) and incubated for 48 h at 37°C. Two presumptively positive colonies were streaked in triplicate soy agar and incubated for 24 h at 37°C, and were subsequently confirmed by biochemical conventional assays (Koneman et al., 1999). Isolates identified as *Salmonella* spp. were serotyped by agglutination according to M. Poppof’s (Clinical and Laboratory Standards Institute, 2009) scheme, using polyvalent and monovalent O and H antisera produced at the Servicio de Antı́genos y Antisueros, Instituto Nacional de Producción de Reactivos y Biológicos—ANLIS “Carlos G. Malbrán.”

**Antimicrobial resistance assay**

Susceptibility of *Salmonella* spp. strains to ampicillin, cephalothin, cefotaxime, norfloxacin, enrofloxacin, nalidixic acid, gentamicin, streptomycin, amikacin, chloramphenicol, fosfomycin, polymixin, tetracycline, nitrofurantoin, and trimethoprim-sulphamethoxazole was established by disk diffusion according to the Clinical Laboratory Standards Institute (Hunter et al., 2005).

**PFGE**

This was carried out following PulseNet standardized protocol from the Centers for Disease Control and Prevention (Ribot et al., 2006). Briefly, the plugs of agarose containing DNA were digested with 30 U of *XbaI* (Promega, Madison, WI). Fragments were separated in a 1% agarose gel (SeaKem Gold; Lonza, Rockland, ME) in 0.5× Tris borate EDTA buffer at 14°C in a contour CHEF-DR III System (Bio-Rad, Hercules, CA). Run time was 18 h, with a constant voltage of 200 V, using linear ramp of 2.2–63.8 sec. Staining was carried out with 0.5 μg/mL of aqueous ethidium bromide solution (Bio-Rad). *Salmonella* Braenderup CDC-H-9812 was included as fragment size marker to analyze the patterns generated with *XbaI*-PFGE (Funk et al., 2001). The images of PFGE gels were obtained by Gel-Doc 2000 System (Bio-Rad), and were analyzed using BioNumerics version 3.5 (Applied Maths, Kortrijk, Belgium). The relationship among the patterns was estimated as the proportion of shared bands applying the Dice coefficient with a 1.5% band position tolerance, and a dendrogram based on the unweighted pair group method with the arithmetic mean method was generated.
Results

At the beginning of the study, eight of the pigs tagged died of causes not related with *Salmonella* spp. infection.

Figure 2 shows the temporal variation of seropositive pigs and positive fecal samples found throughout the study. Briefly, percentage of positive sera clearly decreased from S1 to S4, and subsequently increased from S4 to S7. The percentage of fecal *Salmonella*-positive cultures increased from M1 to M3, and then abruptly declined in M4 and increased in M5; all the samples were negative in M6 and M7. During the study, only three *Salmonella* serovars were identified (Table 1).

While *Salmonella* Bovismorbificans isolates were sensitive to all the antimicrobial agents assayed, *Salmonella* Muenster and *Salmonella* 3,10:e,h:- isolates showed resistance to at least one antibiotic (Table 1).

To determine the genetic relatedness among the isolates belonging to each serovar, strains were analyzed by PFGE. The subtyping results for the 13 *Salmonella* Bovismorbificans isolates showed that three *Xba*I-PFGE patterns were identified. These patterns showed very slight differences in the position of only one band of high molecular weight and were distinct from other *Salmonella* Bovismorbificans isolates present in the *Salmonella* PFGE National Database (Fig. 3). Likewise, the three *Salmonella* Muenster isolates and the *Salmonella* 3,10:e,h:- isolate showed two similar patterns with only one band difference. The *Salmonella* 3,10:e,h:- isolate, recovered from M1 stage, showed an identical pattern to one of the *Salmonella* Muenster isolates, confirming its serovar. The *Xba*I-PFGE patterns obtained were different from those of other *Salmonella* Muenster isolates present in the *Salmonella* PFGE National Database. (Fig. 4).

Discussion

Concerns about pork safety have stimulated research along the food chain in an attempt to determine the effects of various pre- and postharvest processes on the prevalence of *Salmonella* spp. contamination. Because of the temporal variability in prevalence within groups, longitudinal sampling is recommended for evaluating the behavior of *Salmonella* spp. in different farm systems (Funk *et al*., 2005).

In this surveillance, a low number of shedder sows at farrowing was observed. Additionally, stress factors are considered one of the triggers for *Salmonella* spp. shedding process; in this study, farrowing seems not to increase the

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Number of isolates</th>
<th><em>Salmonella</em> serovars</th>
<th>Resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>23</td>
<td>1</td>
<td><em>Salmonella</em> 3,10:e,h:-</td>
<td>Nit, Tet, Nal, Cmp</td>
</tr>
<tr>
<td>M2</td>
<td>11</td>
<td>1</td>
<td><em>Salmonella</em> Muenster</td>
<td>Nit, Tet, Nal</td>
</tr>
<tr>
<td>M3</td>
<td>10</td>
<td>8</td>
<td><em>Salmonella</em> Bovismorbificans</td>
<td>–</td>
</tr>
<tr>
<td>M4</td>
<td>10</td>
<td>1</td>
<td><em>Salmonella</em> Muenster</td>
<td>Nit, Tet</td>
</tr>
<tr>
<td>M5</td>
<td>10</td>
<td>4</td>
<td><em>Salmonella</em> Bovismorbificans</td>
<td>–</td>
</tr>
<tr>
<td>M6</td>
<td>10</td>
<td>0</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td>10</td>
<td>0</td>
<td>Negative</td>
<td></td>
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</tbody>
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Nit, nitrofurantoin; Tet, tetracycline; Nal, nalidixic acid; Cmp, chloramphenicol.
shedding rate. Further, Funk et al. observed a decreased rate of shedding of *Salmonella* spp. between late gestation and early lactation. This reduction in shedding may be explained for certain changes in sow’s environment such as diet, housing, water delivery, and husbandry practices (Hurd et al., 2003; Funk and Gebreyes, 2004; Nollet et al., 2005b). On the other hand, this low shedding rate could be related to the intermittent dissemination of the bacteria (Haye et al., 1981; Griffith et al., 2006) and cohort temporal variation (Kranker et al., 2003). Moreover, the seropositive sow’s rate was 96%, which differs from that reported by Kranker et al. (2003) and Silva et al. (2006). Further, there is no information available regarding the influence of serological status of sows at farrowing avoiding the dissemination of the bacteria. Findings of this study suggest that immune response might have reduced the shedding rate. Further studies are needed to understand the influence of serological status of sows on the epidemiology of *Salmonella* spp. dissemination. The low shedding rate observed at the early lactation stages in this study as well as previous studies suggests that sows play a more important role in *Salmonella* spp. transmission to the newborns than is actually considered (Kranker et al., 2003).

There are different concerns regarding the bacteriological status in the lactation period. In this study, before weaning, 9% of piglets were shedding. This elimination rate is similar to the rate reported by Funk et al. (2001) for the same period (<7%). Other studies found no shedding piglets at weaning (Kranker et al., 2003; Silva et al., 2006). This information complements existing evidence, indicating that *Salmonella* spp. infection can occur in young pigs (Nollet et al., 2005a; Griffith et al., 2006). The percentage of seropositive piglets (48.8%) at 21 ± 3 days old may reflect the passive immune protection against *Salmonella* spp. infection (Davies et al., 1999). Moreover, this high percentage of seropositive piglets explains the moderate percentage of shedding piglets at weaning (Kranker et al., 2003).

At 35 ± 3 days old, midway through the postweaning period, 90% of the fecal samples were positive to *Salmonella* spp. This represents a high percentage compared with studies carried out in Denmark, where only 50% of piglets were

FIG. 3. Dendrogram showing the genetic relationship between *Salmonella* Bovismorbificans isolated from pigs at different stages of production compared with *Salmonella* pulsed-field gel electrophoresis (PFGE) National Database.

FIG. 4. Dendrogram showing the genetic relation between *Salmonella* Muenster isolated from pigs at different stages of production compared with *Salmonella* PFGE National Database.
positive using rectal swabs (Kranker et al., 2003). Rectal swab has less sensitivity than pooled floor fecal samples (Nollet et al., 2005b). Not only could the discrepancies observed be due to the sampling method (Kranker et al., 2003), but also due to the minor differences in the sensitivity of the bacteriological technique used. The high percentages of shedding pigs found in this surveillance might represent the real situation at weaning due to the all-in/all-out policies used. The rapid increase in *Salmonella* spp. prevalence in postweaning might have been triggered by weaning stress and associated factors, such as a change in feed and confinements of litters (Kranker et al., 2003; Griffith et al., 2006). On the other hand, horizontal transmission occurred at late lactation and early weaning increased the percentage of shedding pigs in postweaning period (Kranker et al., 2003).

One week before the pigs left the postweaning unit (65 ± 3 days old) the percentage of culture positive to *Salmonella* spp. fell drastically to 10%. This finding differs from the results of Kranker et al., 2003. This decrease in the number of shedding pigs is related to management practices. The effect of antibiotics on the frequency and length of shedding of *Salmonella* spp. has received little attention in pigs (Griffith et al., 2006). However, the common use of antibiotics as growth promoters or preventive of respiratory diseases could decrease the shedding rate of *Salmonella* spp.

Only 2.04% of the pigs were seropositive at 65 ± 3 days of age. The serological technique used in this study does not distinguish between maternal or pig’s own antibodies. Thus, the fall of colostral antibodies could therefore mask an active immune response of pigs (Beloeil et al., 2003). On the other hand, the shedding rate observed at this point could not be enough to trigger an active immune response in a herd. The late nursery stage seems not to be important in the epidemiology of *Salmonella* spp. (Silva et al., 2006). Moreover, stress factors at the beginning of the nursery period were diminished at this point, and this could be the reason for the low shedding and serological rates observed. However, surveillance at this stage should be considered an important prior step in the early finishing period.

The bacteriological results obtained at the early finishing period showed a significant increase from the late nursery. The farm studied has its finishing units 5 km away from nursery facilities. Thus, relocation, among other stress factors, could be the reason for the increase in the shedding rate observed at 86 days of age (Davies et al., 1999). Further, the low number of positive pigs spreading the bacteria at the late nursery period seems to be the source of infection in early finishing units (Kranker et al., 2003). The *Salmonella* spp. shedding pattern observed in this study at the finishing stage suggests that the early finishing period plays a central role in the infection of finisher pigs. The dissemination pattern observed throughout this period agrees with that obtained by Funk et al. (2001) and Silva et al. (2006). However, we must emphasize that the failure to detect *Salmonella* spp. in middle and late finishing period did not indicate that the previously infected pigs continued harboring the organism (Nielsen et al., 1995).

The serological results obtained at the fattening period suggest an active immune response against the natural infection produced at the beginning of this period (Beloeil et al., 2003). The onset and serological peak has been described between 7 and 30 days postnaturally infection (Cappuccio et al., 2006); it supports the delay observed against the infection and the serological response observed in this surveillance.

Although *Salmonella Typhimurium* had been isolated from a systemic infection in a previous study in this farm (Cappuccio et al., 2006), only *Salmonella Muenster* and *Salmonella Bovismorbificans* were identified, and both are considered to be of low pathogenicity to pigs (Griffith et al., 2006). The absence of *Salmonella Typhimurium* isolation could be due to the low prevalence of this serovar present during the study, as well as the natural competition and competence of multiple serovars circulating concurrently on the farm. The relatively common isolation of multiple serovars from a single swine source is presented in a study from Sweden that documented the incidence of up to seven of concurrent serovars in several farms from 1993 to 1997. Interestingly, neither *Salmonella Muenster* nor *Salmonella Bovismorbificans* were isolated (Boqvist et al., 2003). Moreover, there are few other reports of *Salmonella Muenster* or Bovismorbificans clinically affecting swine, and the majority of case reports are isolates from healthy animals and abattoir carcasses (Lázaro et al., 2004), hence the need to conduct surveillance of these serovars during the growth period (Barber et al., 2002; Hurd et al., 2002). Additionally, both serovars observed in this longitudinal study were not related with clinical disease in pigs, and their presence should be considered a potential contaminant of final products.

Barber (Holmberg et al., 1984) suggested that *Salmonella* spp. are transmitted readily between many ecological compartments within the swine production system. However, more precise genetic analyses of isolates would be needed to support this conclusion. The findings of this study appear to support this notion as was evidenced by PFGE, since for both *Salmonella* serovars identified, a single subtype was found throughout the different stages analyzed. Further, identical or very closely related *Salmonella Muenster* subtypes were identified in the sow and its piglets. For the 3,10:e,h:- isolate, the serovar could not be established because the isolate did not express the second flagellar antigen, but according to the antigenic formula, it is highly probable that this isolate also belonged to the Muenster serovar. This observation was further confirmed with the PFGE results.

In this study, molecular analysis was important to determine that the same subtype was circulating and remained at all stages where *Salmonella* spp. were recovered.

Several studies have indicated an increase in MDR (Douris et al., 2008). Moreover, the extensive use of antibiotics in farms for both therapeutic and growth promotion purposes has been considered to be an important trigger for the emergence of antibiotic-resistant pathogens (Poppe et al., 1990; Threlfall et al., 2000), and their real impact on public health continues to be a matter of debate. Antibiotic-resistant strains have emerged worldwide and have led to increasing consumer concerns about animals for food consumption. Infections caused by resistant strains have shown a higher fatality rate compared to those caused by susceptible microorganisms (Threlfall et al., 2000). MDR were also more frequently associated with certain serovars (Zhao et al., 2007). In this study, three multidrug resistance strains (one belonged to *Salmonella* 3,10:e,h:- and two belonged to *Salmonella Muenster*) were identified. The MDR pattern appears only in the isolates belonging to sows and at 35 ± 3 days old. However, the resistance profile found is different from the one described in...
Brazil for the same serovar (Barber et al., 2002), which most likely reflects both different animal production environments and associated uses of antimicrobials in preventing or treating common diseases (Zhao et al., 2007).

Epidemiologic prevalence and behavior of *Salmonella* spp. infection can differ markedly between different farms and with different management practices. Importantly, this study allows us to determine the critical points of *Salmonella* spp. infection, their serological dynamics, and their shedding patterns. When all-in/all-out management production systems are utilized, residual infection of small numbers of animals within every group of raised pigs in the farrowing and nursery units can be considered a critical point in the persistence of subclinical *Salmonella* spp. infection. The association between stress and *Salmonella* spp. excretion has been previously described (Kranker et al., 2003; Nollet et al., 2005a; Griffith et al., 2006). Although there is no information about the degree of impact of transportation and commingling of litters, in three separate sites with all-in/all-out management systems, it was determined that transportation and litter commingling should be diminished to reduce *Salmonella* spp. excretion from the residual infected pigs. Moreover, further studies should be carried out to evaluate the real impact of other stressors, such as facilities, food usage, or antibiotic usage, which could be acting as a hazard point of *Salmonella* spp. subclinical persistence. Longitudinal periodical studies could allow determination of the optimal point in time in which to take specific control measures, such as feed and/or water acidification, and vaccination or antibiotic therapy, thereby reducing therapy failures, loss of food animals, production costs, or antibiotic resistance problems. Moreover, PFGE allowed determining the clonal relationships among different serovars throughout all the production stages. Antibiotic resistance profiles should be periodically included due to public health concerns.

**Disclosure Statement**

No competing financial interests exist.

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