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Original Article

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 (M/S1) and in 50 offspring at 21 (M/S2), 35 (M/S3), 65 (M/S4), 86 (M/S5), 128 (M/S6), and 165 (M/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* cholerasuis, 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). Enterocolitis 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

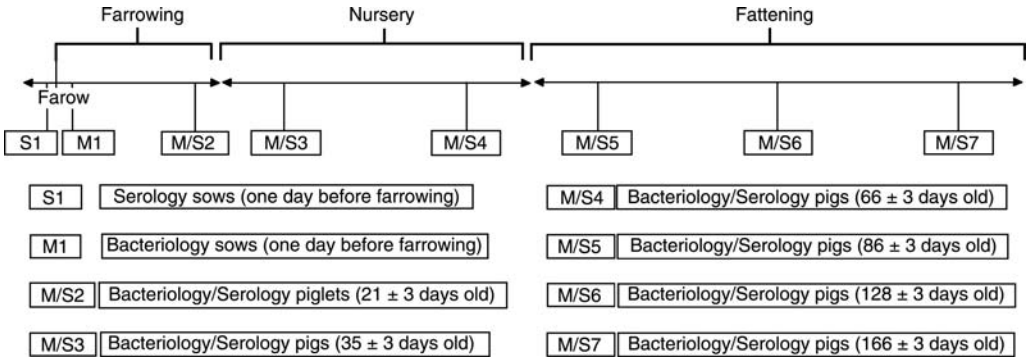


FIG. 1. Longitudinal sampling scheme for serological and bacteriological studies. M, fecal material for bacteriological studies; S, blood sample for serological studies. The samples of M1 and S1 corresponded with 25 randomly selected sows sampled 1 day prefarrowing (M1) and 1 day postfarrowing (S1), respectively. The samples from M2 to M6 and S2 to S6 corresponded with those obtained for bacteriological and serological studies, respectively, in each stage of production in the cohort of studied piglets.

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 *Salmonella* Information Sheet; Idexx Laboratories).

The ODs were calculated as

$$\text{OD} = \frac{\text{OD sample} - \text{OD-negative control}}{\text{OD-positive control} - \text{OD-negative control}}$$

Samples with OD $\geq$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 tripticase 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, 2005) scheme, using polyvalent and monovalent O and H antisera produced at the Servicio de Antígenos 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, polimixin, 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 *Xba*I (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 *Xba*I-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, Kortrij, 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.

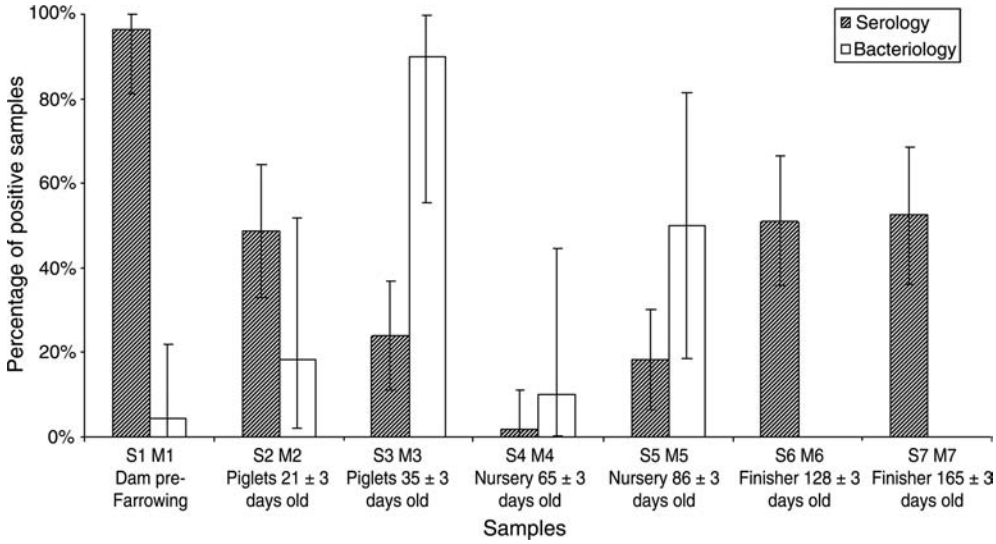


FIG. 2. The bacteriological (M1) and serological (S1) status of the samples throughout the production. Percentage of *Salmonella*-positive samples detected (open bars, bacteriology, M2–M7) and percentages of seropositive animal (shaded bars, serology, S2–S7) are shown. Error bars indicate 95% confidence interval.

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

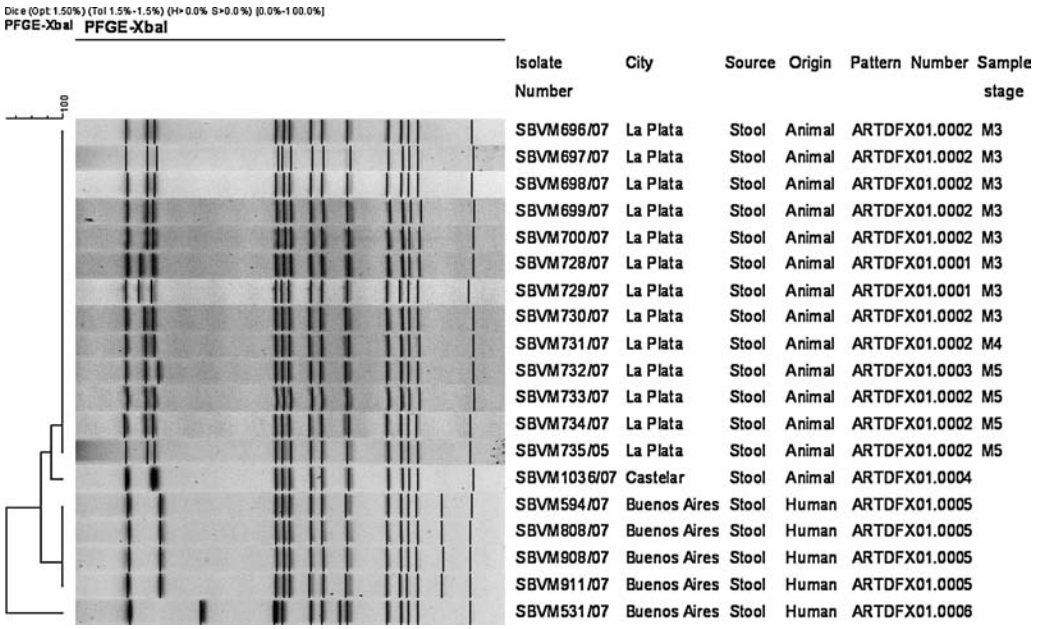
Concerns about pork safety have stimulated research along the food chain in an attempt to determine the effects of various pre- and postharvest processes on the prevalence of *Salmonella* spp. contamination. Because of the temporal variability in prevalence within groups, longitudinal sampling is recommended 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 1. *Salmonella* Serovars and Resistance Profiles of the Isolates Recovered in Each Stage Sampled

Sample	n	*Number of isolates*	Salmonella *serovars*	*Resistance profile*
M1	23	1	*Salmonella* 3,10:e,h:-	Nit, Tet, Nal, Cmp
M2	11	1	*Salmonella* Muenster	Nit, Tet, Nal
M3	10	8	*Salmonella* Bovismorbificans	–
	1	*Salmonella* Muenster	Nit, Tet	
M4	10	1	*Salmonella* Bovismorbificans	–
M5	10	4	*Salmonella* Bovismorbificans	–
	1	*Salmonella* Muenster	Tet	
M6	10	0	Negative	
M7	10	0	Negative	

Nit, nitrofurantoin; Tet, tetracycline; Nal, nalidixic acid; Cmp, chloramphenicol.



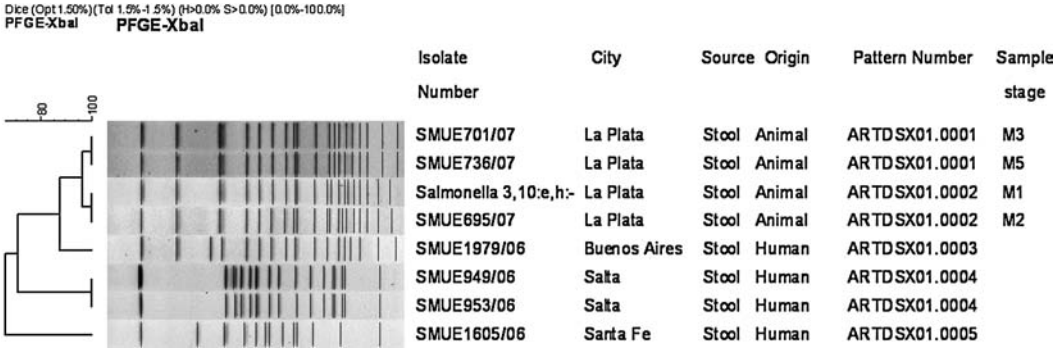
Isolate Number	City	Source	Origin	Pattern Number	Sample stage
SBVM696/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM697/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM698/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM699/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM700/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM728/07	La Plata	Stool	Animal	ARTDFX01.0001	M3
SBVM729/07	La Plata	Stool	Animal	ARTDFX01.0001	M3
SBVM730/07	La Plata	Stool	Animal	ARTDFX01.0002	M3
SBVM731/07	La Plata	Stool	Animal	ARTDFX01.0002	M4
SBVM732/07	La Plata	Stool	Animal	ARTDFX01.0003	M5
SBVM733/07	La Plata	Stool	Animal	ARTDFX01.0002	M5
SBVM734/07	La Plata	Stool	Animal	ARTDFX01.0002	M5
SBVM735/05	La Plata	Stool	Animal	ARTDFX01.0002	M5
SBVM1036/07	Castelar	Stool	Animal	ARTDFX01.0004	
SBVM594/07	Buenos Aires	Stool	Human	ARTDFX01.0005	
SBVM808/07	Buenos Aires	Stool	Human	ARTDFX01.0005	
SBVM908/07	Buenos Aires	Stool	Human	ARTDFX01.0005	
SBVM911/07	Buenos Aires	Stool	Human	ARTDFX01.0005	
SBVM531/07	Buenos Aires	Stool	Human	ARTDFX01.0006	

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.

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



Isolate Number	City	Source	Origin	Pattern Number	Sample stage
SMUE701/07	La Plata	Stool	Animal	ARTDSX01.0001	M3
SMUE736/07	La Plata	Stool	Animal	ARTDSX01.0001	M5
Salmonella 3,10:e,h:-	La Plata	Stool	Animal	ARTDSX01.0002	M1
SMUE695/07	La Plata	Stool	Animal	ARTDSX01.0002	M2
SMUE1979/06	Buenos Aires	Stool	Human	ARTDSX01.0003	
SMUE949/06	Salta	Stool	Human	ARTDSX01.0004	
SMUE953/06	Salta	Stool	Human	ARTDSX01.0004	
SMUE1605/06	Santa Fe	Stool	Human	ARTDSX01.0005	

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 commingling 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 postnatural 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 (Poppof *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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