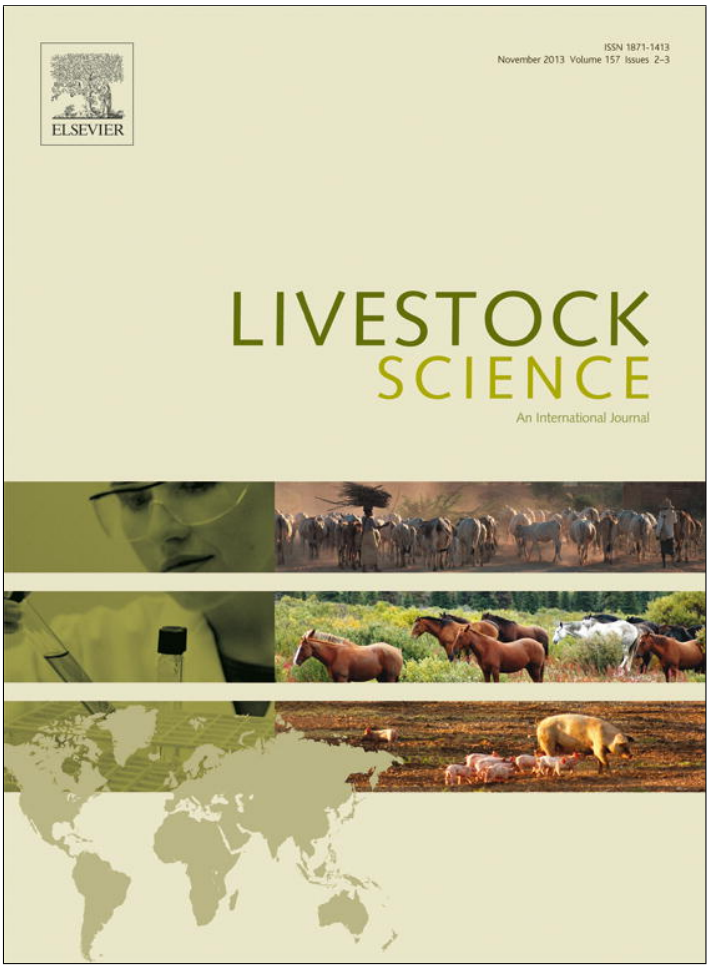

Livestock Science 157 (2013) 605–611

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Salmonella transmission from the gilt to her offspring

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ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form
13 September 2013
Accepted 17 September 2013

Keywords:
Salmonellosis
Swine production
On-farm transmission
Molecular analysis

ABSTRACT

The identification of gilts as a key factor in the salmonellosis dynamics is an important issue to the implementation of specific control programs in herds. This paper aims to assess the transmission of *Salmonella enterica* from the gilt to her offspring. The study was carried out in a multiple sites farrow-to-finish farm, built before the study to house 4500 sows, populated gradually with gilts weaned with less than 9 days of age. To determine the *Salmonella* infection prevalence in gilts, 1000 blood samples, 719 fecal samples and 236 mesenteric lymph nodes were collected from ten groups of gilts at an average age of 150 days. After that, a longitudinal study of the newborn piglets from the breeding herd was carried out for 3 consecutive weeks, which were followed from 10 to 150 days of age by serology (ELISA) and bacteriology (ISO 6579/02). The relatedness among the *Salmonella* isolates recovered was determined by *XbaI*-PFGE. A significant variability in the average of seropositive gilts among groups (from 0.00 to 31.52%) and low *Salmonella* shedding (1.4%) were found in the breeding herd at 150 days of age, but a wide range of *Salmonella* serovars ($n=11$) were isolated from slaughtered gilts. In the serological profile of the offspring, none of the pigs were found seropositive between 35 and 90 days of age, and bacteriology allowed to recover *S.* Derby from pigs only after 90 days of age. This suggests that offspring infection may not be taking place in the farrowing unit. The *S.* Schwarzengrund isolates recovered from gilts showed mainly the same *XbaI*-PFGE pattern, whereas *S.* Derby patterns of the strains obtained from gilts were different and also differed from the single *XbaI*-PFGE pattern isolated from the offspring. All these results suggest that serotype specific passive immunity would protect pigs from infection by *S. enterica* strains present in sows during their stay in the farrowing facilities, but fattening pigs can be infected by *Salmonella* from different sources of infection.

1. Introduction

Salmonella infections in swine is an important issue because of the impact on the production performance caused by the clinical presentation (salmonellosis), and also because of the implications for public health, of human disease attributed to consumption of contaminated pork and pork products (Griffith et al., 2006).

Specifically in swine production, salmonellosis is related to an increase in mortality rates and septicemic episodes, in addition to decrease in the production performance by affecting the daily weight gain and feed conversion, which economic cost has been estimated in £16.200/year/100 sow in United Kingdom (Muirhead and Alexander, 2001).

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http://dx.doi.org/10.1016/j.livsci.2013.09.010

The in-herd prevalence shows important differences between farms. In a meta-analysis based on 46 studies in 15 European and North American countries, Fosse et al. (2009) calculated a herd prevalence estimate of 21.8%, whlie a prevalence estimate of 6.7% was calculated for *Salmonella* shedding.

Most *Salmonella* control programs are based on monitoring systems used to assess the infection prevalence and the possible dynamics of infection in swine herds. These programs include serological testing, as well as, bacteriology testing of both fecal samples (FS) and mesenteric lymph nodes (MLN) (Arnold and Cook, 2009; Funk et al., 2001; Funk, 2003; Nowak et al., 2007; Rostagno et al., 2012).

The detection of specific antibodies against *Salmonella* spp. by ELISA in serum from pigs of different ages allows for the identification of time and level of the herd infection (Baum et al., 2005; Nielsen et al., 1995; Nollet et al., 2005), as well as the passive transfer of immunity from the sow to the newborn piglet (Kranker et al., 2003; Proux et al., 2000). Moreover, the bacteriology of FS allows for detection of pigs shedding *Salmonella enterica*, and the isolates recovered can be serotyped to determines serovars present in the herd. In addition, molecular analysis tools, such as pulsed-field gel electrophoresis (PFGE), can be applied to establish the genetic diversity, to infer or confirm transmission sources and pathways (Magistrali et al., 2008; Mannion et al., 2012; Rao et al., 2010; Weigel et al., 2007).

Through the study of risk factors and the analysis of key components, several aspects related to *Salmonella* transmission in swine herds have been identified, such as the farm biosecurity, hygienic measures, farm staff, animal health, the season and *Salmonella* serovar (Cardinale et al., 2010; Lurette et al., 2009; Lo Fo Wong et al., 2002; Rostagno and Callaway, 2012).

However, at present the role of *Salmonella* infection in sows, and more especially in gilts, related to on –farm transmission and dissemination still remains unclear (Funk and Gebreyes, 2004). Lurette et al. (2009) suggested that early infection, occurring between birth and weaning, is a critical point of *Salmonella* spread within a herd, whereas, to Kranker et al. (2003) the transmission at this stage may not be relevant, due in part to the presence of passive immunity in piglets. More specifically, acquired replacement gilts are considered by several authors to be an important source for introduction and dissemination of new *Salmonella* serovars in a herd (Davies et al., 2000; Silva et al., 2006), although, others consider it unlikely (Penmetchsa et al., 2009).

The identification of gilts as a key factor in the salmonellosis dynamics is a very important issue for the implementation of specific control programs in herds and swine production. Therefore, this paper aims to assess the transmission of *S. enterica* from the gilt to her offspring, by molecular subtyping and serology.

2. Materials and methods

The study was carried out in a multiple sites farrow-to-finish farm that had strict biosecurity measures. The farm had been recently built before the study to house 4500 sows and had a site used only for boars. The farm was populated gradually with groups of 760 gilts per week, with a total of 10 groups included in the study. All the gilts were brought from the same farm, they shared the same genetics and had been weaned with less than 9 days of age. Previous to the weaning, each piglet was medicated with tulatromicine (Draxxin, Pfizer Animal Health), following to the manufacturer´s recommendations. Each weekly group was taken to an individual room equipped to house the gilts from 9 to 150 days of age, at this time, the gilts that would constitute the breeding herd were selected. The gilts that were not selected were sent to slaughter.

2.1. Determination of Salmonella infection prevalence in the gilts

The software *WIN Episcope* 2.0 (www.clive.ed.ac.uk/winepiscope) was used to determine the number of gilts to be sampled, necessary to estimate the prevalence of the gilts shedding *Salmonella* spp. in every group. It is known that *Salmonella* shedding in infected animals is intermittent (Nielsen et al., 1995), which could lead to the shedding prevalence at certain time to be lower than the number of infected animals (Fosse et al., 2009). Because of that, we considered an expected prevalence of 8%, with 95% confidence level and 5% error, resulting in $n=100$ animals per group. The 100 gilts were selected at random in every group, at an average age of 150 days. Rectal fecal samples and blood samples (5 ml) were collected from each pig. The blood was placed in tubes, where the serum was separated and then kept at −20 °C. The fecal samples were refrigerated until they were processed. A total number of 1000 blood samples from the 10 groups and 719 fecal samples from 8 gilt groups were collected during a 10-week sampling period.

Mesenteric lymph nodes (MLN) were taken from 236 gilts that were sent to the slaughterhouse, following the procedures proposed by the European Union (Anonymous, 2006).

2.2. Determination of Salmonella infection prevalence in the offspring

A longitudinal study of the newborn piglets from the selected breeding herd was carried out for three consecutive weeks (weeks 1, 2 and 3). Blood samples from 10 pigs were taken at 10, 35, 56, 90, 120 and 150 days of age in each week. The number of samples necessary to detect pigs infected with *Salmonella* spp., was estimated at an expected prevalence of 25%, with 95% confidence level. At least 4–6 pigs were randomly selected and necropsied on each sampling day. MLN and contents of cecum (CC) samples were collected from each pig.

2.3. Sample processing

2.3.1. Serology

All serum samples were analyzed for the presence of *Salmonella* antibodies using a commercially available indirect mix-ELISA (Herd-Check Swine *Salmonella*, IDEXX Laboratories, Inc., Maine, USA) according to the manufacturer´s instructions. This kit combines different LPS

antigens from serogroups B, C1 and D of *S. enterica*. Each sample was classified by relating the absorbance value at 650 nm to the positive control mean by calculating the Sample to Positive (*S*/*P*) ratio. Results were evaluated at different cut-off values: Optic density 10% (*S*/*P*=0.25), 20% (*S*/*P*=0.5) and 40% (*S*/*P*=1), following the recommendations of the manufacturer. Different cut-off values allow modifying the sensitivity of the ELISA according to the aim of the study.

2.3.2. Bacteriology

All the FS (in pools of 5), the MLN and the CC were processed, within 24 h after they were collected, according to ISO 6579/2002 standard. Briefly, every sample was diluted 1:10 in Buffered Peptone Water (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37 ± 1 °C for 18 ± 2 h. Afterwards, the pre-enrichment culture was diluted 1:100 in Rappaport-Vassiliadis broth (Biokar Diagnostics, Allonne, France) and incubated at 42 ± 1 °C for 24 h. Each culture was streaked onto Brilliant Green Agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated for 18–24 h at 37 ± 1 °C. The colonies with a typical *Salmonella* morphology were identified by biochemical (ISO 6579/2002) and PCR to detect the *invA* gen, according to Malorny et al. (2003).

For the identification of *Salmonella* spp. serovars, the somatic and flagellar antigens were determined using specific antisera produced at the Instituto Nacional de Producción de Biológicos – ANLIS "Dr Carlos G. Malbrán" (Buenos Aires, Argentina). Serotyping was performed according to the White-Kauffmann-Le Minor Scheme (Institut Pasteur, Paris, France).

2.3.3. Genetic comparison

The genetic relatedness among *Salmonella* isolates recovered from MLN or FS in gilts, and MLN or CC in the offspring was determined by pulsed filed gel electrophoresis (PFGE) analysis with restriction enzyme *XbaI* (Fermentas, Burlington, Ontario, Canada), performed according to the CDC PulseNet protocol (Ribot et al., 2006). PFGE patterns were analyzed with BioNumerics v.4.0 software (Applied Math, Sint-Martens-Latem. Belgium).

3. Results

3.1. Salmonella infection prevalence in the gilts

No clinical symptoms, compatible with salmonellosis, were reported in the farm during the study.

A total of 963 serum samples from the 10 groups of gilts were analyzed by ELISA. Descriptive statistics of *S*/*P* values of the gilts analyzed from each group are shown in Fig. 1.

The bacteriology and serology results for each group of gilts are shown in Table 1.

A total of 211 (22.93%), 97 (10.54%) and 43 (4.67%) sera were found positive for the cut-off values of (*S*/*P* ratio) 0.25, 0.5 and 1, respectively (Table 1).

From the 719 FS processed from gilts at 150 days of age (144 pools), *S. enterica* was isolated in 2 pools (1.4%) belonging to the same group (Table 1). The isolates were identified as *S. typhimurium* (1 pool) and *S. schwarzengrund* (1 pool) (Table 1).

From 9 of the 10 gilt groups studied, 236 MLN were analyzed, isolating *Salmonella enterica* in 44 lymph nodes (18.6%). The most frequent serovars identified among these isolates were *S. schwarzengrund* (15), *S. bredeney* (13),

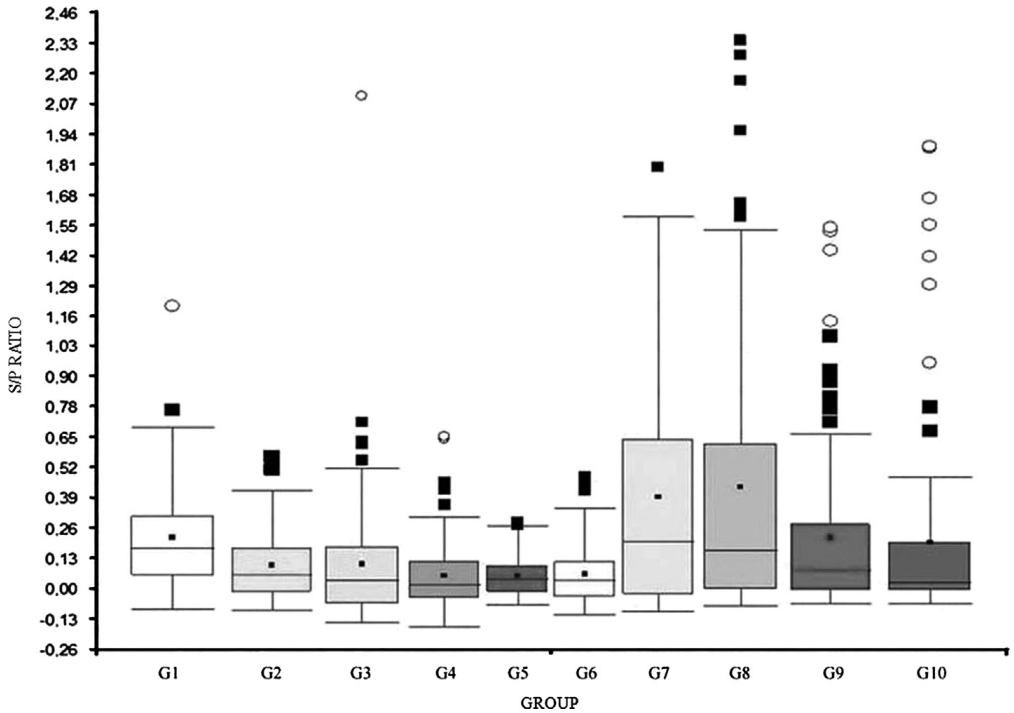

Fig. 1. Box plot representing the distribution of *S*/*P* ratio (by ELISA for *Salmonella* spp.) within each group of gilts. Squares represent outlier included between 1 and 2 confidence intervals, and circles are outlier higher than 2 confidence intervals.

Table 1
Results reported as percentages (positive/analyzed), obtained by serology at different cut-off, and bacteriology of MLN and FS for *Salmonella* spp., and isolated serovars in MLN and FS of each group of gilt. n/d=not determined.

Group	%+ELISA			%+ FS	Serovar recovered from FS (number of isolated)	%+ MLN	Serovar recovered from MLN (number of isolated)
	$S/P \geq 0.25$	$S/P \geq 0.5$	$S/P \geq 1$				
1	37.0	9.8	1.0	0	–	8.3	*S. bovismorbificans* (2)
2	12.0	2.0	0.0	0	–	0	–
3	19.5	6.5	1.0	0	–	n/d	n/d
4	9.8	2.2	0.0	n/d	–	27.0	*S. saintpaul* (3); *S. bredeney* (2); *S. derby* (2); *S. schwarzengrund* (2); *S. typhimurium* (1)
5	4.4	0.0	0.0	n/d	n/d	12.0	*S. infantis* (1); *S. derby* (1); *S. schwarzengrund* (1)
6	9.8	0.0	0.0	0	–	27.8	*S. bredeney* (9); *S. rissen* (1)
7	47.9	31.5	15.2	11.8	*S. schwarzengrund* (1); *S. typhimurium* (1)	16.7	*S. bredeney* (2); *S. saintpaul* (1); *S. schwarzenground* (1); *S. sandiego* (1)
8	41.3	29.3	18.4	0	–	20.0	*S. infantis* (1); *S. schwarzengrund* (1)
9	29.0	14.0	5.0	0	–	25.7	*S. schwarzengrund* (7); *S. livingstone* (1) *S. subesp* IO (1)
10	19.0	9.0	6.0	0	–	11.1	*S. schwarzengrund* (3)

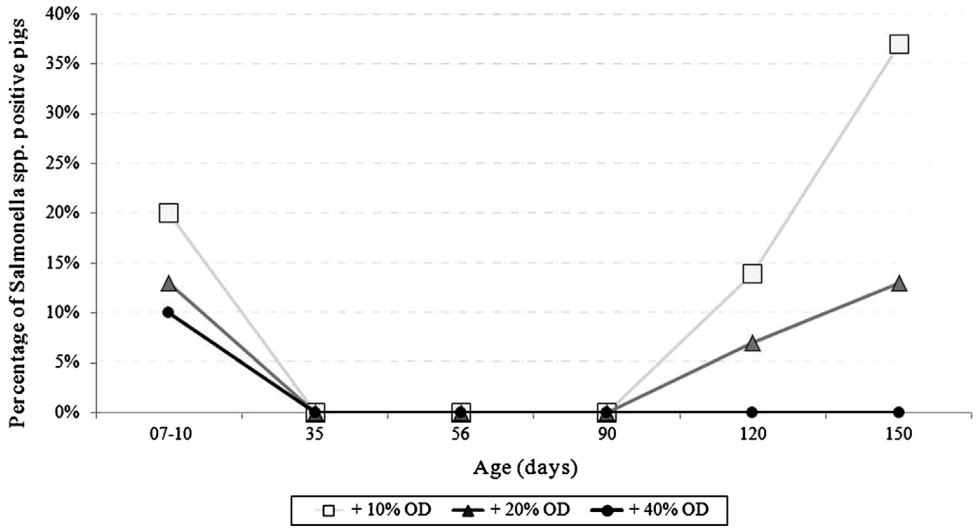

Fig. 2. Serological profile of the offspring; representing the percentage of *Salmonella*-positive sera in all 3 weeks sampled by pig age, analyzed using different cut-off values.

S. saintpaul (4), *S. derby* (3), and *S. infantis* (2) (Table 1). All the serovars isolated, except for *S. rissen* (G2) and *S. bovismrbificans* (C2), belonged to serogroup B and C1 of *S. enterica*.

3.2. *Salmonella* infection prevalence in the offspring

From the 6 different ages studied in each of the 3 weeks, 180 sera samples were processed. The percentage of *Salmonella*-positive samples in all 3 weeks by pig age are shown in Fig. 2.

Among the samples analyzed from 76 pigs necropsied in all three weeks, four were positive for *Salmonella* (Table 2); all these strains were classified as *S. derby*.

3.3. Genetic comparison

The *S. schwarzengrund* isolates recovered from FS and MLN of gilts from the Group 7 and from MLN of gilts from Group 9 showed the same *XbaI*-PFGE pattern (Fig. 3).

Table 2
Number of pigs necropsied (*N*) and *Salmonella enterica* positive pigs (*P*) in all 3 weeks sampled by pig age.

	Age				
	10 days *N/P*	56 days *N/P*	90 days *N/P*	120 days *N/P*	150 days *N/P*
Week 1	9/0	5/0	–	2/1	6/1
Week 2	10/0	7/0	5/0	5/0	5/0
Week 3	9/0	5/0	4/2	4/0	–

However, the isolate recovered from MLN of a gilt from group 4 presented a different PFGE patterns (Fig. 3)

The *S. derby* PFGE patterns of the strains obtained from MLN of Groups 4 and 5 differed, and were different from the single *XbaI*-PFGE pattern showed by the isolates recovered from the offspring (Fig. 3).

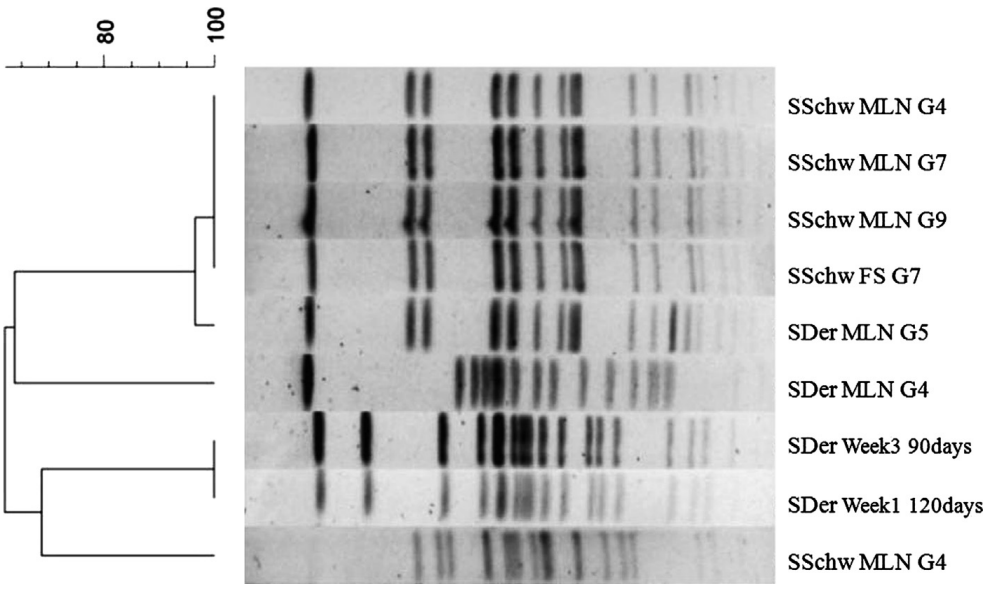

Fig. 3. *Xbal*-PFGE patterns of *S. schwarzengrund* (SSchw) and *S. derby* (SDer) isolated from mesenteric lymph nodes (MLN) and fecal samples (FS) of different groups (G) of gilts, and from the offspring.

4. Discussion

Global health programs in pig farms implemented to reduce *Salmonella* spp. infection in pork, may be affected by the fact that farms replace between 35 and 45% of their breeding herd every year with gilts from genetic companies. This has been demonstrated as an important risk factor in the dynamics of different pig pathogens (Griffith et al., 2006), although there is no conclusive information about the role of gilts in the introduction and spread of *S. enterica* in a farm. Although our results are limited by the type of farm and population conditions, this study presents sensitive information regarding *Salmonella* infections from the gilt to her offspring.

These data represent one farm with strict biosecurity measures, populated with very young gilts from the same genetic farm, all which may limit external validity of the study. On the other hand, to our understanding, these conditions may be important to minimize the effect of other variables in the results, and the findings of this study were consistent with previous reports. Moreover, the population methodology used in the present study is known to be useful to build up a new high health farm (as SPF farms).

The significant variability in the average of seropositive gilts found in the present study among the different groups (from 0.00 to 31.52%), is consistent with the reports from Kranker et al. (2003) and Rostagno et al. (2012), and suggest a particular prevalence for each batch of animals in a farm, which indicates the complexity of the *Salmonella* infection dynamics due to the presence of different subpopulations in a herd. Moreover, the average of *S*/*P* values increased and showed greater dispersion within the successive groups, which could be related to the exposure of the gilts as time passed, to an environment with increasing contamination, and thus enabling *Salmonella* infection and dissemination in the herd.

Low rates of *Salmonella* shedding (1.4%) were detected by the bacteriology methodology applied. Similar results were reported by Bonde and Sorensen (2012), 0.8%; Funk et al. (2001), 1.1%; and Penmetchsa et al. (2009), 2.2%. However, it is difficult to determine whether this level actually reflects the prevalence of shedding animals, since the pooled sample approach has been reported to both overestimate (Alban et al., 2012; Arnold and Cook, 2009), or underestimate the individual prevalence. This latter possibility suggested because an intestinal pathogen can increase the growth of the total *Enterobacteriaceae* population (Guenther et al., 2010), therefore, the dilution of a positive sample with high bacterial load can lead to decrease the sensitivity of the test, and therefore, as it has been shown previously, pooling could underestimate the shedding prevalence (Arnold et al., 2011).

The higher number of gilts infected with *S. enterica* detected in MLN at slaughter compared to fecal shedding prevalence, could represent the real number of infected and potential shedders gilts in the herd. However, it is also important to note that the higher number of infected gilts and the diversity of *S. enterica* serovars (11 different) recovered from MLN, might have also been due to acquisition of new infection during transportation and lairage before slaughter, especially considering that it has been shown to be a major risk factor for *Salmonella* infection (Erdman et al., 2003; Mannion et al., 2012; Vyt et al., 2006). Along these lines our results are in agreement with Magistrali et al. (2008) that reported the presence of different *S. enterica* serovars recovered from pigs in the farm compared to pigs at slaughter, and the fact that serovars *S. bredeney*, *S. saintpaul*, *S. bovismorbificans*, also found in the current study, were the most frequently isolated from transport trucks. Although the presence of serovars other than typhimurium in the gilts may explain the low but variable level of antibodies detected in gilts of the different groups (van Winsen et al., 2001), the absence of them in the FS taken in farm, support the hypothesis of truck/lairage contamination. However, it cannot be underestimated in the present study because no environmental

samples were taken from the farm facilities to confirm their presence or not in the farm.

According to the prevalence of *Salmonella* infected gilts and the study of their offspring, *Salmonella* status showed a differential pattern according to the subpopulation, which could be related with each group of animals, generally from the same production phase, having some risk factors in common (for example pen hygiene, feed, other diseases, weight). *S. schwarzengrund* seem to be responsible for gilts infection, whereas *S. derby* was the unique serovar present in finishing pigs. Consistent with the serological profile of the offspring, although some piglets were seropositive, no pig was found positive between 35 and 90 days of age, suggesting that piglet infection is not taking place in the farrowing unit, critical point reported by Lurette et al. (2009). This is in agreement with other previous studies in which the importance of passively transferred protection against *Salmonella* to reduce piglet infection was highlighted (Hur and Lee, 2010; Kranker et al., 2003). This might be explained by the presence of both IgG and IgA in sow's colostrum, and IgA in sow's milk, and taking into account the general consensus that there is little cross-protection between serovars, the piglet protection would be specific to the *Salmonella* serovar present in the sow.

The possible passive protection of sucking piglets against the serovar present in sows seems to be in contrast to a few previous studies which have shown that it is possible to prevent *Salmonella* infection by segregated early weaning (Nietfeld et al., 1998). On the other hand, the exposure to new *Salmonella* serovars during transport or at the nursery environment, in addition to weaning stress could lead to a rapid increase in *Salmonella* prevalence in the nursery as demonstrated previously by Kranker et al. (2003). The fact that some of the gilts in the current study, which were weaned before 9 days of age and then transported to a new farm, were found positive for *Salmonella* at the sampling age provides additional support to the idea that segregated weaning might not, per se, be successful.

Another factor to consider is the lack of active humoral immune response in pigs naturally infected with some serovars of *Salmonella enterica*, reported by previous studies (Nielsen et al., 1995), which tends to increase the risk for piglets from non reactive sows (Kranker et al., 2003). The *Salmonella* antibodies titers detected in suckling piglets were lower than those reported by Proux et al. (2000), where all piglet were seropositive at the highest cut-off. In the present study just one in five were classified as positive for the lowest cutoff value ($S/P=0.25$). One possible explanation is that Proux worked in farms contaminated with *S. typhimurium* which has been demonstrated as one of the most invasive serovar, which could increase antibody titers in gilts, to be then transferred to their offspring. In our case, *S. schwarzengrund* was the main serovar isolated in gilts, and it is not considered as virulent as typhimurium, which could explain that just few piglets were seropositives with a relatively low S/P ratio.

Despite this assumption of relatively poor maternal protection, no fattening pigs were found to be infected by *Salmonella* until 90 days of age, or were seropositive until 120 days of age; these data strongly suggest that *Salmonella* infections occurred during the late fattening period, which is consistent with Silva et al. (2006). Nevertheless, the fact that environmental samples were not included in this study design could be taken as a limitation.

According to Nollet et al. (2005), despite the hormonal changes and the decreased immunity in the periparturient sows, the authors did not observe any difference in the shedding pattern of *Salmonella* in sow during this period, but interestingly they found a significant increase in the number of *Salmonella* shedding sows seven days after weaning. Finally, authors conclude that the higher number of *Salmonella* shedding sows in the mating unit compared with the farrowing unit might contribute to the maintenance of *Salmonella* infection in a pig herd. The infection dynamics of *Salmonella* in the breeding herd has been well documented by Rao et al. (2010), found that *Salmonella* infection was distributed in group of sows with a certain special distribution within the barns, where transmission occurs in short periods and generally limited to neighboring pens. All this support our results about the importance of the different herd subpopulations in *Salmonella* infection dynamics.

An extensive variety of risk factors associated with *Salmonella* infection in pig herds have been proposed by several authors, for example farm staff, contaminated feed, and the presence of insects or rodents (Cardinale et al., 2010; Funk and Gebreyes, 2004; Lo Fo Wong et al., 2002; Rostagno and Callaway, 2012). Also, previous studies have shown that incoming gilts have been found as an important factor in the epidemiology of salmonellosis in a herd, either by activation of latent infection from the farm of origin or by acquisition of infections in the new breeding herd (Davies et al. 2000; Penmetchsa et al., 2009). This is consistent with the presence of the same *S. schwarzengrund* genotype in different groups of gilts of 150 days of age in the current study, although, there was no evidence of this serovar or genotype in the fattening pigs, were a different serovar was found. This suggests that incoming gilts could be important to *Salmonella* transmission into the breeding herd, at least when they are introduced directly in the breeding barns.

5. Conclusion

In our study, in a multi-site farm with subclinical *Salmonella enterica* infections gilts introduced in different groups were found positive for *S. enterica* infection. The genetic subtypes that circulated in different subpopulations at the breeding herd, differed from those present in fattening pigs. This may be related to the serotype specific passive immunity which would protect pigs from infection by *S. enterica* strains present in sows during their stay in the farrowing facilities, and the infection in fattening pigs would be related to different sources of infection.

Conflict of interest statement

No competing financial interests exist.

Acknowledgments

Authors wish to thank Dr. M.I. Caffer and to the Instituto Nacional de Producción de Biológicos – ANLIS "Dr Carlos G. Malbrán", Buenos Aires, Argentina, for the contribution in the serotyping of *Salmonella* isolates.

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