Longitudinal study of fecal *Salmonella* shedding by sows

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Summary

Objectives: To compare fecal excretion of *Salmonella* in sows of different parities and stages of reproduction.

Materials and methods: A total of 166 sows at two farrow-to-finish farms in Italy were tested for *Salmonella* shedding at four stages of reproduction. Sows were divided into three groups: primiparous (farrowed one litter), pluriparous (two to five litters), and old sows (> 5 litters). Fecal samples were collected approximately 2 weeks before parturition (Late Gestation), 1 and 3 weeks after parturition (Postpartum One and Two), and 30 to 60 days postpartum (Postweaning). Environmental samples were collected from farrowing rooms, farrowing crates, and gestation pens before placement of sows.

Results: The prevalence of *Salmonella* was 0.6 % in Late Gestation, 1.9% in Postpartum One, 4.3% in Postpartum Two, and 26.5% in Postweaning, and 33.3% in primiparous, 28.8% in pluriparous, and 4.6% in old sows. *Salmonella* was isolated from environmental samples in farrowing rooms (8%) and gestation pens (23%). *Salmonella* serovar Muenchen and *Salmonella* serovar Typhimurium were isolated both from sows and environmental samples on Farm One, while on Farm Two, *Salmonella* serovar Choleraesuis and *Salmonella enterica* serovar 4,5,12:i- were identified in fecal samples, and *Salmonella* serovar 4,5,12:i- and *S* Typhimurium var Copenhagen were recovered from environmental samples.

Implications: Young sows are more likely to shed *Salmonella* than older animals. The postweaning period is the high-risk period for excretion of *Salmonella*. Environmental contamination and poor hygiene may play a role in the higher *Salmonella* risk in weaned sows.

Keywords: swine, *Salmonella*, sow, production cycle

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Resumen - Estudio longitudinal de excreción de *Salmonella* fecal en hembras

Objetivos: Comparar la excreción fecal de *Salmonella* en hembras de diferentes paridades y etapas de reproducción.

Materiales y métodos: Se realizaron pruebas a un total de 166 hembras en dos granjas de ciclo completo en Italia, en busca de la excreción de *Salmonella* en cuatro etapas de reproducción. Las hembras fueron divididas en tres grupos: primíparas (parieron una camada), multíparas (dos a cinco camadas) y hembras viejas (> 5 camadas). Se recolectaron muestras fecales aproximadamente 2 semanas antes del parto (Final de la Gestación), semanas 1 y 3 después del parto (Postparto Uno y Dos), y 30 a 60 días postparto (Después del destete). Se recolectaron muestras medioambientales de las salas de parto, jaulas de parto, y corrales de gestación antes de la entrada de las hembras.

Resultados: La prevalencia de *Salmonella* fue de 0.6 % al Final de la Gestación, 1.9% en el Postparto Uno, 4.3% en Postparto Dos, y 26.5% en el Postdestete, y 33.3% en primíparas, 28.8% en multíparas, y 4.6% en hembras viejas. Se aisló *Salmonella* de muestras medioambientales en salas de parto (8%) y corrales de gestación (23%). Se aislaron *Salmonella* serovar Muenchen y *Salmonella* serovar Typhimurium tanto de las hembras como de las muestras medioambientales en la Granja Uno; mientras que en la Granja Dos, se identificaron la *Salmonella* serovar Choleraesuis y la *Salmonella enterica* serovar 4,5,12:i- en muestras fecales y se recuperaron *Salmonella* serovar 4,5,12:i- y *S* Typhimurium var Copenhagen de muestras medioambientales.

Implicaciones: Las hembras jóvenes tienen más posibilidad de excretar *Salmonella* que los animales adultos. El periodo postdestete es el periodo de alto riesgo para la excreción de *Salmonella*. La contaminación medioambiental y la falta de higiene pueden jugar un papel en el mayor riesgo de *Salmonella* en hembras destetadas.

Résumé - Étude longitudinale sur l'excrétion fécale de *Salmonella* par des truies

Objectifs: Comparer l'excrétion fécale de *Salmonella* chez des truies de différentes parités et à différents moments du cycle de la reproduction.

Matériels et méthodes: Un total de 166 truies dans deux fermes de type naisseur-finisseur en Italie ont été vérifiées pour l'excrétion de *Salmonella* à quatre moments du cycle de la reproduction. Les truies ont été séparées en trois groupes: primipares (mise-bas de une portée), multipares (deux à cinq portées), et vieilles truies (> 5 portées). Des échantillons de fèces ont été prélevés environ 2 semaines avant la parturition (Fin de gestation), 1 et 3 semaines après la mise-bas (Postpartum Un et Deux), et 30 à 60

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jours postpartum (Post-sevrage). Des échantillons environnementaux ont été prélevés des chambres et cages de mise-bas et des enclos de gestation avant l'entrée des truies.

Résultats: La prévalence de *Salmonella* était de 0.6% en Fin de gestation, 1.9% à Postpartum Un, 4.3% à Postpartum Deux, et 26.5% au Post-sevrage, et de 33.3% chez les primipares, 28.8% chez les multipares, et 4.6% chez les vieilles truies. *Salmonella* sérovar Muenchen et *Salmonella* sérovar Typhimurium ont tous les deux été isolés des truies et de l'environnement sur la Ferme Un alors que sur la Ferme Deux, *Salmonella* sérovar Cholerasuis et *Salmonella enterica* sérovar 4,5,12:i- ont été identifiés dans les échantillons de fèces, et *Salmonella* sérovar 4,5,12:i- et *S* Typhimurium var Copenhagen ont été retrouvés dans des échantillons environnementaux.

Implications: Les jeunes truies sont plus susceptibles d'excréter *Salmonella* que des animaux plus âgés. La période post-sevrage est la période à risque élevé pour l'excrétion de *Salmonella*. La contamination environnementale et une mauvaise hygiène pourraient jouer un rôle dans le risque plus élevé associé à *Salmonella* chez les truies au sevrage.

Salmonella still represents one of the major agents of foodborne diseases in humans. In Europe, salmonellosis, with 151,995 cases reported in 2007, is the second most common zoonosis, after campylobacteriosis.[1] Pork, after chicken and eggs, is considered one of the most relevant sources of infection.[2] In a 2007 survey, 1.1% of fresh pork meat samples collected in Europe were positive for *Salmonella*, while occurrence of *Salmonella* in mesenteric lymph nodes of swine at slaughter was 10.3% across Europe and 16.5% in Italy.[3] With the goal of progressive reduction of salmonellosis in the European Union, several control measures were taken into consideration at different steps along the meat-production chain, since a holistic approach is required to finally reduce the bacterial load in the final products. Numerous studies have been carried out at different levels (preharvest,[4,5] transport,[6,7] slaughter,[8-10] processing and distribution[11-13]) in order to clarify the epidemiology of *Salmonella* infection and identify the most efficacious control measures. Several preharvest transmission routes have been described in pigs, and significant roles were demonstrated both for direct and indirect transmission (environmental contamination).[14] The role of sows in maintenance of the infection has been discussed. Recently, the prevalence of *Salmonella* in breeders has been evaluated as a dominating risk factor influencing the presence of positive pigs at slaughter.[15] The role of sows may be direct, with transmission to piglets, or indirect, by contamination of the environment.[15] This is particularly important where breeders and finishing pigs are housed in the same environment,[16] even though the prevalence in sows is usually lower than in finishing pigs, at least in Europe, where *Salmonella* is only occasionally detected in sow units.[17,18] This role is more relevant in farrow-to-finish farms, where cross-contamination between groups (reproduction and finishing) seems more frequent.[5,18] In these farms, another significant risk factor for *Salmonella* spreading and being maintained in the environment is represented by restocking gilts, since increased excretion of *Salmonella* has been observed in gilts after their introduction into new herds.[19] On the basis of this epidemiological data, intervention strategies in the European Union are increasingly focused on prevention, particularly in breeder farms, to prevent the risk of introducing *Salmonella* by replacement animals.[20] More recently, a risk assessment study has been carried out in Europe to assess the relative contribution of *Salmonella* infection in breeder pigs to the final prevalence in swine at slaughter, concluding that, in high-prevalence countries, a 90% reduction of breeder-pig herd prevalence could result in a reduction of approximately two-thirds in slaughter lymph-node prevalence.[15]In Italy, intervention in breeder farms seems particularly important, because a high serological prevalence of *Salmonella* infection (93.8% to 100.0%) has been reported in breeding-pig herds.[17] To better clarify the dynamic of infection in breeders, fecal samples were collected from sows reared in farrow-to-finish pig farms in central Italy to evaluate the prevalence of excretion of *Salmonella* in feces in relation to the stage of the reproductive cycle and sow parity.

Materials and methods

The non-invasive nature of the fecal sampling process did not require the study protocol to be approved by the Istituto Zooprofilattico Sperimentale Umbria Marche Ethical Committee.

Animals

Farm registries were used to randomly select 166 animals at the beginning of a period of study (December 2006 to November 2007). Of these, only 102 were actually sampled for the whole period of the study, as some subjects had died or were culled or pregnancy was not confirmed. The animals were individually ear tagged, divided into groups as reported in Table 1, and scheduled for sampling on the basis of the expected date of parturition.

Housing and management

The animals were housed on two farrow-to-finish farms in central Italy, with 450 (Farm One) and 800 sows (Farm Two). These farms had similar management systems that were typical of farms in this geographical area: external replacement, weaning at 28 to 30 days of age, growing and finishing in separate buildings, and feed supplied from commercial suppliers. The farms were managed according to the European Council (EC) legislation concerning pig welfare.[21-23] During late gestation and post weaning, the animals were managed in a continuous flow and housed in multiple pens with slatted flooring and with 20 to 30 sows per pen. Approximately 1 week before expected parturition, the sows were moved to farrowing rooms managed in an all-in, all-out system, with 10 to 20 farrowing crates per room. Cleaning and disinfection were applied only in these rooms prior to sow placement.

Sampling process

The sows were divided into parities: primiparous (first litter), pluriparous (two to five parities), and old (> 5 parities). Individual fecal samples, approximately 25 g per sample, were collected from the rectum of each animal at Late Gestation (approximately 2 weeks before parturition), Postpartum One (1 week after parturition), Postpartum Two (approximately 3 weeks after parturition), and Postweaning (30 to 60 days after parturition). The temporal relationship between the occurrence of *Salmonella* in the environment and positivity in the sows was also investigated. For this purpose,182 environmental samples were collected from farrowing rooms (n = 62) and gestation rooms (n = 120). At least five pre-moistened sponge bags (Solar-cult Pre-moistened Sponges; Solar Biologicals Inc, Ogdensburg, New York) were swabbed on surfaces (walls, floors, farrowing crates, nipple drinkers, and feeders) in each room, without identifying a standard area. A room was classified *Salmonella*-positive when at least one sample was positive for *Salmonella*.

Table 1: Proportion of sows positive for *Salmonella* by fecal culture, divided by parity group and stage of reproduction in a study in two farrow-to-finish farms in Italy*

Time of sampling	No. of sows positive/no. of sows tested (%)			
	Primiparous	Pluriparous	Old	Total
Late Gestation	1/45 (2.2)	0/52 (0.0)	0/65 (0.0)	1/162 (0.6)
Postpartum One	3/42 (7.1)	0/51 (0.0)	0/61 (0.0)	3/154 (1.9)
Postpartum Two	1/38 (2.6)	4/51 (7.8)	1/49 (2.0)	6/138 (4.3)
Postweaning	11/35 (31.4)	13/44 (29.6)	3/23 (13.0)	27/102 (26.5)

* Primiparous: first litter; Pluriparous: parities 2-5; Old: parity > 5; Late Gestation: 14 days before parturition; Postpartum One: 1 week after parturition; Postpartum Two: 20 days after parturition; Postweaning: 30-60 days post partum.

Bacterial isolation and identification

All samples were stored in sterile containers and maintained at 4°C until processed (maximum 24 hours). For *Salmonella* detection, fecal samples were processed according to the ISO method.[24] Briefly, 25 g of feces was diluted in 225 mL of buffered peptone water (Conda-Pronadisa, Madrid, Spain) used as pre-enrichment medium, and incubated for 18 hours at 37°C. Enrichment was further performed on modified semisolid Rappaport-Vassiliadis medium (Biokar Diagnostics, Pantin, France) incubated for 24 to 48 hours at 41.5°C. Suspect turbid zones were plated out for single colonies on a selective solid medium, xylose lysine deoxycolate agar (Biolife Italiana, Milan, Italy), and chromogenic RAPID *Salmonella* Agar (Bio-Rad Laboratories SRL, Segrate, Milan, Italy) and incubated at 37°C for 24 to 48 hours. *Salmonella* suspect colonies were further confirmed biochemically (Api Rapid 20E; Biomerieux Italia, Bagno a Ripoli, Florence, Italy) and serologically by polyvalent antiserum (*Salmonella* Test Serum; Siemens Healthcare Diagnostics SRL, Milan, Italy). Environmental samples were soaked in 90 mL of buffered peptone water (Conda-Pronadisa) used as a pre-enrichment medium and then processed as described. Isolates of *Salmonella* from fecal or environmental positive samples were serotyped according to the Kauffmann-White scheme. *Salmonella* Typhimurium, *Salmonella* Muenchen, and *Salmonella enterica* serovar 4,5,12:i- isolated from both the environment and the animals were further discriminated using pulsed-field gel electrophoresis (PFGE), performed according to the Salm-gene protocol.[25] Deoxyribonucleic acid restriction was performed using the XbaI enzyme (Promega Corporation, Madison, Wisconsin). Dendrogram and cluster analysis were performed using algorithms available within the BioNumerics software package version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium). The percent similarity between different chromosomal fingerprints was scored by the Dice coefficient. The unweighted pair group method with arithmetic means, with a 1.00% tolerance limit and 1.00% optimization, was used to obtain the dendrogram. Profiles differing by one or more DNA fragments were considered to be distinct patterns.

Statistical analysis

McNemar's chi-square was used to compare *Salmonella* shedding prevalence rates among sows at different times during the production cycle and among age groups. Differences in prevalence were considered significant at $P < .05$, and 95% confidence intervals (CI) were calculated. The association between the risk factors (different periods of the production cycle and age group) and outcome was determined using a prevalence ratio (PR) with 95% CI. A chi-square test was also used to compare the presence of *Salmonella* in samples from gestation pens and farrowing rooms. Statistical analysis was performed using Stata11.1 for Windows XP (Stata Corp, College Station, Texas).

Results

Overall, 6.6% of the fecal samples collected were positive for *Salmonella*, and the data stratified by parity group of animals and stage of reproductive cycle are summarised in Table 1. Prevalence ratio was significantly higher among pluriparous sows than among old sows (PR = 6.25; 95% CI, 1.91-20.44) and PR was significantly higher in primiparous sows than in old sows (PR = 7.22; 95% CI, 2.22-23.50).

The prevalence rates observed were 0.6% (95% CI, 0-1.8) in Late Gestation, 1.9% (95% CI, 0.0-4.2) in Postpartum One, 4.3% (95% CI, 0.8-7.8) in Postpartum Two, and 26.5% (95% CI, 17.7-35.2) in Postweaning. The animals in Postpartum Two and Postweaning were more likely to excrete *Salmonella* ($P < .05$) than those in Late Gestation, and sows in Postweaning were more likely to excrete *Salmonella* than those in Postpartum One and Postpartum Two ($P < .05$).

The PR calculated from prevalence at different times during the sow reproductive cycle indicates that the prevalence of infection in sows in Postpartum Two was seven times greater than that in Late Gestation sows (PR = 7.04; 95% CI, 1.17-42.46). The prevalence of infection in Postweaning sows was 42 times greater than that in Late Gestation sows (PR = 42.88; 95% CI, 14.11-130.26), 13 times greater than that in Postpartum One sows (PR =13.5; 95% CI, 5.71-31.87), and six times greater than that in Postpartum Two sows (PR = 6.1; 95% CI, 2.96-12.52).

Salmonella Muenchen and *S* Typhimurium were identified in Farm One, while *Salmonella* serovar 4,5,12:i- and *S* Choleraesuis were identified in Farm Two. Five of 62 samples from farrowing rooms (8%) and 28 of 120 samples from gestation rooms (23%) were culture-positive ($P < .05$). Different clones could not be discriminated among isolates of *Salmonella* serovar 4,5,12:i-, repeatedly isolated in Farm Two: genetic similarity ranged from 92% to 100%. The same outcome was obtained for *S* Typhimurium in Farm One, where isolates from the environment (gestation rooms) and those from sows sampled 1 week later (Postweaning) belonged to a single clone. A temporal relationship between environmental contamination and subsequent isolation of *Salmonella* from sows was also observed for *S* Muenchen, which was isolated from gestation pens and, 10 days later, from sows housed in those pens (Postweaning). Again, the isolates were indistinguishable by PFGE.

Discussion

The epidemiology of *Salmonella* infection in swine breeding stock is still not completely elucidated; however, an assessment by the

European Food Safety Authority, based on quantitative microbiological risk assessment, has recently confirmed a role of this category of animals in the transmission of *Salmonella* along the production chain.[15] The prevalence of *Salmonella* in sows is usually below 10%.[1,26] In this study, significant variation of *Salmonella* excretion by sows was observed, depending on stage of reproductive cycle and parity, with markedly higher prevalence of excretion by sows after weaning.

The increase in prevalence occurring after weaning was numerically greatest in primiparous sows and declined with increasing parity. This association of *Salmonella* excretion with parity might be explained by a different immune status in older sows, as previously described by Nollet et al[27] and Letellier et al.[4] Our observation of increased *Salmonella* prevalence after weaning is consistent with that in a previous study.[27] Moreover, in other studies, a low prevalence of *Salmonella* shedding was found in sows prior to weaning.[28,29] Several factors could explain the postweaning increase in excretion, including the greater load of *Salmonella* in the environment where the sows were housed post weaning, the stress linked to weaning, and the major reduction of intake of feed and water after weaning.[28,29] Given the magnitude of the effect, and that the highest prevalence coincided with the time when most sows would be culled and enter the food supply,[29] further research into this postweaning increase is warranted.

Our results suggest that environmental contamination could have played a major role in the epidemiology of *Salmonella* on these Italian farms. The environmental effect may be attributable to poorer management and hygiene in gestation rooms than in farrowing rooms. All-in, all-out protocols were adopted for the farrowing rooms, while gestation pens were in a continuous flow.

The role of *Salmonella* contamination of the pen and the presence of positive pigs has been investigated by other authors,[30] and it is generally accepted that the probability of infection depends on the quantity of *Salmonella* in the farm environment.[26] Moreover, in a recent study,[16] residual *Salmonella* contamination of rooms had a great impact on *Salmonella* seroprevalence over time. In some cases during this study, a temporal succession was observed between the isolation of specific serotypes from the environment and later from the sows moved into this environment. These results seem to confirm the role of the environment as the major source of infection. This is particular evident for *S* Muenchen and *S* Typhimurium, where *Salmonella* was previously isolated from the gestation rooms and, after few days, from the postweaning sows housed in those rooms. Pulsed-field gel electrophoresis analysis confirmed the clonality of *S* Typhimurium and *S* Muenchen from animals and rooms. This temporal succession was not evident for *Salmonella* serovar 4,5,12:i-, which was repeatedly isolated both from environment and animals. In conclusion, the results suggest that parity influences *Salmonella* excretion, and therefore management of replacement gilts might be of help to reduce contamination on breeding farms. In addition, biosecurity measures applied to gestation pens should be taken into major consideration to reduce *Salmonella* spreading in closed pig herds.

Implications

- Younger sows constitute a high-risk group for *Salmonella* excretion on sow farms.
- Under the conditions of this study, the postweaning period (30 to 60 days after parturition) is the period of greatest risk for *Salmonella* excretion by sows.
- Environmental contamination and poor hygiene may play a role in the increase in *Salmonella* risk seen in weaned sows.

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Conversion tables

Weights and measures conversions

Weights and measures

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^2	ft^3 to m^3	0.03
35.3 ft^3	1 m^2	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°C	°F
0	32
10	50
15.5	60
16	61
18.3	65
21.1	70
23.8	75
26.6	80
28	82
29.4	85
32.2	90
38.8	102
39.4	103
40.0	104
40.5	105
41.1	106
100	212

°F = (°C × 9/5) + 32

°C = (°F - 32) × 5/9

Conversion chart, kg to lb (approx)

Pig size	Kg	Lb
Birth	1.5 – 2.0	3.3 – 4.4
Weaning	3.5	7.7
	5	11
	10	22
Nursery	15	33
	20	44
	25	55
	30	66
Grower	45	99
	50	110
	60	132
Finisher	90	198
	100	220
	105	231
	110	242
	115	253
Sow	135	300
	300	661
Boar	360	794
	363	800

1 tonne = 1000 kg

1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne

1 ppm = 1 mg/L