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***Salmonella* in sows: a longitudinal study in farrow-to-finish pig herds**

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Abstract – Besides finishing pigs, sows are also believed to be important in the epidemiology of *Salmonella*. The study objective was to investigate the prevalence of *Salmonella* excretion in sows during an entire reproductive cycle. In 3 farrow-to-finish herds, groups of 34, 40 and 32 sows, respectively, were sampled serially. Faecal samples, environmental swabs and feed samples were taken and submitted to a qualitative *Salmonella* isolation. All isolates were characterised using RAPD and a representative number of isolates was serotyped. The prevalence of *Salmonella* excretion was < 10% during gestation, around farrowing and during lactation, but a significant increase in the number of *Salmonella* excreting sows was found in herds A ($p < 0.01$) and C ($p = 0.02$) after weaning. *S. Infantis* was the most prevalent serotype in herd A, *S. Derby* in herds B and C. Except for the *S. Infantis* group in herd A, all isolates within each group of the RAPD analysis belonged to the same serotype. Three sows in herd A and 1 sow in herd C shed different serotypes at different time points. The present results indicate that sows can maintain *Salmonella* infections in farrow-to-finish herds and that culled sows, leaving the herd after weaning, may constitute a substantial risk for contamination of their carcasses with *Salmonella*.

***Salmonella* / farrow-to-finish herds / sows / culled sows**

1. INTRODUCTION

For many years, *Salmonella* has been recognised as one of the most important foodborne pathogens [1, 2, 32] causing more than 10 000 human infections in Belgium yearly [25]. Besides eggs and poultry meat, pork is one of the most important sources of human infection [21, 36]. In order to prevent human disease due to the consumption of *Salmonella* contaminated

pork, many studies have focussed on the epidemiology and the control of *Salmonella* in finishing pigs [5, 18, 23, 34, 35]. Also many studies have focussed on the lairage and the slaughterhouse in order to prevent contamination of pig carcasses as much as possible [4, 7, 8, 31]. One of the general conclusions of these studies is that the *Salmonella* problem has to be tackled during the different steps of the production cycle, starting at the herd level.

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Many risk factors for a high *Salmonella* prevalence in pigs suggest different routes of infection of pigs. *Salmonella enterica* is known to survive well in the environment [29], and the direct and/or indirect transmission of *Salmonella* from the environment to pigs is believed to play an important role in the infection of pigs. As demonstrated in other studies [3, 10, 12], *Salmonella* shedding can also be detected in sows and the role of the sow in the direct transmission to the piglets has been investigated. However, it has never been demonstrated before what the importance of the sow is in the epidemiology and maintenance of *Salmonella* infections in farrow-to-finish pig herds. In Belgium, the majority of the pig herds are single site herds in which all production stages, from the sows (mating unit, gestation unit, farrowing units) until the finishing pigs (nursery, growing and finishing unit) are located at the same site. One can suggest that, if sows are excreting *Salmonella*, the sow unit can be continuously contaminated with *Salmonella* and thus might be an important source for (in)direct transmission of *Salmonella* infections to other animals present in the herd.

The aims of the present longitudinal study were to investigate the prevalence of *Salmonella* excretion in sows in three farrow-to-finish pig herds at different stages of one reproductive cycle, and to elucidate time-points with a higher risk for *Salmonella* excretion. Additionally, the serotype distribution and the strain diversity of isolates found in sows and in the environment are described.

2. MATERIALS AND METHODS

2.1. Study population

Three unrelated Belgian farrow-to-finish herds were included in the study. The selection criteria were a herd size between 250 and 500 sows, the use of a group management system for the sows and the presence of *Salmonella enterica* in the herd. The lat-

ter was detected during preliminary sampling and analysis for the presence of *Salmonella*.

In each herd, one group of sows with the same expected farrowing date was selected. Each group consisted of sows of different parities. From each of the sows, the following data were collected: individual sow number, breed, parity and date of farrowing. Thirty-four, 40 and 32 sows were selected in herds A, B and C, respectively.

Herd A was a two-site farrow-to-finish herd with 450 cross-bred sows raised in the herd. The average group size was 30–35 sows, which were managed in a 2-week system. Seven days before the expected farrowing date, the sows were moved to cleaned and disinfected farrowing units, which were already empty for 10 days. The piglets were weaned at 26 days of age and the sows were moved to the mating unit on the same day. Two days after artificial insemination (A.I.), inseminated sows were moved to the gestation unit, which was part of the same stable as the mating unit. Sows received three different pelleted feeds during gestation, lactation and from weaning until 2 days after insemination, respectively. All feed was purchased from the same feed company.

Herd B was a one-site farrow-to-finish herd with a herd size of 280 hybrid sows. Gilts were purchased from a commercial breeding herd. The average group size in the 3-week management system was 35–40. One week before the expected farrowing date, the sows were moved to cleaned and disinfected farrowing units. Twenty-six days after farrowing, the piglets were weaned and the sows were moved to the mating unit. After pregnancy diagnosis at ± 30 days after A.I., the sows were moved to the gestation unit. Both the mating and gestation unit were located in the same room. The sows were fed different pelleted feeds during gestation (0–9 weeks, 9–15 weeks) and early lactation (from 5 days before until 2 days after farrowing). From then on, a lactation diet (pellets) was given until insemination.

Table I. Sampling scheme of the sows (faecal and blood samples) and the environment (overshoes) in 3 Belgian farrow-to-finish herds. Thirty-four, 40 and 32 sows in herds A, B and C, respectively were serially sampled throughout one reproductive cycle. An “x” means that the respective sample was taken at that time point.

	Time point Days to / after farrowing	Sample type		
		Faeces	Blood	Environment (overshoes)
Late gestation	–37	x	x	
	–7	x		x (farrowing unit)
Farrowing	–2	x		
	4	x		
	7	x	x	
Weaning	25	x	x	x (mating unit)
	34	x		
Gestation	64	x	x	x (gestation unit)

All feed types were purchased from the same feed company.

Herd C was a one-site farrow-to-finish herd with 500 hybrid sows and a traditional one-week management system was applied. Gilts were purchased from a commercial breeding herd. Because the weekly farrowing group size was only 15–20 sows, two consecutive groups were included in the study. The sows were moved to the cleaned and disinfected farrowing unit 7 days before the expected farrowing date and they stayed there until 26 days after farrowing. At the day of weaning, all sows were moved to the mating unit. After pregnancy diagnosis, approximately 25 days after A.I., the sows were moved to the gestation unit. Two different pelleted feeds from the same feed company were provided during gestation and lactation.

In each herd an all in/all out system in the farrowing, nursery and finishing units was used. All sows were housed individually in each unit, on fully slatted floors in the mating and gestation unit and on partially slatted floors in the farrowing unit. All sows were vaccinated against Aujeszky disease (pseudorabies), *E. coli* and atrophic rhinitis. Additional vaccination against porcine reproductive and respiratory syndrome was

applied in herds A and C, against porcine parvovirus and erysipelas in herds B and C, against influenza in herd B and against *Mycoplasma hyopneumoniae* in herd C. The sows were treated with anthelmintics twice, once and three times a year in herds A, B and C, respectively. All herds were free from the Aujeszky disease wild virus. No antimicrobial growth promoters, antibiotics or organic acids were incorporated in the feed or drinking water of any of the herds.

2.2. Collection of the samples

The sampling scheme is shown in Table I. The blood samples were taken by puncture of the jugular vein (Bovi-Vet disposable veterinary injection needles, 2.1 × 80 mm, Kruuse, Marslev, Denmark). Faecal samples were collected rectally and further processed individually. Swabs of the environment in different units (gestation unit, farrowing unit, mating unit) were collected by means of overshoes (non-woven white overshoes CEMH01038, Novolab, Geraardsbergen, Belgium) the day before the sows were moved to that unit. In each unit, five zones with a higher probability of oral contact by the sows were selected. During each visit, feed samples were taken in the feed storage room.

2.3. Sample analyses

2.3.1. Blood samples

The blood samples were centrifuged at $1\,400 \times g$ for 10 min and the serum samples were stored at $-20\text{ }^{\circ}\text{C}$ until further analysis. They were analysed using a commercial indirect mix-ELISA, according to the recommendations of the manufacturer (Herd-Check Swine *Salmonella* Antibody Test Kit, Idexx Laboratories, Inc., Maine, USA). Optical densities were measured and expressed as a percentage of a known positive control (OD%). The samples were considered positive if the OD% was equal to or higher than 10%.

2.3.2. Faecal and feed samples and environmental swabs

Salmonella was isolated from faecal and feed samples and environmental swabs using a qualitative isolation method. Briefly, faecal and feed samples were weighed and diluted 1:9 (w/w) with Buffered Pepton Water (BPW). Approximately 225 mL of BPW was added to the environmental swabs until they were submerged. All samples were incubated for pre-enrichment during 16–20 h at $37\text{ }^{\circ}\text{C}$, followed by selective enrichment on Modified Semisolid Rappaport-Vassiliadis (MSRV) agar plates for 24 h at $42\text{ }^{\circ}\text{C}$. If migration zones were present on the MSRV plates, a loopful of the culture edge of the migration zones was streaked on a Xylose Lysine Desoxycholate (XLD) agar plate and the plates were incubated for 24 h at $37\text{ }^{\circ}\text{C}$. XLD plates were examined for the presence of typical colonies. After biochemical confirmation of suspected colonies, one colony of each *Salmonella* positive identified sample was randomly picked and subcultured on Tryptone Soya Broth (TSB) (Oxoid, CM131) and stored at $-20\text{ }^{\circ}\text{C}$ until further examination.

2.4. Characterisation of the isolates

The selected isolates were grown in Tryptone Soya Broth (TSB) (Oxoid,

CM0129) at $37\text{ }^{\circ}\text{C}$ for 24 h in an aerobic atmosphere. Template DNA was extracted from the bacterial cells using the AquaPure Genomic DNA Kit (Bio-rad, 732-6340) according to the manufacturer's instructions. A 5 μL volume of each DNA preparation was size separated by electrophoresis in order to analyse the integrity of the DNA extracted. The concentration of the DNA templates was determined spectrophotometrically at A_{260} and adjusted to a concentration of 25 $\text{ng}/\mu\text{L}$. The isolates were genotyped by the Random Amplified Polymorphic DNA (RAPD) assay using each of three primers 23L (5'-CCGAAGCTGC-3'), OPB17 (5'-AGGGAACGAG-3') and P1254 (5'-CCGCAGCCAA-3') [19]. DNA extractions of isolates from the same herd were grouped and analysed in the same PCR run to decrease fingerprint heterogeneity due to PCR-linked variations. All PCR amplifications were performed using 1 μL of DNA template, 25 pmol of primer with Ready-To-Go RAPD Analysis Beads (Amersham Pharmacia Biotech, Uppsala, Sweden), containing premixed, pre-dispensed *AmpliTaq* DNA polymerase, as well as all the necessary buffer ingredients and nucleotides. The cycling parameters were as follows: denaturing at $95\text{ }^{\circ}\text{C}$ for 30 s, annealing at $36\text{ }^{\circ}\text{C}$ for 1 min, and extension at $72\text{ }^{\circ}\text{C}$ for 2 min, for a total of 45 cycles. Prior to cycling, the samples were heated at $94\text{ }^{\circ}\text{C}$ for 5 min. All amplifications were performed in 25 μL volumes. In order to identify patterns of genetic relatedness among isolates originating from the same herd, computer based normalisation and interpolation of the DNA profiles, and numerical analysis using the Pearson product moment correlation coefficient with 1% position tolerance, were performed using the GelCompar 4.2 software package (Applied Maths, Belgium) for each RAPD assay. A dendrogram was constructed for the composite 3-primer RAPD assay using the unweighted pair group linkage analysis method. For convenience, the correlation level was expressed as a percentage similarity. DNA patterns that differed in one or

more DNA fragments were considered to represent different types. Whenever type differences relied on only one band, a repeat analysis was performed (including a repeat DNA extraction) to confirm the reproducibility of the fingerprint.

At least two representatives of each RAPD type were further characterised by Pulsed Field Gel Electrophoresis (PFGE) using *Xba*, *Spe* and *Not I* as restriction enzymes (Invitrogen, Paisley, UK). These isolates were grown for 18 h at 37 °C in TSB. Following incubation, the suspensions were adjusted to an OD₆₀₀ value of 0.8. For preparing the plugs, 200 µL 1.4% InCert agarose (Biowhittaker Molecular Applications, Rockland, USA) was mixed with 200 µL of the cell suspension. The plugs were lysed for 2 h at 37 °C in 1 M NaCl, 10 mM Tris-HCl pH8, 200 mM Na₂EDTA, 0.5 % N-lauroylsarcosine, 0.2% deoxycholic acid, 2 µL/mL RNase and 1 mg/mL lysozyme (Roche Diagnostics GmbH, Mannheim, Germany) and incubated for 18 h at 56 °C in 0.5M Na₂EDTA pH9, 1% N-lauroylsarcosine and 0.65 mg/mL proteinase K (Roche Diagnostics GmbH, Mannheim, Germany). The plugs were rinsed with ddH₂O, treated twice with TE containing 100mM phenyl-methyl-sulphonyl fluoride, rinsed with ddH₂O and rinsed twice with TE. A plug slice of each isolate was digested for 4 h with 40 U of *Xba*, *Spe*, and *Not I* each. DNA fragments were separated by Chefmapper in a 1% Seakem Agarose (Biowhittaker Molecular Applications, Rockland, USA) gel. The running conditions were 6 V/cm at 14 °C in 0.5 × TBE buffer for 25 h with a ramping time from 4 to 40 s for *Xba* and *Spe* and from 2 to 12 s for *Not I*. The gel images were analysed as described above.

2.5. Serotyping

From each RAPD type, at least two isolates were sent to the Belgian reference laboratory (Veterinary and Agrochemical Research Centre, Ukkel, Belgium) for serotyping following the Kaufmann-White scheme [26].

2.6. Statistical analyses

The number of sows with at least one positive faecal isolation and with at least one positive serological result, and the prevalence of different serotypes and genotypes were evaluated descriptively. A Mc Nemar test was used to compare the number of shedding sows and the number of seropositive sows, both at different time points. The comparison between the mean OD% of the serum samples at different time points was done using a one-way ANOVA with the post hoc Tuckey test. A univariate logistic regression model was used to compare the number of shedding sows per parity, with 3 categories of parities: 1st parity sows (sows in first gestation), 2–5 parities, > 5 parities. First parity sows were suspected to be more susceptible to *Salmonella* infections and therefore considered as a separate group in the analysis. The limit between the two other categories was set arbitrarily at the 5th parity, to obtain groups of similar size. All statistical analyses were performed using SPSS 11.0.

The mean OD% of the serum samples in the three categories of parities were compared in a linear mixed model using the sow nested in herd as the random factor and an autoregressive correlation structure of the 1st order (S plus).

3. RESULTS

3.1. Bacteriological results

In herd A, there were 20/34 sows (59%) with at least 1 *Salmonella* culture positive faecal sample. Six/40 (15%) and 12/32 sows (38%) were at least once *Salmonella* culture positive in herds B and C, respectively. Two sows in herd A were found *Salmonella* culture positive at two different time points, two sows at three different time points and one sow was found *Salmonella* culture positive at six out of the nine samplings. In herd B, none of the sows was more than once *Salmonella* culture positive.

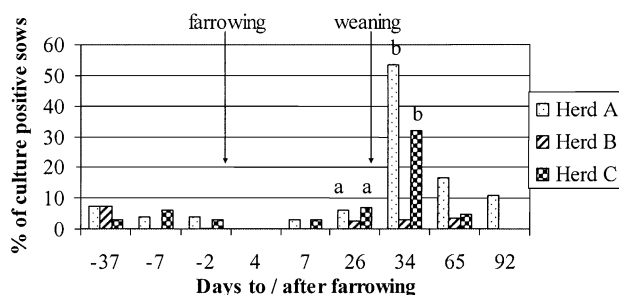


Figure 1. The percentage of *Salmonella* culture positive sows at different time points based on faecal sampling in 3 Belgian farrow-to-finish herds. The number of *Salmonella* shedding sows the day before weaning (day 26) and 7 days after weaning (day 34) were compared with each other. Data with different superscripts are significantly different from each other.

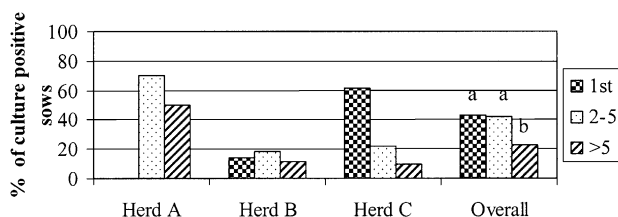


Figure 2. The percentage of sows in each parity group with at least one positive *Salmonella* isolation during the study period for 3 Belgian farrow-to-finish herds. Data with different superscripts are significantly different from each other ($p < 0.01$).

In herd C, one sow was found *Salmonella* culture positive twice and 2 sows were found *Salmonella* culture positive three times. The percentage of *Salmonella* shedding sows at different time points is shown in Figure 1. During late gestation, around farrowing and during lactation, the prevalence of *Salmonella* shedding was lower than 10% in all herds. The increase at day 34 after farrowing, or 7 days after weaning, in comparison with the day before weaning was found to be significant in herd A ($p < 0.01$) and C ($p = 0.04$). In herd B, no significant increase was observed ($p = 1.00$).

The percentage of *Salmonella* shedding sows per parity is given in Figure 2. When taking all sows from the 3 herds together, the number of shedding sows in the group with more than five parities (9 sows of the

39) was significantly lower than in the other groups (9 sows of the 21 and 19 sows of the 45 in the first parity group and the group with 2–5 parities, respectively) ($p < 0.01$).

None of the feed samples was found to be culture positive. None of the swabs taken in the farrowing units was found positive. In herd A, one swab was positive in the gestation unit. All swabs collected in the gestation unit, and three of the five taken in the mating unit were *Salmonella* culture positive in herd B. In herd C, no *Salmonella* could be detected in the environmental swabs.

3.2. Serological results

In herd A, all 34/34 (100%) sows were at least once seropositive. In herds B and C,

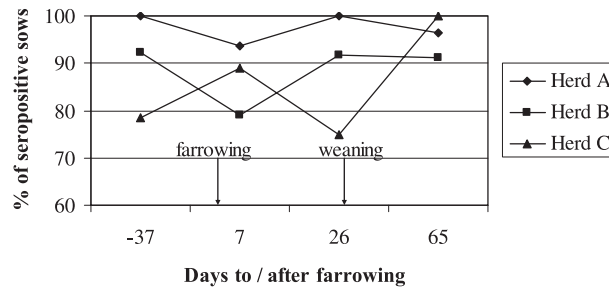


Figure 3. The percentage of *Salmonella* seropositive sows at different time points and for the different herds. In herds A, B and C, 34, 40 and 32 sows were included, respectively. No significant differences could be demonstrated between the different time points in any of the herds.

39/40 (97.5%) and 30/32 (93.8%) sows had at least one *Salmonella* serological positive result during the entire study period. The percentage of sows that was seropositive at the different time points is shown in Figure 3. No significant differences were found in the number of seropositive sows at the different time points ($p > 0.05$). There was no significant difference between the mean OD% of the different categories of parities ($p = 0.17$).

3.3. Characterisation of the isolates

The results of the characterisation and the serotyping of the isolates are shown in Table II. In all herds, cluster analysis of the composite 3-primer RAPD assay resulted in clear delineation of the groups of isolates. Pearson correlation coefficients of 94% and higher were obtained for isolates with identical or slightly different fingerprints. Pearson correlation coefficients lower than 80% were obtained with isolates that differed in three or more fragments in at least one RAPD assay.

In herd A, three groups of isolates were delineated, which corresponded with three serotypes (Tab. II). Within each group, all isolates belonged to the same serotype. Only within the *S. Infantis* group, genotypic differences were observed. One of the 16 isolates slightly differed in all three RAPD fingerprints as well as in PFGE analysis. This isolate represented another strain.

Within the other 15 *S. Infantis* isolates with identical RAPD and PFGE profiles, a small difference in only one fragment of 1100 bp was found and was obtained with the RAPD primer 23L. Three of the five sows with recurrent *Salmonella* positive isolations shed different serotypes at different time points. An *S. Infantis* strain was isolated from the faeces of the same sow at different time points only once, but all strains were genetically identical. The isolate from the environmental swab taken in the gestation unit was serotyped as *S. Derby*, and was genotypically identical to the *S. Derby* strain isolated from the sows.

In herd B, also three groups of isolates were delineated, representing three serotypes, but no different genotypes were found within a group (Tab. II). Typing based on genotypic differences was not able to distinguish the phenotypic difference of the two *S. Typhimurium* isolates, respectively *S. Typhimurium* O5- and O5+. The eight environmental isolates were characterised as one strain, and serotyped as *S. Infantis*. This strain was not isolated from the faeces of the sows.

In herd C, one *S. Derby* strain was isolated from 15 faecal samples from 11 sows. One of those sows shed an *S. Typhimurium* O5+ strain at another time point. One *S. Livingstone* was isolated from another sow (Tab. II).

Table II. The results of serotyping and genetic characterisation of *Salmonella* isolated in faecal samples in sows taken at different time points during one reproductive cycle in 3 Belgian farrow-to-finish herds.

Days to / after farrowing	Number of sows positive / number of sows sampled	<i>Salmonella</i> serotype (N)	Genotype ^a (N)
Herd A			
-37	2/34	<i>S. Derby</i> (2)	D1
-7	1/34	<i>S. Derby</i> (1)	D1
-2	1/34	<i>S. Derby</i> (1)	D1
4	0/34	–	–
7	1/34	<i>S. Derby</i> (1)	D1
25	2/34	<i>S. Derby</i> (1) <i>S. Infantis</i> (1)	D1 I1
34	18/31	<i>S. Infantis</i> (11)	I1 (10) I2 (1)
64	5/31	<i>S. Infantis</i> (2) <i>S. Goldcoast</i> (3)	I1 G1
92	3/31	<i>S. Infantis</i> (2) <i>S. Derby</i> (1)	I1 D1
Herd B			
-37	3/40	<i>S. Derby</i> (3)	D2
-7	0/40	–	–
-2	0/40	–	–
4	0/40	–	–
7	0/40	–	–
25	1/40	<i>S. Goldcoast</i> (1)	G2
34	1/36	<i>S. Typhimurium</i> O5– (1)	T1
64	1/36	<i>S. Typhimurium</i> O5+ (1)	T1
92	0/36	–	–
Herd C			
-37	2/32	<i>S. Derby</i> (1) <i>S. Livingstone</i> (1)	D3 L1
-7	3/32	<i>S. Derby</i> (2) <i>S. Typhimurium</i> O5+ (1)	D3 T2
-2	0/32	–	–
4	0/32	–	–
7	1/32	<i>S. Derby</i> (1)	D3
25	1/32	<i>S. Derby</i> (1)	D3
34	9/26	<i>S. Derby</i> (9)	D3
64	0/26	–	–
92	1/26	<i>S. Derby</i> (1)	D3

^a The genotypes are coded with a letter followed by a number. The letter corresponds to the first letter of the serotype; all genotypes within a serotype are numbered consecutively.

No differences were observed between the serotypes or genotypes found in the sows of different categories of parities.

4. DISCUSSION

Most intervention measures for reducing the prevalence of *Salmonella* in pig herds are focussing on finishing pigs. As generally accepted, pigs become orally infected with *Salmonella* [11, 30], and the major sources are a contaminated environment and direct contact with *Salmonella* shedding pen-mates [30]. Since sows and finishing pigs are present at the same site in farrow-to-finish herds, it seems acceptable that sows may also play a role in the maintenance of *Salmonella* in a herd. In the present study, one group of 30–40 sows in each of three Belgian farrow-to-finish herds was serially sampled with emphasis on the farrowing and the weaning period. These 2 periods were highlighted because farrowing [16, 33] and weaning [27] are known to be associated with stress. Due to stress, immunity is decreased [33] and consequently changes in the shedding pattern of *Salmonella* have been reported before in feeder calves [9] and in finishing pigs [6].

Because only 3 herds were examined in the present study, one should be aware of making conclusions for all Belgian sow herds. However, the herds included in the study were typical Belgian farrow-to-finish herds, regarding herd size, housing, feeding and management of the sows and could therefore give a reasonable indication about the incidence of *Salmonella* during the production cycle in sows in general.

During late gestation, around farrowing and during lactation, the prevalence of *Salmonella* shedding in the present study was lower than 10% in all herds. Comparable low prevalences were seen in other studies [12, 17, 28]. Despite the hormonal changes like the rise in plasma cortisol [16], and the decreased immunity in the periparturient sow [33], a change in the shedding pattern of *Salmonella* in sows could not be demonstrated during these periods.

Seven days after weaning, a significant increase in the number of *Salmonella* shedding sows was demonstrated in two of the three herds. During this period, different hormonal changes take place in the sow resulting in follicular growth, ovulation and oestrus behaviour [15]. Simultaneously, a rise in adrenocorticotrope hormone, indicating stress, was seen. Due to stress, sows are more susceptible to new *Salmonella* infections and carrier sows, harbouring the pathogen in the intestines or the mesenteric lymph nodes, are more likely to start shedding the pathogen [30].

Some authors explain the low prevalence of *Salmonella* in the farrowing unit by environmental factors such as housing, feeding and water delivery [12]. Also in the present study, housing and feeding conditions were different between the farrowing and the gestation unit. All sows were housed in individual farrowing crates, in which they had no contact with neighbouring sows. The source of the drinking water was the same for the entire herd. In the farrowing unit as well as in the mating and the gestation unit, individual drinking nipples and troughs were provided. However, the troughs in the farrowing units were empty, cleaned and disinfected which is in contradiction with the troughs in the mating unit and the gestation unit. In the latter units, the troughs were not cleaned or disinfected and remaining feed could have been present, being a possible source for *Salmonella* infections. Given the low incidence of *Salmonella* during the farrowing and the lactation period in the present study, the hygienic measures taken in the farrowing units might have been effective in the control of *Salmonella* in sows. Weaned sows were moved to the mating unit which was not cleaned or disinfected, suggesting that the environment of the mating unit could have been contaminated with *Salmonella*. However, only the environmental swabs taken in the mating unit in herd B were positive for the presence of *Salmonella* but they were other serotypes than those found in the sows. The latter is probably due to the low number of

Salmonella culture positive sows. Since faecal samples have a low sensitivity for detecting infected pigs [14], it is possible that some of the sows were infected with *S. Infantis* but not shedding at the time of sampling. In herds A and C, only 1 and none of the swabs, respectively, were *Salmonella* positive. The *S. Derby* isolate found in the environment in herd A was genetically identical to the isolates found in the sows. Despite the difficulties to demonstrate the link between the excretion of *Salmonella* in sows and the contamination of the environment, we still believe that the higher number of *Salmonella* shedding sows in the mating unit compared with the farrowing unit may contribute to the maintenance of *Salmonella* infections in pig herds. Based on the findings that multiple serotypes were isolated from the same sow at different occasions, one can presume that re-infections occurred when the sows were moved to another unit and consequently, that sows seemed to remain sensitive to new infections. If the pig farmer uses the same materials (shovels, brooms, buckets, etc.) and wears the same boots and clothes in the sow units and the nursery or finishing units, indirect transmission of *Salmonella* from sows to piglets and finishing pigs is possible [10]. However, these results should be interpreted with care given the limited number of positive samples, especially in herd B.

Although the majority of the sows in the present study had antibodies against *Salmonella*, they still were potential *Salmonella* shedders. It is known that humoral immunity is less effective against facultatively intracellular bacteria, such as *Salmonella* [13]. *Salmonella enterica* can survive and multiply within the macrophages, where they cannot be reached by antibodies [13, 37].

The calculation of discrepancies between the serological and the bacteriological results was not the aim of the present study, but has been described in a previous paper [24]. Those results showed that the correlation between both diagnostic tests was weak, particularly at the individual level,

and that the serological reaction depended on the serotype isolated. In the present study, the incidence of shedding was rather low apart from the increase after weaning in 2 herds. By contrast, 98% of the sows did seroconvert, and the number of seropositive sows did not vary significantly between the different time points. Since no seroconversion was observed, it is difficult to conclude whether the serological result was a reaction on the *Salmonella* serotype isolated in that sow during the study period or whether the antibodies were already present before the start of the study.

Given the fact that in Belgium, about 11×10^6 pigs of which 250 000 are culled sows are slaughtered on a yearly base [22], the increase of *Salmonella* shedding in sows after weaning may not be neglected. Culled sows generally leave for slaughtering shortly after weaning and thus are at a moment of high risk for *Salmonella* shedding, as seen in the present results. During transport to the slaughterhouse, stress can even increase the number of *Salmonella* shedding animals [6, 20]. If these sows are slaughtered, the risk for contamination of the carcasses may not be neglected. Also the cross-sectional study by Davies et al. [10] has suggested that culled breeding stock may be an important source for food-borne infections. Although the dry sow unit has been seen before as the unit with the highest prevalence of *Salmonella* excretion¹, the period of risk for a high incidence in *Salmonella* shedding shortly after weaning has never been demonstrated before in a longitudinal study, to our knowledge.

No significant differences were found between the mean OD% in sows from different parities. However, the number of *Salmonella* shedding sows was significantly lower in the group of sows with more than five parities in comparison to the younger sows. Older sows are probably more

¹ Van Schie F.W., Some epidemiological and nutritional aspects of asymptomatic *Salmonella* infections in pigs, Ph.D. thesis, Utrecht, Chapter 3, 1987.

immune against *Salmonella* infections and might be less susceptible to stress than younger sows. Despite the fact that the first parity sows were introduced in the breeding population only 5 months before the start of the study, no significant difference in the number of shedding animals was seen between this group and the group of 2–5 parities.

Although the link between the excretion of *Salmonella* in sows and the contamination of the environment is hard to demonstrate, the present study shows that sows may play an important role in the maintenance of *Salmonella* infections in farrow-to-finish herds. Due to stress and housing conditions, sows are susceptible to new infection cycles after weaning resulting in a rise in the number of *Salmonella* excreting sows. The role of the sow in the transmission of *Salmonella* to piglets is the subject of current research at the author's department. If culled sows are sent to the slaughterhouse shortly after weaning, as is commonly practised, they form a substantial risk for contamination of carcasses. Intervention measures to reduce the prevalence of *Salmonella* are not only necessary in finishing pigs but should also focus on sows.

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REFERENCES

- [1] Altekruse S.F., Cohen M.L., Swerdlow D.L., Emerging foodborne diseases, *Emerg. Infect. Dis.* 3 (1997) 285–293.
- [2] Baird-Parker A.C., Foodborne Salmonellosis, *Lancet* 336 (1990) 1231–1235.
- [3] Beloeil P.A., Chauvin C., Proux K., Rose N., Queguiner S., Eveno E., Houdayer C., Rose V., Fravallo P., Madec F., Longitudinal serological responses to *Salmonella enterica* of growing pigs in a subclinically infected herd, *Prev. Vet. Med.* 60 (2003) 207–226.
- [4] Beloeil P.A., Chauvin C., Proux K., Madec F., Fravallo Ph., Alioum A., Impact of the *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter, *Vet. Res.* 35 (2004) 513–530.
- [5] Beloeil P.A., Fravallo P., Fablet C., Jolly J.P., Eveno E., Hascoet Y., Chauvin C., Salvat G., Madec F., Risk factors for *Salmonella enterica* subsp. *enterica* shedding by market-age pigs in French farrow-to-finish herds, *Prev. Vet. Med.* 63 (2004) 103–120.
- [6] Berends B.R., Urlings H.A.P., Snijders J.M.A., Van Knapen F., Identification and quantification of risk factors in animal management and transport regarding *Salmonella* in pigs, *Int. J. Food Microbiol.* 30 (1996) 37–53.
- [7] Berends B.R., van Knapen F., Snijders J.M.A., Mossel D.A.A., Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses, *Int. J. Food Microbiol.* 36 (1997) 199–206.
- [8] Botteldoorn N., Heyndrickx M., Rijpens N., Grijspeerdt K., Herman L., *Salmonella* on pig carcasses: positive pigs and cross contamination in the slaughterhouse, *J. Appl. Microbiol.* 95 (2003) 891–903.
- [9] Corrier D.E., Purdy C.W., De Loach J.R., Effects of marketing stress on fecal excretion of *Salmonella* spp. in feeder calves, *Am. J. Vet. Res.* 51 (1990) 866–869.
- [10] Davies P.R., Bovee F.G.E.M., Funk J.A., Morrow W.E.M., Jones F.T., Deen J., Isolation of *Salmonella* serotypes from feces of pigs raised in a multiple-site production system, *J. Am. Vet. Med. Assoc.* 212 (1998) 1925–1929.
- [11] Fedorka-Cray P.J., Kelley L.C., Stabel T.J., Gray J.T., Laufer J.A., Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine, *Infect. Immun.* 63 (1995) 2658–2664.
- [12] Funk J.A., Davies P.R., Nichols M.A., Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems, *Vet. Microbiol.* 83 (2001) 45–60.
- [13] Haesebrouck F., Pasmans F., Chiers K., Maes D., Ducatelle R., Decostere A., Efficacy of vaccines against bacterial diseases in swine: what can we expect? *Vet. Microbiol.* 100 (2004) 255–268.
- [14] Hurd H.S., Mc Kean J.D., Griffith R.D., Rostagno M.H., Estimation of the *Salmonella enterica* prevalence in finishing swine, *Epidemiol. Infect.* 132 (2003) 127–135.
- [15] Hein K.G., Allrich R.D., Influence of exogenous adrenocorticotrophic hormone on estrus behaviour in cattle, *J. Anim. Sci.* 70 (1992) 243–247.

- [16] Jarvis S., Lawrence A.B., McLean K.A., Chirnside J., Deans L.A., Calvert S.K., The effect of environment on plasma cortisol and -endorphin in the parturient pig and the involvement of endogenous opioids, *Anim. Reprod. Sci.* 52 (1998) 139–151.
- [17] Kranker S., Alban L., Boes J., Dahl J., Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds, *J. Clin. Microbiol.* 41 (2003) 2282–2288.
- [18] Leontides L.S., Grafanakis E., Genigeorgis C., Factors associated with the serological prevalence of *Salmonella enterica* in Greek finishing swineherds, *Epidemiol. Infect.* 131 (2003) 599–606.
- [19] Lin A.W., Usera M.A., Barrett T.J., Goldsby R.A., Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*, *J. Clin. Microbiol.* 34 (1996) 870–876.
- [20] Mc Kean J.D., Hurd H.S., Larsen S., Rostagno M., Griffith R., Wesley I., Impact of commercial pre-harvest processes on the prevalence of *Salmonella enterica* in cull sows, *Berl. Münch. Tierärztl. Wschr.* 114 (2001) 353–355.
- [21] Mousing J., Thode Jensen P., Halgaard C., Bager F., Feld N., Nielsen B., Nielsen J.P., Bech-Nielsen S., Nation-wide *Salmonella enterica* surveillance and control in Danish slaughter swine herds, *Prev. Vet. Med.* 29 (1997) 247–261.
- [22] NIS, Nationaal Instituut voor de Statistiek, [Online] (2004) http://statbel.fgov.be/figures/d51_nl.asp [Consulted 22 September 2004].
- [23] Nollet N., Maes D., De Zutter L., Duchateau L., Houf K., Huysmans K., Imberechts H., Geers R., de Kruif A., Van Hoof J., Risk factors for the herd-level bacteriologic prevalence of *Salmonella* in Belgian slaughter pigs, *Prev. Vet. Med.* 65 (2004) 63–75.
- [24] Nollet N., Maes D., Duchateau L., Hautekiet V., Houf K., Van Hoof J., De Zutter L., de Kruif A., Geers R., Discrepancies between the isolation of *Salmonella* from mesenteric lymph nodes and the results of serological screening in slaughter pigs, *Vet. Res.* 36 (2005) 545–555.
- [25] NRRS – National Reference Centre for *Salmonella* and *Shigella*. Annual report on *Salmonella* and *Shigella* in Belgium in 2003, Institute of Public Health. [Online] (2004) http://www.iph.fgov.be/bacterio/iframes/rapports/SalmShig_2003_NL.pdf [Consulted 23 September 2004].
- [26] Popoff M.Y., Le Minor L., Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France, 1992.
- [27] Roth J.A., The immune system, in: D'Allaire S. (Ed.), *Diseases of swine*, Iowa State University Press, Ames, Iowa, USA, 1999, pp. 799–820.
- [28] Rowe T.A., Leonard F.C., Kelly G., Lynch P.B., Egan J., Quirke A.M., Quinn P.J., *Salmonella* serotypes present on a sample of Irish pig farms, *Vet. Rec.* 153 (2003) 453–456.
- [29] Sandvang D., Jensen L.B., Baggesen D.L., Baloda S.B., Persistence of a *Salmonella enterica* serotype Typhimurium clone in Danish pig production units and farmhouse environment studied by pulsed field gel electrophoresis, *FEMS Microbiol. Lett.* 187 (2000) 21–25.
- [30] Schwartz K.J., Salmonellosis, in: Taylor D.J. (Ed.), *Diseases of swine*, Iowa State University Press, Ames, Iowa, USA, 1999, pp. 535–551.
- [31] Swanenburg M., Berends B.R., Urlings H.A.P., Snijders J.M.A., van Knapen F., Epidemiological investigations into sources of *Salmonella* contamination of pork, *Berl. Münch. Tierärztl. Wschr.* 114 (2001) 356–359.
- [32] Tauxe R.V., Emerging foodborne diseases: an evolving public health challenge, *Emerg. Infect. Dis.* 3 (1997) 425–434.
- [33] Van de Ligt J.L.G., Lindemann M.D., Harmon R.J., Monegue H.J., Cromwell G.L., Effect of chromium tripicolinate supplementation on porcine immune response during the periparturient and neonatal period, *J. Anim. Sci.* 80 (2002) 456–466.
- [34] Van der Wolf P.J., Bongers J.H., Elbers A.R.W., Franssen F.M.M.C., Hunneman W.A., van Exsel A.C.A., Tielen M.J.M., *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection, *Vet. Microbiol.* 67 (1999) 263–275.
- [35] Van der Wolf P.J., Wolbers W.B., Elbers A.R.W., van der Heijden H.M.J.F., Koppen J.M.C.C., Hunneman W.A., van Schie F.W., Tielen M.J.M., Herd level husbandry factors associated with the serological *Salmonella* prevalence in finishing pig herds in The Netherlands, *Vet. Microbiol.* 78 (2001) 205–219.
- [36] Van Loock F., Ducoffre G., Dumont J.M., Libotte-Chasseur M.L., Imberechts H., Gouffaux M., Houins-Roulet J., Lamsens G., De Schrijver K., Bin N., Moreau A., De Zutter L., Daube G., Analysis of foodborne disease in Belgium in 1997, *Acta Clin. Belg.* 55 (2000) 300–306.
- [37] Wick M.J., Living in the danger zone: innate immunity to *Salmonella*, *Curr. Opin. Microbiol.* 7 (2004) 51–57.