TRANSMISSION OF *SALMONELLA* FROM SOWS TO PIGLETS: A LONGITUDINAL STUDY

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Abstract The study objective was to investigate the probability of transmission of *Salmonella* from sows to their offspring. In each of 3 farrow-to-finish herds (A, B and C), one cohort of sows (n=34, n=40, n=32, respectively), together with 3 piglets of their offspring (n=102, n=120, n=96, respectively) were selected. Individual faecal and blood samples were taken from the sows during late gestation and lactation, and from the piglets from weaning until slaughter. Blood samples were analysed in an indirect mix-ELISA detecting *Salmonella* antibodies. Faecal samples were submitted to a qualitative *Salmonella* isolation. Isolates were characterised using RAPD and PFGE. The direct role of the sow in the transmission of *Salmonella* to her offspring was expressed by means of risk ratios (RR).

Direct transmission from the sows to the piglets could not be demonstrated. The similarities between the isolates found in the sows and those during the nursery and finishing period suggest indirect transmission.

Introduction To prevent human salmonellosis due to the contamination of pork, intervention measures are to be taken in the pork production chain, starting with the prevention of *Salmonella* infection at the herd level (Berends *et al.*, 1998; Swanenburg *et al.*, 2001).

The most common route of infection is the oral-faecal route (Fedorka-Cray *et al.*, 1994; Schwartz *et al.*, 1999) and pigs can get infected by contaminated feed, contact with infected penmates or through a contaminated environment. As the majority of pig herds in Belgium are single site herds in which all production stages (from the sow unit to the finishing pig unit) are located at the same site, also sows might be an important source for (in)direct transmission of *Salmonella* infection to other animals in the herd. *Salmonella* shedding in sows has been investigated in different longitudinal studies (Funk *et al.*, 2001; Kranker *et al.*, 2003; Nollet *et al.*, 2005). Comparable results were achieved in these studies, with a low (<10%) prevalence of *Salmonella* shedding during late gestation, around farrowing and during lactation. In the study by Nollet *et al.* (2005), a significant increase in the proportion of shedding sows was demonstrated after weaning, which can be explained by the stress of weaning. Another reason may be that at weaning, sows are moved to the mating room which is in most herds only rarely cleaned and thus is supposed to be *Salmonella* contaminated.

The aim of the present study was to investigate the role of the sow in the direct and indirect transmission of *Salmonella* to her offspring.

Materials and Methods Three unrelated farrow-to-finish herds were included in the study. In each herd, one group of sows with the same expected farrowing date was selected. From every sow, three piglets of their offspring were randomly selected. Thirty-four, 40 and 32 sows and 102, 120 and 96 piglets were selected in herd A, B and C, respectively. Herd data are described in detail in Nollet *et al.* (2005).

The sampling scheme is shown in Table 1. Blood samples were taken by puncture of the jugular vein. Faecal samples were collected rectally and further processed individually.
The blood samples were centrifuged and the serum was analysed in an indirect mix-ELISA following the recommendations of the manufacturer (HerdCheck Swine Salmonella Antibody Test Kit, Idexx Laboratories, Inc., Maine, USA). Samples were considered positive if the OD% was equal to or higher than 10%.

Salmonella was isolated from faecal samples using a qualitative isolation method with pre-enrichment in buffered peptone water (BPW), enrichment on modified semisolid Rappaport-Vassiliasis (MSRV) agar plates followed by selective enrichment on xylose lysine desoxycholate (XLD) agar plates and biochemical confirmation. One colony of each *Salmonella* positive identified sample was stored at -20°C until further examination.

The selected isolates were grown in Tryptone Soya Broth (TSB) (Oxoid, CM0129) at 37°C for

24 h. Template DNA was extracted using the AquaPure Genomic DNA Kit (Bio-Rad 732-6340) according to the manufacturer's instructions. The isolates were genotyped by three consecutive random amplified polymorphic DNA (RAPD) assays using the primers 23L, OPB17 and P1254. Isolates from the same herd were analysed in the same PCR run to decrease fingerprint heterogeneity due to PCR-linked variations. DNA patterns that differed in one or more DNA fragments were considered to represent different types. Whenever type differences relied on one band only, 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). RAPD and PFGE analyses were carried out as described in detail by Nollet *et al*. (2005). 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 (Popoff and Le Minor, 1997).

The direct role of the sow in the transmission of *Salmonella* to her offspring was expressed by means of risk ratios (RR). RR's were calculated for a litter to be culture or serologically positive for *Salmonella* given the respective sow had been *Salmonella* positive or serologically positive during late gestation and/or lactation. A litter was defined as positive if at least one pig of the litter was defined as positive. Exact 95% confidence intervals were calculated by means of multinomial parametric bootstrapping (@risk 4.5).

Results Twenty-five, 36 and 22 sows in herds A, B and C, respectively were serologically positive for *Salmonella* at 5 weeks before the expected farrowing date. During lactation, 32, 35 and 30 sows in herds A, B and C, respectively, were serologically positive for *Salmonella*. Thirty-three, 38 and 30 sows were serologically positive for *Salmonella* at least once during late gestation or lactation in herd A, B and C, respectively. Two sows in herd A and three sows in herds B and C were found *Salmonella* positive during late gestation. During lactation, three, one and two sows were *Salmonella* positive in herds A, B and C, respectively. Three, four and five sows were *Salmonella* positive at least once during one of both periods in herds A, B and C.

The results from the faecal and the blood samples of the piglets in herd A, B and C are shown in Figure 1a and 1b, respectively.

The RR's for a litter to be *Salmonella* positive or serologically positive given the respective sow was *Salmonella* positive or serologically positive during late gestation and lactation are shown in Table 2. Piglets originating from sows seropositive during lactation had a significant lower risk to be *Salmonella* positive during the nursery period. Piglets originating from *Salmonella* excreting sows during late gestation or lactation did not have a significant higher risk for *Salmonella* excretion during the nursery period.

In herd A, 3 serotypes were isolated from the sows: S. Derby (D1), S. Infantis (I1) and S. Goldcoast (G1). D1 was also found during the finishing period. A genetically closely related strain, D1', differed in only one extra fragment of 1050 bp in the RAPD assay using primer 23L and was recovered during the finishing period. Additionally, isolates recovered from the fattening pigs during the nursery and the finishing period were serotyped as *S*. Typhimurium. These isolates could be subdivided into 2 genotypes (T1 and T2). Other strains (T1') were genetically closely related to T1 but differed in only 1 reproducible fragment in the RAPD assay (primer 23L).

The three serotypes found in the nursery, the growing unit, and the finishing unit of herd B were also isolated in the sows or in the sow unit (D2, G2, T3). Genotypes D2 and T3 were isolated from the nursery period onwards until the end of the finishing period. From the growing period on, a new genotype of *S*. Derby (D3) could be isolated next to D2 in the growers and finishing pigs but not in the sows.

In herd C, the isolates recovered from the sows' faeces were serotyped as *S*. Derby (D4), *S*. Typhimurium (T4) and *S*. Livingstone (L1).

Discussion Most intervention measures for reducing the prevalence of *Salmonella* in pig herds are focusing on finishing pigs. However, as they can be shedder of *Salmonella* (Davies *et al*., 1998; Nollet *et al*., 2005), sows might be an important source of infection of the finishing pigs with *Salmonella*.

Direct transmission of *Salmonella* from sows to their offspring could not be demonstrated in the present study. The low prevalence of *Salmonella* shedding in the piglets might however be

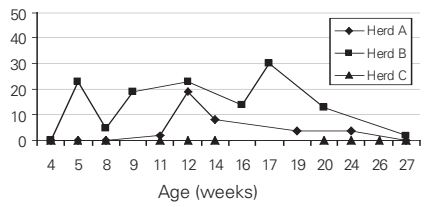

FIGURE 1a: The number of *Salmonella* culture positive pigs during a longitudinal study in 3 Belgian farrow-to-finish herds. Three piglets from one cohort of 34, 40 and 32 sows were serially sampled in herd A (n=102), B (n=120) and C (n=96), respectively.

underestimated because rectal swabs were taken which are of lower sensitivity in comparison with higher amounts of faeces (Funk *et al*., 2000). Remarkable is that seropositive sows seemed to protect their offspring, since those piglets were at lower risk of shedding *Salmonella* during the nursery period. *Salmonella* infections in sows might thus indirectly lead to protection of piglets, although the protection by maternal antibodies is limited to the nursery period.

Based on the characterisation of the isolates, similarities were found between the isolates originating from the sows and those recovered from the fattening pigs during the nursery, growing or finishing period. Since direct transmission could not be demonstrated, the authors assume that indirect transmission occurred via the farmer's boots or clothes, utensils, visitors… Earlier research (Nollet *et al.*, 2005) demonstrated that sows can maintain *Salmonella* infections in pig herds and might consequently be a source of infection for other pigs in the herd.

Conclusion Despite *Salmonella* infections in sows can lead to maternal protection of their offspring during the nursery period, sows seem to be an indirect source for *Salmonella* infection of other pigs in the herd. Intervention measures on farrow-to-finish herds should not only focus on the reduction of *Salmonella* in finishing pigs but also in sows.

References

Berends B.R., F. Van Knapen, D.A.A. Mossel, S.A. Burt, J.M.A. Snijders. 1998. Impact on human health of *Salmonella* spp. on pork in the Netherlands and the anticipated effects of some currently proposed control strategies. *Int. J. Food Microbiol.* 44: 219-229.

Davies P.R., Bovee F.G.E.M., Funk J.A., Morrow W.E.M., Jones F.T., Deen J. 1998. Isolation of *Salmonella* serotypes from feces of pigs raised in a multiple-site production system. *J. Am. Vet. Med. Assoc.* 212: 1925-1929.

Fedorka-Cray P.J., S.C. Whipp, R.E. Isaacson, N. Nord, K. Lager. 1994. Transmission of *Salmonella* Typhimurium to swine. *Vet. Microbiol.* 41: 333-344.

Funk J.A., P.R. Davies, M.A. Nichols. 2000. The effect of fecal sample weight on detection of *Salmonella enterica* in swine feces. *J. Vet. Diagn. Invest.* 12: 412-418.

Funk J.A., Davies P.R., Nichols M.A. 2001. Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems. *Vet. Microbiol.* 83: 45-60.

Kranker S., Alban L., Boes J., Dahl J. 2003. Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds. *J. Clin. Microbiol.* 41 2282-2288.

Nollet N., K. Houf, J. Dewulf, A. de Kruif, L. De Zutter, D. Maes. 2004. *Salmonella* in sows: a longitudinal study in farrow-to-finish pig herds. *Vet. Res.*, in press.

Popoff M.Y., L. Le Minor. 1992. Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on Salmonella, Institute Pasteur, Paris, France. 142pp.

Schwartz K.J., Salmonellosis. In Taylor D.J., ed. *Diseases of swine*, Iowa State University Press: Ames, Iowa, USA, 1999, 535-551.

Swanenburg M., Berends B.R., Urlings H.A.P., Snijders J.M.A., van Knapen F., 2001. Epidemiological investigations into sources of *Salmonella* contamination of pork. *Berl. Münch. Tierärztl. Wschr.* 114: 356-359.

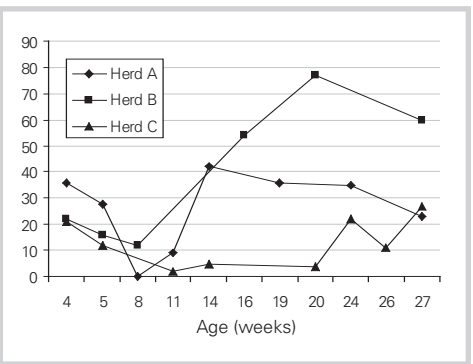

FIGURE 1b: The number of *Salmonella* seropositive pigs during a longitudinal study in 3 Belgian farrow-to-finish herds. Three piglets from one cohort of 34, 40 and 32 sows were serially sampled in herd A (n=102), B (n=120) and C (n=96), respectively.

Time before/after farrowing	Sows						Fattening Pigs					
	F	B	F	B	F	B	F	B	F	B	F	B
	Herd A		Herd B		Herd C		Herd A		Herd B		Herd C	
-37 days	x	x	x	x	x	x						
-7 days	x		x		x							
-2 days	x		x		x							
4 days	x		x		x							
7 days	x	x	x	x	x	x						
26 days	x	x	x	x	x	x	x	x	x	x	x	x
5 weeks							x	x	x	x	x	x
8 weeks							x	x	x	x	x	
9 weeks									x			
11 weeks							x	x			x	x
12 weeks							x		x		x	
14 weeks							x	x			x	x
16 weeks									x	x		
17 weeks									x			
19 weeks							x	x				
20 weeks									x	x	x	x
24 weeks							x	x			x	x
26 weeks											x	x
27 weeks							x	x	x	x	x	x

Table 1: Sampling scheme for the sows and the fattening pigs. Three fattening pigs from each sow of cohorts of 34, 40 and 32 sows were serially sampled in herd A (n=102), B (n=120) and C (n=96), respectively in three Belgian farrow-to-finish pig herds. F=faecal sample, B=blood sample

	RR for a *Salmonella* positive litter	RR for a *Salmonella* seropositive litter
Salmonella positive sow during late gestation	-[1]	0.90 (0.00-1.68)
Salmonella positive sow during lactation	1.18 (0.00-5.05)	1.21 (0.00-2.69)
Salmonella positive sow during late gestation or lactation	0.52 (0.00-1.90)	0.79 (0.22-1.53)
Salmonella seropositive sow during late gestation	-	4.12 (0.85-7.79)
Salmonella seropositive sow during lactation	0.25 (0.09-0.93)[2]	1.30 (0.51-3.16)
Salmonella seropositive sow during late gestation or lactation	-	-

Table 2: Risk ratios (RR) for a litter to be *Salmonella* positive or serologically positive in the nursery (from 26 days of age until 11 weeks of age) given the respective sow was *Salmonella* positive or serologically positive. A litter was defined as positive if one or more of the pigs of the respective litter were positive.
[1] RR could not be calculated because one of the cells in the 2x2 table was equal to zero
[2] significant (P<0.05)