Relationship between *Salmonella* shedding at the slaughter and pig *Salmonella* status during the fattening period.

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**Abstract**

In this study, the pig’s risk of shedding at slaughter with regard to its previous *Salmonella* status was estimated. A total of 133 pigs were serologically monitored monthly to determine their *Salmonella* status during the fattening period. Mesenteric lymph nodes (MLN) and faecal (FEC) samples were collected further at slaughter for bacteriology. Pigs were grouped as non-infected pigs (group A); animals likely infected at the end of the fattening period (B); and animals infected at least two weeks before the end of the fattening period (C). The probability of shedding in group A was high (36.5%) and likely related to *Salmonella* exposure during transport or lairage, but also to possible false-negative bacteriological results. The odds of shedding for groups B and C was 3.1 (95%CI=1.1-9.2) and 8.2 (1.4-48.6) times higher, respectively, when compared to group A. Overall most of the infected pigs from groups B and C showed same serotype in MLN and FEC samples (61% and 77%, respectively) and PFGE analysis confirmed majority were genetically related. In conclusion, the control of *Salmonella* infection along the fattening period appears to be of utmost importance to prevent slaughter contamination.

**Introduction**

In the EU salmonellosis is ranked as the second most important zoonosis transmitted through the consumption of food (EFSA, 2015), and pigs are considered the second major source of human infection (EFSA, 2015; Pires et al., 2011). The environmental contamination of the fattening unit is considered a major risk factor of *Salmonella* infection of slaughter pigs (Blaha, 2010). *Salmonella*-infected pigs are usually asymptomatic and, when arriving to slaughter, they usually show higher likelihood of shedding *Salmonella* than pigs at farm (Beloeil et al., 2004). Stress factors such as the transport to the abattoir and/or the mixing at lairage and environmental contamination of trucks and the abattoir may be responsible of this situation (Berends et al., 1996; Hurd et al., 2001). The aim of this study was to assess the pig’s risk of shedding at slaughter with regard to its previous *Salmonella* status at the farm in order to better understand the importance of these factors.

**Material and Methods**

Selected pigs belonged to a farm consistently showing a high prevalence of *Salmonella* spp. infection. Pigs came from 5 cohorts that acted as control groups for different field trials carried out by our research team for other purposes. The animals were tagged and serologically monitored monthly (4 bleedings) for the presence of antibodies to determine their *Salmonella* status during the fattening period. Sera were analysed with the Herd-Check® Swine *Salmonella* ELISA (IDEXX Laboratories, USA) and a cut-off value of OD%≥40 was used to deem a pig as seropositive. Once at slaughter, mesenteric lymph nodes (MLN) and faecal (FEC) samples were collected for bacteriology and *Salmonella* culture was performed according to the EN ISO 6579:2002/A1:2007. Pigs were then classified in three major groups according to serology and MLN infection: A) non-infected pigs, i.e. pigs always seronegative (OD%<40) during the fattening period and also negative for bacteriology on MLN at slaughter (N=52); B) animals likely infected at the end of the fattening...
period (within the last 2 weeks before slaughter), i.e. always seronegative during the fattening period but positive for bacteriology on MLN at slaughter (N=35); and C) animals infected at least two weeks before the end of the fattening period, i.e. pigs were seropositive in at least one of the last 3 samplings and positive for bacteriology on MLN (N=46). The relationship between these three groups and Salmonella shedding at slaughter was assessed by a logistic regression analysis (STATA, StataCorp, L.P., USA) where the outcome variable was the presence/absence of Salmonella spp. in feces (shedding), and the explanatory variable was the group (A, B and C). The cohort (1 to 5) was also included in the model as a potential confounder factor. A large proportion of Salmonella isolates from MLN and FEC samples from pigs belonging to groups B and C were serotyped at the National Reference Laboratory for Animal Salmonellosis (Madrid, Spain). When the same serotype was found in MLN and FEC samples for a given pig, both isolates were genotyped by pulsed-field gel electrophoresis (PFGE) (Ribot et al., 2006) to assess their genetic relatedness.

Results and discussion

Figure 1 shows the proportion of pigs in each group shedding Salmonella spp. at slaughter.

Figure 1. Relationship between Salmonella shedding at slaughter and Salmonella status (based on serology and mesenteric lymph node infection) at the fattening unit.

The prevalence of shedding in group A (36.5%) was significantly (P<0.05) lower than that for groups B (63%) and C (87%). The odds of shedding for group B and C was 3.1 (95%CI= 1.1-9.2) and 8.2 (1.4-48.6) times higher, respectively, when compared to group A. A high proportion of Salmonella non-infected pigs ended up shedding Salmonella at slaughter. This percentage could not be exclusively attributed to a pig misclassification due to the diagnostic techniques used. In fact, the sensitivity of Salmonella on MLN is quite high (≥87%; Mainar-Jaime et al., 2013). Besides, although some false-negative serological results could be expected given the high cut-off value used, the observed repeated negative results for the pigs in this group and the fact that similar results were found (not shown) when a lower cut-off value was used (OD%≥20%), suggested that the impact of serological misclassification was probably minor. It is more likely this result was the consequence of the exposure of the non-infected pigs to highly Salmonella-contaminated environments during transport or lairage and the subsequent circulation of the ingested salmonellae through the pig’s guts. Overall, it is very likely that a significant proportion of shedders in group A may have their origin from the contaminated trucks and abattoir premises. Pigs exposed to highly contaminated environments could get infected in a few hours (Berends et al., 1996; Hurd et al., 2001), thus animals from group B could be considered as recently infected with Salmonella, either at farm or during transport/lairage. The proportion of shedders within this group was high (63%) but significantly lower than that for the group of pigs that were infected along the fattening period (as detected by serology), which showed the highest probability of shedding the bacterium (87%; P<0.01). Considering that recently Salmonella-infected pigs are at the greatest risk of shedding (Scherer et al., 2008), this result emphasizes the importance that stress factors may have on pigs previously infected with Salmonella, and the need for developing strategies to prevent the infection at the fattening unit and minimize stress. Overall most of the infected pigs showed same serotype in MLN and FEC samples (61% in group B and 77% in group C). Results from the PFGE analysis confirmed that 100% of the Salmonella isolates from FEC were genetically related to those from MLN in group B (88% in group C). The observation that in some pigs different serotypes at MLN and FEC were found suggested the presence of simultaneous infections by different Salmonella serotypes within a pig (Garrido et al., 2014). In conclusion, in this study where the prevalence of Salmonella infection at the farm was high, the role that farm had on Salmonella shedding at slaughter appeared to be more important than that of transport and/or lairage. However, had the farm conditions be different (i.e. low prevalence of infection), transport and lairage might be considered as important risk factors for shedding. Salmonella-risk characterization at farm level and logistic transport and slaughter are therefore advisable.

References


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