Isolation of Salmonella spp. in pigs during transport, lairage, slaughterline and quartering

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Abstract
The aim of this study was to determine the prevalence of Salmonella in different points of the chain (transport, lairage, slaughterline and quartering). From eight consecutive assays a total of 134 Salmonella isolates out of 1180 different samples (11.3%) were recovered. The highest percentage of isolates were detected at the point of pre-scalding (30/80, 37.5%), caecal contents (18/80, 22.5%), trucks (13/60, 21.6%), tonsils (15/80, 18.7%), ileocaecal lymph nodes (13/80, 16.2%) and lairage (9/80, 11.2%). In the remaining points of sampling the number of isolates was minimal, being remarkable of 21 isolates obtained from different environmental samples (knives and surface of tables) (21/320, 6.5%) and 5 isolates in the quartering plant samples (5/80, 6.25%). Two more assays enhancing the pre-operational use of disinfectant on surfaces and irrigating ozone on carcasses were carried out. Serotypes more frequently isolated from four selected assays (64 isolates) were Bredeney, Rissen, Typhimurium, Montevideo, Israel, Derby, Emek, Choleraesuis, Durban, Kentucky, London and Sandiego. S. Typhimurium phage types U311, 193 and 104b were identified. Except for one assay, where Salmonella Bredeney was isolated from transport to quartering, different serotypes were isolate from the stages of sampling (trucks, lairage, slaughterline or quartering), which point to a different epidemiological source of those isolates. Although a lack of an evident cross-contamination was observed, our results have shown a high rate of isolates from transport, lairage and slaughterline environment which represent important critical points to acquire Salmonella infection both before and after slaughter of Iberian pigs.

Introduction
Salmonella is an important foodborne pathogen worldwide commonly recovered from pigs and pork in the European Union (EFSA, 2010). Spain occupies the second place in both pig and pork production and is considered as the highest pork consumer between the 27 member states of Europe (Marquer, 2010). Pigs may acquire the infection during transport and lairage and pig products may become contaminated during slaughterline and quartering process; stress factors during transport and lairage may induce Salmonella carrier pigs to shed the pathogen at a higher rate and increase the susceptibility of Salmonella-free pigs to infection (Mannion et al., 2010). Others studies have reported differences on Salmonella serotypes isolated and prevalence between farms and slaughterhouses (Hurd et al., 2001) suggesting that the transport and lairage are relevant risk factors in pigs at slaughter. Furthermore, cross-contamination in slaughterline has been reported as an important source of Salmonella contamination for pig carcasses (Swanenburg et al., 2001).

The aim of this research was to determine the presence of Salmonella spp. during Transport, Laiage, Slaughterline and Quartering (TLSQ) to improve Standard Sanitation Operates Procedures (SSOP) throughout the food production chain of Iberian pigs.

Material and Methods
During 2009 and 2010 eight systematic sampling (TLSQ1 to TLSQ8 assays) from ten Iberian pigs production units (5 Iberian commercial fed and 5 Iberian acorn-fed) were carried out. Ten finishing pigs from each pig herd were followed through one abattoir. Six different stages of the chain were tested: (i) trucks at its arrival to the slaughterhouse and after cleaning and disinfection (C+D), (ii) lairage prior entry of the pigs, and after departure to slaughter, (iii) ten pig
carcasses in different stages of slaughter-dressing, (iv) tonsils, ileocaecal lymph nodes and caecal contents, (v) environmental samples (slaughterline and quartering), and (vi) quartering samples (ham, shoulder and back). Moreover, two more assays (TLSQ9 and TLSQ10) were carried out as previously cited after a specific pre-operative disinfection program on quartering tables surfaces (dectocide H21R), and after an ozone treatment during the carcasses washing stage, respectively.

All samples were processed in peptone water (37 °C, 24 hours) and then transferred onto MSRV (Oxoid) (42 °C, 24-48 hours) according to ISO 6579: 2002. Finally, we conducted a double culturing on XLD and Chromogenic media (Oxoid) (37 °C, 24 hours). Moreover, sixty four isolates - from 4 out of 8 selected TLSQ assays - were serotyped and S. Typhimurium strains were phage typed by the National Reference Laboratory of Salmonella (Madrid, Spain).

**Results**

A total of 134 Salmonella isolates from 1180 different samples (11.3%) (Table 1) were recovered. The highest percentage of isolates were detected at the point of pre-scalding (30/80, 37.5%), caecal contents (18/80, 22.5%), trucks (13/60, 21.6%), tonsils (15/80, 18.7%), ileocaecal lymph nodes (13/80, 16.2%) and lairage (9/80, 11.2%). In the remaining points of sampling the number of isolates was minimal, being remarkable the isolation of 21 isolates from different environmental samples (knives and surface of tables) (21/320, 6.5%) and 5 isolates in the quartering plant from ham/shoulder/loin samples (5/80, 6.25%). Moreover, the higher number of isolates was obtained coinciding with the higher workload of the slaughterhouse, when more than 400 pigs were slaughtered. Two more specific assays, using disinfectant on quartering surfaces and ozone on carcasses, were performed at the end of our study (TLSQ9 and TLSQ10, respectively); only three and four isolates were detected, respectively, from caecal contents (5 isolates), ileocaecal lymph nodes (1 isolate), and carcass at post-scalding stage (1 isolate).

The distribution of serotypes isolated from environmental and pig samples (TLSQ3, 4, 6 and 7) was as follow: Bredeney (28 strains), Rissen (11), Typhimurium (8), Montevideo (4), Israel (4), Derby (2), Emek (2), Choleraesuis (1), Durban (1), Kentucky (1), London (1) and Sandiego (1). S. Typhimurium phage types U311 (5 strains), 193 (1) and 104b (2) were identified.

**Table 1. Number of Salmonella isolates and prevalence levels (%) during Transport, Lairage, Slaughterline and Quartering process (TLSQ1 to TLSQ8) in the Iberian pig processing plant**

<table>
<thead>
<tr>
<th>TLSQ1</th>
<th>TLSQ2</th>
<th>TLSQ3</th>
<th>TLSQ4</th>
<th>TLSQ5</th>
<th>TLSQ6</th>
<th>TLSQ7</th>
<th>TLSQ8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (C+D)</td>
<td>T2 (C+D)</td>
<td>L1 (entry)</td>
<td>L2 (exit)</td>
<td>S1 (pre-scalding)</td>
<td>S2 (post-scalding)</td>
<td>S3 (post-chilling)</td>
<td>S4 (post-evisceration)</td>
<td>S5 (post-washing)</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>12</td>
<td>17</td>
<td>22</td>
<td>19</td>
<td>21</td>
<td>23</td>
<td>134</td>
</tr>
</tbody>
</table>

Key: T1, truck prior C+D; T2, truck after C+D; L1, lairage prior entry of the pigs; L2, lairage after exit of the pigs; Slaughterline (carcasses samples): S1 (pre-scalding), S2 (post-scalding), S3 (post-chilling), S4 (post-evisceration), S5 (post-washing); To, tonsils; In, ileocaecal lymph nodes; C, caecal contents; E, environmental samples; Q, quartering plant (ham, shoulder and loin)

**Discussion**

Our results show the potential role of transport, lairage and slaughterline for contamination of carcasses in the pre- and post-slaughter environment. Recently Mannion et al. (2010) reported the role of transport, lairage and slaughterline equipment in the dissemination of Salmonella in pigs in the pre- and post-slaughter environments in the Republic of Ireland. Three processing plants with a total of eight production units (sixteen finishing pigs each one) were investigated. From
911 samples 153 isolates were detected (16.8%) including environmental (71/177, 40%) and pig samples (62/734, 8.5%) [Mannion et al., 2010]. Our results are in agreement with those detected in Ireland (11.3% vs 16.8%) although are different with respect to environmental samples (9.3% vs 40%), overcoat from lairage samples prior entry of the pigs (15% vs 80%).

The phenotypic characterization of the 64 selected isolates from TLSQ3, 4, 6 and 7 showed that except for one assay (TLSQ4) where Salmonella Bredeney was isolated ‘from transport to quartering’, pointing to a potential cross contamination between environmental and pig samples, different serotypes were obtained from each stage sampled (trucks, lairage, slaughterline or quartering), assuming that in our study the serotypes isolated at the different stages of the chain belonged to a different epidemiological source.

Several serotypes were ‘uncommon’ and different from those found in studies in free-range Iberian pigs and pigs in feedlots in Spain (Astorga et al., 2007; Gómez-Laguna et al., 2010): Montevideo, Israel, Emek, Durban, Kentucky and Sandiego. Previous studies have reported a disparity between the Salmonella serotypes isolated from slaughters and production units (Hurd et al., 2011). Mannion et al. (2010) reported different serotypes from two selected farms at abattoir, as follow: Derby, Infantis, Typhimurium, Bredeney, Panama and Give; being phage type 104b of S. Typhimurium the most frequently detected.

**Conclusion**

In conclusion this study showed the isolation of different serotypes of Salmonella spp. from samples of different source, which constitute a great risk for Iberian pigs both before and after slaughter. Thus, the HACCP must focus on intensive cleaning and disinfection programs in the pre-slaughter environment and the inclusion of new chemical agents or treatments which allow decreasing or eliminating the risk of Salmonella spp. infection or recontamination from the environment, which should be intensified when a higher workload is present.

**References**


ISO 6579:2002/Amendment 1: 2007: Appendix D. Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage.

