Occurrence and epidemiology of Salmonella enterica in two slaughterhouses and cutting plants in Spain

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Abstract
This study aimed at investigating the Salmonella occurrence in two Spanish pig slaughterhouses analysing the lairage, slaughter line, carcasses and the cutting plants thereof. The results obtained showed a decrease of Salmonella prevalence from the lairage to the cutting plant. The high levels of contamination in holding pens and several points of the slaughter line point them out as the major risk sources of Salmonella spreading within the slaughterhouse. The main Salmonella serotypes previously reported in Spanish finishing pig farms were found at both slaughterhouses. S. Typhimurium isolates recovered from different points of the slaughter chain were typed by MLVA.

Introduction
Salmonella is one of the major concern food-borne pathogens worldwide. Although eggs or poultry products are the main sources of contamination, Salmonella is also often associated with the consumption of pork and products thereof [1]. The high Salmonella prevalence in Spanish swine production [2] together with the forthcoming Spanish swine Salmonella Control Programme entail the establishment of suitable control measures. Finishing farms as well as slaughterhouses should be the main targets where these measures should be implemented since several studies have demonstrated the importance of the slaughter process in the spreading of Salmonella [3, 4]. There is no current data available regarding Salmonella prevalence and serovars in carcasses and slaughterhouses in Spain. The objective of this study, carried out in two Spanish slaughterhouses, was to improve the knowledge of the prevalence and serotypes in holding pens, different points of the slaughter line, cutting plants and also in carcasses before and after chilling process in order to find critical control points at slaughter level.

Materials and Methods
Two slaughterhouses, identified as slaughterhouse A and B which processed 400 and 375 pigs per hour respectively were investigated at the beginning, middle and end of the work week. Holding pens, the slaughter line and randomly selected carcasses at the end of the slaughter line (before chilling), were sampled on Monday, Wednesday and Friday while the cutting plant of each slaughterhouse and the same carcasses after chilling and cooling procedures were sampled the following working day (Tuesday, Thursday and the following Monday respectively). Holding pens were sampled in four rounds: (I) before the entry of pigs, (II) at half of the working day, (III) at the end of the working day and (IV) after cleaning procedures. Several points of the slaughter line and cutting plant (Table 1) were sampled throughout the working day.
Sample collection and processing were carried out as previously described [5]. Samples were processed by following the ISO standard methodology 6579:2002/Amd1:2007. A single isolate from each positive sample was serotyped by slide agglutination according to Kauffmann-White scheme using commercial antisera. S. Typhimurium and S. 4,[5],12:i:- isolates were further typed by MLVA.

Results
Holding pens. Each sampling day, eleven holding pens were evaluated in both slaughterhouses. The overall results show that 26 out of 66 (39.4%) of the holding pen floor surfaces were already contaminated by Salmonella before the staying of the pigs. Contamination was higher during the working day, with 98.5% and 80.3% of Salmonella positive samples among those collected in the holding pens at half and end of the working day respectively. After the cleaning procedures, Salmonella was recovered from the floor surface in 42 out of 66 holding pens (63.6%). It is noteworthy that no

Salmonella contamination was recorded in the sampling carried out on Monday in the slaughterhouse A while the eleven holding pens evaluated in the same visit to slaughterhouse B were contaminated by Salmonella. Moreover, in those sampling days in which the number of positive pens was high at the start of the working day, a higher number of positive pens were found during the other three sampling rounds.

Slaughter line. Ten different points within the slaughter line were evaluated (Table 1), most of them at both slaughterhouses. In total, 66 samples were collected in each of the three visits to slaughterhouse A. In slaughterhouse B, 46 samples were collected from the slaughter line in two of the sampling days while only 38 were recovered during the first visit to this abattoir. Mean prevalence of Salmonella positive samples was 33% (66 positive samples out of 198) in slaughterhouse A and 47.7% (62 positive samples out of 130) in slaughterhouse B. No statistically significant differences were found among different visits to slaughterhouse A (χ^2= 2.9, p= 0.23). However, the prevalence of Salmonella contamination varied among different visits in slaughterhouse B (χ^2= 6.3, p= 0.04) where it is remarkable that 60.9% of the samples collected had a positive result in the sampling performed at the middle of the work week.

Carcasses. Salmonella was recovered from 179 out of 446 carcasses evaluated before chilling (40.4%). No statistical differences were found in the prevalence of contaminated carcasses between both slaughterhouses although there were significant differences among different visits to the same slaughterhouse. In a similar way, 445 carcasses were evaluated after chilling and 48 gave a positive result (10.8%). The prevalence of Salmonella contamination was compared before and after chilling in 323 carcasses that were sampled at both stages. In those carcasses evaluated twice, Salmonella contamination was reduced from 45.8% to 10.8%, which means a significant decrease in Salmonella contamination after chilling (χ^2= 95.64, p< 0.001).

Cutting plant: Seventeen points, most of them in both slaughterhouses, were analysed. They were classified in three groups including machinery (surface and organic matter from saws and derinding machines), operators (hands and implements) and surfaces (conveyor belt at different processing points) (Table 2). Salmonella was identified in 17 out of 174 (9.8%) samples collected in slaughterhouse A and 30 out of 172 (17.4%) collected in slaughterhouse B. Contamination was more frequent in machinery (7 positive samples out of 54) (13%) followed by surfaces (4 positive samples out of 60) (6.7%) and operators (3 positive samples out of 90) (3.3%) in slaughterhouse A. On the other hand, Salmonella was detected in 20 out of 96 surface samples (20.8%) as well as in 3 out of 23 machinery samples (13%) and 5 out of 36 operator samples (13.9%) in slaughterhouse B.

Nine different serotypes were found; S. Rissen was the predominant serotype detected in 36.3% of the positive samples followed by S. 4,[5],12:i:- (17.6%), S. Typhimurium (14.8%) and S. Derby (14.5%). Main Salmonella serotypes identified in holding pens, slaughter line, carcasses and cutting plants are shown in Table 2.

A total of 8 and 6 different MLVA profiles were identified in the second and third visits to slaughterhouse A. Isolates with the same MLVA profile were found in several points of the slaughter chain and also one of the patterns was shared by isolates from both visits.

Discussion

Several studies have indicated the role that the slaughter process can play in the spreading and dissemination of Salmonella [3, 4, 5]. The proposal of this study was to identify critical points surrounding the slaughter process by analysing Salmonella prevalence from the lairage to the cutting plant at the beginning, middle and end of the work week. The evaluation of the lairage showed high levels of Salmonella contamination in holding pens. This contamination is consequence of the faecal shedding of Salmonella by the pigs housed there and it increased during the working day. What is more, we were able to demonstrate that the routinely cleaning procedures used in both abattoirs were unsatisfactory. These results are in agreement with previous studies [6] and confirm that the lairage can constitute an important source of new infections particularly when cleaning and disinfection procedures are not carried out properly. An improved cleaning protocol including the use of disinfectants and a proper instruction of the personnel are the main tools to decrease Salmonella contamination at this stage.

Several points of the slaughter line were included in the study to find out which ones were the most relevant in the dissemination of Salmonella. The prevalence of Salmonella contamination was high in those points where actions were performed by hands and implements of operators: the evisceration and the kidney, lard, fat and tonsil removal points. At some of these points, a tendency towards an increase in contamination throughout the working day was observed, probably related to an accumulation of Salmonella in implements. In slaughterhouse B, some samples collected after the sterilization gave a positive result showing the inefficiency of the cleaning and disinfection protocols carried out.

Taking into account the high prevalence of Salmonella infection in Spanish slaughter pigs [2], a relatively high prevalence

of Salmonella contamination in carcasses was expected. Mean prevalence was similar between both slaughterhouses. Nevertheless, the fact that it varied significantly among different visits to the same slaughterhouse demonstrates that carcass prevalence can be affected by the pig status and also by several internal factors of the slaughtering process. Moreover, a reduction in the percentage of Salmonella contaminated carcasses was observed in both slaughterhouses after their chilling and cooling. According to this, it seems that these chilling and cooling procedures should be done before carcass processing in order to limit Salmonella contamination in the latest steps of the pork production chain. What else, this result reveals the importance of taking into consideration the time of the sampling when comparing contamination results among different slaughterhouses or countries.

Also, different points of the cutting plant were analysed including machinery, operators (hands and implements) and surfaces, particularly conveyor belts. Salmonella was also isolated from several of these samples although, in general, a lower frequency of contamination in comparison with previous facilities of the same slaughterhouse was observed.

Main serotypes found during the present study were similar to those previously reported as the most frequent in Spanish fattening pigs [2] S. Rissen, a serotype particularly related to pigs from the Iberian peninsula, was the most prevalent one in all investigated stages followed by S. 4,[5],12:i:-, S. Typhimurium and S. Derby. It is noteworthy that S. Bredeney was the predominant serotypes found in one of the sampling days in the slaughterhouse B showing that Salmonella population within the slaughterhouse varies. MLVA profiles showed the spreading of particular types from the lairage to the cutting plant. The variability of serotypes and MLVA profiles within a sampling day and among different visits indicates that Salmonella population at the slaughterhouse is in constant change.

Conclusion

The present study aimed at investigating Salmonella contamination during the slaughter process in two Spanish abattoirs. The high contamination detected in lairages and slaughter lines implies that they constitute risk points and actions to reduce the contamination at both stages should be taken. Carcass contamination was frequent at the end of the slaughter line but it decreases after chilling and cooling procedures. Finally the main serovars found in Spanish farms are also found in the abattoir indicating a Salmonella flow from farm to the slaughterhouse and its spreading in the slaughter process.

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Table 1. Salmonella contamination at different points of the slaughter line and cutting plant of two commercial Spanish pig slaughterhouses.

	Slaughterhouse A		Slaughterhouse B	
	No positive samples	% positive samples	No positive samples	% positive samples
Slaughter line				
De-hairing	-	-	1	8.3
Bung Dropper	4[a]-0[b]	10[a]-0[b]	4[a]-3[b]	33.3[a]-25[b]
Evisceration	15	33.3	7	58.3
Kidney extraction	14	46.7	7	58.3
Lard removal	17	37.8	14	82.7
Trimming	16	53.3	6	50
Splitting saw				
Saw	1	11.1	-	-
Water	-	-	0	0
Organic matter	2	22.2	7	87.5
Mammals removal	-	-	4	50
Tonsils removal	-	-	5	62.5
TOTAL	66	33.3	58	49.1
Cutting plant				
Machinery				
Organic Matter	7	38.9	2	11.1
Surface	0	0	1	1.7
Operators	4	4.4	5	13.9
Working surface	4	6.7	96	20.8
TOTAL	17	8.3	30	17.4

a Sample collected before sterilization
b Sample collected after sterilization

Table 2. Main Salmonella serotypes recovered in different facilities of two commercial Spanish pig slaughterhouses. Number and percentage of isolates in each evaluated level are reported.

Serotype[1]	Holding pens	Slaughter line	Carcass non-chilled	Carcass chilled	Cutting plant	TOTAL
Bredeney	24 (14.6%)	17 (10.7%)	23 (15.0%)	6 (15.4%)	3 (10%)	73 (13.3%)
Rissen	55 (33.5%)	58 (36.5%)	61 (39.7%)	15 (38.5%)	9 (30%)	198 (36.3%)
Derby	43 (26.2%)	13 (8.2%)	19 (12.3%)	2 (5.1%)	2 (6.7%)	79 (14.5%)
4,[5],12:i:-	16 (9.8%)	48 (30.2%)	19 (12.3%)	5 (12.8%)	8 (26.7%)	96 (17.6%)
Typhimurium	22 (13.4%)	13 (8.2%)	28 (18.2%)	11 (28.2%)	7 (23.3%)	81 (14.8%)
TOTAL	164	159	154	39	30	546

[1]S. Mkamba, S. Clackamas, S. Anatum and S. Agama were the other serotypes found in less than 2% of the positive samples.

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