Is the *Salmonella* contamination of swine carcasses at slaughter related to the *Salmonella* load in caecum?

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Abstract

The aim of this study was to examine the relationship between the load of *Salmonella* spp. in caeca and the carcass contamination in an Italian slaughterhouse. The sampling scheme was designed to be representative of the pigs slaughtered in a day and to estimate a 12% prevalence of pigs highly contaminated by *Salmonella* spp. (HCP, cecal load ≥3log). Environmental swabs were taken before slaughter. Cecal contents and carcass swabs were collected from the same pig. *Salmonella* MPN were estimated according to ISO6579-2:2012/A1 and ISO7218:2007/E. The overall *Salmonella* prevalence were 34.64% and 7.19% for ceca and carcasses respectively, with *S.* Derby and *S.* 4,[5],12:i:- being the prevalent serotypes. The HCP prevalence was 11.44%. 7/59 environmental swabs tested positive; when the same serotype was isolated from the environment and from carcasses, the samples were excluded from further analysis. Statistical analysis was performed to investigate the relationship between *Salmonella* spp. loads in the cecum and contamination of the carcass of the same pig and the prevalence of HCP and the contamination of carcasses on the same day. For this purpose, the days were classified as "high prevalence days" depending on the proportion of caeca resulted positive (≥36%) and as "high load" days depending on the prevalence of HCP (≥10%). A correlation between the contamination of carcasses and the cecal *Salmonella* loads of the same animal was found (Spearman's correlation coefficient: 0.2254; p-value=0.0001). No correlation was found between the contamination of carcasses and the categorization of the day of sampling as "high prevalence day". Conversely, a correlation was found between the contamination of carcasses and the "high load" category of the sampling day (Wilcoxon test, p=0.0011). Notably, not the prevalence of pigs carrying *Salmonella* spp. but the prevalence of highly contaminated pigs was shown to be related to the contamination of carcasses.

Introduction

Salmonellosis is still one of the most important foodborne diseases in the EU. Even though the layer hen reservoir remains the most important source of human salmonellosis in the EU, in some Countries, including Italy, pork is considered the first contributor to the infection (Pires, 2011). The risk of *Salmonella* infection in man is not only associated to its presence on carcasses, but also to the bacterium load, since high levels of *Salmonella* can increase the consumer's exposure. However, most data on the *Salmonella* prevalence in the pig production chain are only qualitative, and this can impair quantitative risk assessment (EFSA, 2008). At the slaughterhouse, the source of contamination of carcasses can be from the same animal, from other pigs, or from the environment, a process known as cross-contamination. *Salmonella* can contaminate the environment either in a persistent way, being present as a 'house flora' of the slaughter plant, or in a transient way, by cross contamination from animals slaughtered on the same day (Smid, 2014). In this complex scenario, the intestinal content and the faeces of carrier pigs are, directly or indirectly, the predominant source of *Salmonella* for carcasses (van Hoeck, 2011). Our hypothesis was that there was a positive association between the *Salmonella* load in the intestinal content and the contamination of carcasses at slaughter. This association was studied on individuals, correlating the amount of *Salmonella* in caecum and the *Salmonella* load of the carcass of the same pig. Then, to unveil the role of intestinal load in cross-contamination, the correlation between the prevalence of highly contaminated pigs and the contamination of carcasses on the same working day was investigated.

Materials and Methods

Sampling: This study was carried out in a slaughterhouse located in Central Italy, with a capacity of 2000-2200 pigs per day, operating for two days every week. The sampling scheme was designed to be representative of the pigs slaughtered in one day and to estimate a 12% prevalence of pigs highly contaminated by *Salmonella* spp. (HCP, cecal load ≥3log), with a 10% precision and 95% confidence level. The expected prevalence was calculated using the data of a pilot study. On each working day, the pigs to be sampled were chosen using simple randomization. Cecal contents and carcass swabs were taken from each pig. Carcass swabs were taken before the final washing, according to the UNI EN ISO 17604:2003/E procedure, in five different points for each half carcass (distal hind limb, hind limb, lateral abdomen, medial abdomen, mid-dorsal region) using 100 cm^2 sterile square templates and pre-moistened sponge bags. Overall, 1000 cm^2 of each carcass were sampled. At the slaughterhouse, environmental samples were collected at the beginning of each sampling day, before the first pig was slaughtered. Eight pre-moistened sponge bags were swabbed on surfaces (scald tank, carcass chute, containers for viscera, hooks, two carcass splitters and two sets of knives, at the beginning and at the end of the slaughter line), in each working day. All samples were immediately put in sterile containers, maintained at +4°C until processing and cultured within the following 24 hrs.

Culture: The microbiological analysis of cecal contents and carcass swabs was carried out using a miniaturized technique, according to ISO/TS 6579-2:2012/A1 protocol. This technique provides an estimate of the *Salmonella* spp. load, following the most probable number (MPN) method. 5 g of the cecal content and carcass swabs were diluted 10 fold in peptone water BPW (Oxoid Ltd., UK). 2.5 ml of this initial suspension was then used to perform a series of 1:5 dilutions carried out by systematically transferring an aliquot of 0.5 ml of each successive dilution in 2 ml of BPW. Each dilution was then incubated and processed as described in the procedure. The MPN values and their 95% CI were calculated using the MPN calculator, available on the website http://standards.iso.org/iso/ts/6579/-2. Isolates of *Salmonella* from positive samples were further serotyped according to the Kauffmann-White scheme (Popoff, 2003). Environmental swabs were analyzed only qualitatively, following the ISO/TS 6579 procedure, after an initial suspension in 225 ml of BPW.

Categorization of pigs and working days: Pigs were classified as highly contaminated by *Salmonella* spp. if the MPN of the cecal content was three logs or higher (HCP, cecal load ≥3log). Subsequently, the working days were categorized into “high load” and “low load” days depending on the prevalence of HCP. Finally, the working days were categorized into “high prevalence” days, if the proportion of cecal contents testing positive for *Salmonella* was 36% or higher. Working days with a proportion of positive cecal contents lower than 36% were classified as “low prevalence” days. This threshold was chosen according to previous data on *Salmonella* prevalence in intestinal contents at slaughter in Italy (Bonardi *et al*., 2003).

Statistical analysis: When two isolates collected on the same working day, one from a carcass swab and the other from an environmental swab, belonged to the same serotype, the contamination was presumed to originate from the slaughterhouse environment. Therefore, the results of the carcass swab and corresponding cecal content were excluded from the statistical analysis.

We tested data to determine if it was or was not normally distributed.

The statistical analysis was performed to evaluate the following hypotheses

i) No correlation between the *Salmonella* load in cecum and the *Salmonella* load in the corresponding carcass, evaluated by Spearman’s rank analysis;

ii) No difference between the contamination of carcasses on “high load” and on “low load” working days, evaluated by Wilcoxon’s test;

iii) No difference between the contamination of carcasses on "high prevalence" and on "low prevalence" working days, evaluated by the Pearson's chi-squared test. The strength of association was measured using the Odd Ratio (OR).

A p≤0.05 level of significance was set for all statistical tests.

Results

Three hundred and six (306) carcass swabs, 306 cecal contents and 59 environmental swabs, on seven working days, from April to November 2014, were collected during this study. The *Salmonella* prevalence was estimated as 34.64% (CI95% 29.37%-40.30%) in cecal contents and 7.19% (CI95% 4.66%-10.84%) in carcass swabs. Seven out of 59 (11.9%) environmental swabs tested positive for *Salmonella*. The most common serotypes detected were the monophasic variant of *Salmonella* Typhimurium (4,[5],12:i:-) and S. Derby; data on the proportion of *Salmonella* serotypes recovered from different sample types are shown in Table 1.

The bacterial load in caeca ranged from <2log UFC/g, to >6log UFC/g, which was the upper detection limit of the test. In carcasses, the amount of *Salmonella* varied between <2,5 UFC/cm^2 and 25 UFC/ cm^2.

Table 1: *Salmonella* spp. isolates from cecal contents, carcass swabs and environmental samples, divided according to the serotype. Only the serotypes isolated more than once are shown.

	S. 4,[5],12:i:-	S. Derby	S. Rissen	S. Goldcoast	S. Infantis	S. London	S. Panama	S. Stanley	S. Anatum	Total
Cecal content	38 (36%)	29 (27%)	15 (13%)	5 (5%)	5 (5%)	5 (5%)	2 (2%)	2 (2%)	2 (2%)	106 (100%)
Carcass swab	3 (14%)	3 (14%)	6 (27%)	1 (4.5%)	1 (4.5%)	2 (9%)	6 (27%)	-	-	22 (100%)
Env. Swab	3 (43%)	2 (29%)	1 (14%)	1 (14%)	-	-	-	-	-	7

The prevalence of highly contaminated pigs (HCP) was 11.44% (IC95% 8.20%-15.67%). Following the criteria stated above, five carcass swabs and the corresponding five cecal contents were excluded from further analysis because the same serotype was isolated from environmental swabs on the same working day. The results of statistical analysis, divided into the three objectives of the study, were as follows:

i) After analysis by Shapiro-Wilk test, the data were not normally distributed so the analysis was performed using Spearman's rank analysis. A Spearman's rank correlation coefficient of 0.2254 (p= 0.0001) was calculated and the null hypothesis of an absence of correlation between the Salmonella load in cecum and the Salmonella load in the corresponding carcass was rejected.

ii) A difference between the contamination of carcasses on "high load" and on "low load" working days was shown (table 2). Again, the data were not normally distributed and they were evaluated by Wilcoxon's test, using a p≤0.05 level of significance.

Table 2: Distribution of samples from 'high load' and 'low load' working days.

	Carcass swabs		
	positive	negative	total
'High load' working days	14 (10.6%)	118 (89.4%)	132 (100%)
'Low load' working days	3 (1.77%)	166 (98.23%)	169 (100%)

iii) No difference was found between the contamination of carcasses on "high prevalence" and on "low prevalence" working days, evaluated by the Pearson's chi-squared test (p=0.7970). The strength of association was measured using the Odd Ratio (OR), and it was estimated as 0.88 (IC95%:0.28-2.64).

Discussion

In literature, few data are available on the *Salmonella* load in ceca of pigs at slaughter. In a study carried out in Denmark, only 0.18% ceca showed more than 670 UFC of *Salmonella*/g (Nauta, 2013), while we estimated a 12% prevalence of pigs with at least 1000 UFC/g. This difference can be partially justified by the different *Salmonella* prevalences in these two EU Countries. In our study, the prevalence of *Salmonella* in caeca was 35%, compared to the 2.6% reported by Nauta (2013). The amount of *Salmonella* recovered from carcasses in our study was low and near to the lower detection limit, which is in accordance with other authors' reports (Nauta, 2013; Delhalle, 2009). In this study, the contamination of the slaughterhouse environment was linked to the contamination of carcasses. In approximately 23% of cases, the same serotype was identified from the house flora and from the carcasses of pigs slaughtered on the same day. The role of environmental contamination varies in different slaughterhouses, however the proportion we observed here is close to that already described in literature, which indicates house flora as responsible for approximately one-third of carcass contamination (van Hoeck, 2011).We showed a weak correlation between the contamination of the carcass and the *Salmonella* load in the cecum of the same pig. According to Berends (1997), up to 70% of carcass contamination originates from the animal itself; however, this hypothesis is not supported by other studies (Nauta, 2013). More importantly, we found that the prevalence of pigs harbouring high *Salmonella* loads in ceca influenced the proportion of carcasses contaminated by *Salmonella* on the same day, suggesting a role of highly contaminated pigs in cross-contamination. The same effect was not observed dividing the working days according to the prevalence of pigs simply harboring *Salmonella* in caeca. Therefore, our data confirm the hypothesis that the amount of *Salmonella* in cecum is linked to the probability of the contamination of carcasses at slaughter.

Conclusion

In this study, we showed a correlation between the prevalence of highly contaminated pigs and the contamination of carcasses. The same correlation with carcass contamination was not found with the prevalence of pigs simply carrying *Salmonella* spp. If confirmed, these findings suggest that a control strategy based on the reduction of highly contaminated pigs may be effective in preventing the contamination of carcasses.

References

- *Anonymous. EN ISO 17604:2003/E "Microbiology of food and animal feeding stuffs – Carcass sampling for microbiological analysis" ISO 17604:2003/E.*
- *Anonymous. EN ISO 6579-2:2012/A1. "Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp. – Amendment 1: Annex D: Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage".*
- *Berends BR1, Van Knapen F, Snijders JM, Mossel DA.Identification and quantification of risk factors regarding Salmonella spp. on pork carcasses. Int J Food Microbiol. 1997 May 20;36(2-3):199-206.*
- *Bonardi S., Brindani F., Pizzin G., Lucidi L., S'Incau M., Liebana E., Morabido S. (2003) "Detection of Salmonella spp., Yersinia enterocolitica and verocytotoxin-producing Escherichia coli O157 in pigs at alaughter in Italy" International Journal of Food Microbiology, 2003 Aug 15;85(1-2):101-10.*
- *Delhalle L, Saegerman C, Farnir F, Korsak N, Maes D, Messens W, De Sadeleer L, De Zutter L, Daube G. (2009) Salmonella surveillance and control at post-harvest in the Belgian pork meat chain. Food Microbiol. 26(3):265-71.*
- *Nauta M, Barfod K, Hald T, Sørensen AH, Emborg HD, Aabo S. (2013) Prediction of Salmonella carcass contamination by a comparative quantitative analysis of E. coli and Salmonella during pig slaughter. Int J Food Microbiol. 166(2):231-7.*
- *Pires, S.M., de Knegt L. and Hald T. (2011) Scientific/technical report submitted to EFSA 'Estimation of the relative contribution of different food and animal sources to human Salmonella infections in the European Union'. Available on line at:http://www.efsa.europa.eu/en/supporting/doc/184e.pdf.*
- *Popoff M.Y., Bockenmuhl J., Gheesling L.L. (2003) supplement 2001 (no 45) to the Kauffmann-White Scheme. Research in Microbiology, 154: 173-174.*
- *Smid JH, van Hoek AH, Aarts HJ, Havelaar AH, Heres L, de Jonge R, Pielaat A. (2014) Quantifying the sources of Salmonella on dressed carcasses of pigs based on serovar distribution. Meat Sci. 96(4):1425-31.*

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