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Short communication

Association between pigs with high caecal *Salmonella* loads and carcass contamination

M. Pesciaroli, L. Cucco, S. De Luca, F.R. Massacci, C. Maresca, L. Medici, M. Paniccià, E. Scoccia, M. Staffolani, G. Pezzotti, C.F. Magistrali *

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, via Salvemini 1, 06126 Perugia, Italy

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ABSTRACT

Contaminated pork is a significant source of foodborne *Salmonella* infections. Pork is contaminated at the slaughterhouse; however, the mechanisms driving *Salmonella* contamination of carcasses are still poorly understood. The aim of this study was to investigate whether the amount of *Salmonella* carried by slaughtered pigs in their guts has an influence on carcass contamination. On that account, we tested whether the number of carcasses contaminated during a slaughter day was associated with the prevalence of highly contaminated pigs (HCP: *Salmonella* caecal loads ≥3log/g), or with the prevalence of pigs that simply carry *Salmonella* spp. in their guts. Three hundred and six pigs were sampled in a slaughterhouse from Central Italy. *Salmonella* loads in the caecum and on the carcass of each pig were estimated by the most probable number (MPN) technique. The overall prevalence of *Salmonella* was 34.64% and 7.19% for the caeca and carcasses, respectively. *S.* Derby and *Salmonella enterica* 4,[5],12:i:– were the most frequently isolated serovars. The prevalence of HCP was 11.44%. We found a higher number of contaminated carcasses on days of high prevalence of HCP than on days of low prevalence of HCP ($p = 0.0011$). Conversely, carcass contamination did not vary with the prevalence of pigs that simply carried *Salmonella* spp. in their guts ($p = 0.7970$). Therefore, the prevalence of HCP, but not the prevalence of pigs carrying *Salmonella* spp., was related to carcass contamination. Taken together, these findings suggest that reduction of *Salmonella* loads in the guts of slaughtered pigs would result in fewer contaminated carcasses, and consequently, help to minimise the risk of human infection due to the consumption of contaminated pork.

1. Introduction

Salmonellosis is still one of the most important foodborne diseases in the EU (EFSA, 2015). Even though the laying hen reservoir remains the most important source of human salmonellosis in the EU (EFSA, 2015), in some European countries, including Italy, pork is considered the main contributor to the infection (Graziani et al., 2013; Pires et al., 2011). Pork gets contaminated during the slaughtering process, and the intestinal content and the faeces of carrier pigs are, directly or indirectly, the predominant source of *Salmonella* for carcass contamination (van Hoek et al., 2012). In fact, *Salmonella* microorganisms present on a carcass can originate from the same animal, other pigs, or the environment (cross-contamination). *Salmonella* can contaminate the environment either in a persistent way, being present as part of the 'house flora' of the slaughterhouse, or in a transient way, by cross contamination from animals slaughtered on the same day (Smid et al., 2014). For this reason, the *Salmonella* status of the animals delivered to the slaughterhouse plays a crucial role in this complex scenario (Hill et al., 2010). Studies on the dynamics of carcass contamination are based on the prevalence of *Salmonella* infection estimated by qualitative microbiological cultures of gut content or of lymph nodes of slaughter pigs (Botteldoorn et al., 2003), but little or no information is available on the amount of *Salmonella* they carry in their guts. In fact, quantitative cultural methods for *Salmonella* enumeration are costly and time-consuming, and hence not performed on a routine basis (Malorny et al., 2008). The hypothesis of the present study is that pigs carrying large amounts of *Salmonella* in their guts drive carcass contamination. This hypothesis was tested by investigating whether the contamination of carcasses occurring during a slaughter day was associated either with the prevalence of pigs carrying high loads of *Salmonella* or with the prevalence of pigs that simply carry *Salmonella* spp. in their guts.

2. Materials and methods

2.1. 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 per week. Three hundred and six (306) carcass swabs, 306 caecal contents, and 59 environmental swabs, were collected on seven

* Corresponding author at: Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, via Salvemini 1, 06126 Perugia (PG), Italy.
E-mail address: c.magistrali@izsum.it (C.F. Magistrali).

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Table 1
Isolation of *Salmonella* spp. from the different types of samples.

	Positive	Negative	Total
Caecal content	106 (34.6%)	200 (65.4%)	306 (100%)
Carcass swab	22 (7.2%)	284 (92.8%)	306 (100%)
Environmental swab	7 (11.9%)	52 (91.1%)	59 (100%)

working days between April and November 2014. Sampling was designed to be representative of the pigs slaughtered in a day and to estimate the prevalence of pigs highly contaminated with *Salmonella* spp. (HCP, caecal load ≥ 3 log), with 10% precision, and a 95% confidence level. The expected prevalence (12%), was determined using the data of a pilot study carried out previously. Slaughtered pigs were divided into batches, and each batch originated from a different farm. So, on each working day, approximately 45 pigs from an average of 20 (16–21) farms were sampled by systematic randomization. Caecal 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 (2003) procedure. Briefly, five different points on each half carcass (distal hind limb, hind limb, lateral abdomen, medial abdomen, mid-dorsal region) were sampled using 100 cm^2 sterile square templates and pre-moistened sponges. Overall, 1000 cm^2 area of each carcass was sampled. Environmental samples from the slaughterhouse 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, one at the beginning and one at the end of the slaughter line). All samples were immediately placed in sterile containers, maintained at 4 °C until processing, and cultured within the following 24 h.

2.2. Microbiological culture

The microbiological analysis of caecal contents and carcass swabs was carried out using a miniaturised most probable number (MPN) technique, according to the ISO/TS 6579-2:2012/A1 (2012) protocol. This technique provides an estimate of the *Salmonella* spp. load, using the most probable number (MPN) method. Briefly, 5 g of the caecal contents and carcass swabs were diluted 10-fold in buffered peptone water (BPW) (Oxoid Ltd., UK). This initial suspension was then used to perform 12 serial dilutions in a 1:5 ratio, carried out by systematically transferring an aliquot of 0.5 ml of each successive dilution into 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* spp. from positive samples were serologically identified according to the Kauffmann-White scheme (Popoff, 2003). Environmental swabs were analysed only qualitatively, following the ISO/TS 6579 procedure, after an initial suspension in 225 ml of BPW.

2.3. Categorization of pigs and working days

A pig was categorised as highly contaminated by *Salmonella* spp. if the MPN estimated a load of 3 logs or higher per gram of caecal content (HCP: caecal load of ≥3 log/g). Subsequently, the working days were categorised into "high load" and "low load" days depending on the prevalence of HCP: ≥10% or <10%, respectively. Finally, the working days were categorised into "high prevalence" days, if the proportion of caecal contents testing positive for *Salmonella* was ≥36%. Working days with a proportion of positive caecal contents lower than 36% were classified as "low prevalence" days. This threshold was chosen according to previous data on *Salmonella* prevalence in intestinal contents of pigs at slaughterhouses in Italy (Bonardi et al., 2003).

2.4. 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 and showed the same pulsotype, after analysis with pulsed-field gel electrophoresis (PFGE), according to the Salmgene protocol (Peters et al., 2003), the contamination was presumed to originate from the slaughterhouse environment (house flora). Therefore, the results from the carcass swab and the corresponding caecal content were excluded from the statistical analysis. The overall *Salmonella* prevalence, the prevalence of HCP, and the prevalence of *Salmonella*-contaminated carcasses and their 95% confidence interval (CI 95%) were calculated. Data were evaluated by the Shapiro-Wilk test to determine whether they were normally distributed.

Statistical analysis was performed to evaluate the following:

i) A correlation between the *Salmonella* load in caecum and the *Salmonella* load in the corresponding carcass, evaluated by Spearman's rank analysis;
ii) The difference between the contamination of carcasses on "high load" and on "low load" working days, evaluated by Wilcoxon's test;
iii) The difference between the contamination of carcasses on "high prevalence" and on "low prevalence" working days, evaluated by the Pearson's chi-squared test.

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

3. Results

Salmonella spp. were isolated from 106 caecal contents and 22 carcass swabs, and their prevalence was estimated as 34.6% (CI 95% 29.3%–40.3%) and 7.2% (CI 95% 4.6%–10.8%), respectively (Table 1). Seven out of 59 (11.9%) environmental swabs tested positive for *Salmonella* spp. (Table 1). The most frequently detected serotypes were the monophasic variant of *Salmonella typhimurium* (4,[5],12:i:–) and *Salmonella* Derby; data on the proportion of *Salmonella* serotypes recovered from different sample types are shown in Table 2.

Table 2
Serotypes of the *Salmonella* spp. isolates isolated from caecal contents, carcass and environmental swabs. 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	Tot
Caecal 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%)
Environmental swab	3 (43%)	2 (29%)	1 (14%)	1 (14%)	–	–	–	–	–	7