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Impact of the sampling method and chilling on the *Salmonella* recovery from pig carcasses

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ABSTRACT

Differences in recovery of *Salmonella* on pig carcasses using non-destructive and destructive sampling methods is not well understood in respect to the chilling processes applied in slaughterhouses.
Therefore, in two slaughterhouses, four strains at two different concentrations were inoculated onto pork skin. Inoculated skin samples were sampled before and after chilling with two sampling methods: swabbing and destruction. Both slaughterhouses were visited three times and all tests were performed in triplicate. All samples were analysed using the ISO-method and recovered isolates were confirmed by PFGE.
The chilling system (fast or conventional cooling) nor the sampling step (before and after chilling) did not significantly influence the recovery of *Salmonella*. However, swabbing after chilling leads to an underestimation of the real number of contaminated carcasses. Therefore, destructive sampling is the more designated sampling method after chilling.

1. Introduction

Salmonella causes the second highest number of bacterial zoonotic illnesses in Europe. The major source of human infection, associated with up to 57% of the reported cases of salmonellosis in the EU, is the consumption of contaminated pork (EFSA and ECDC, 2013). The presence of meat contamination with human pathogenic bacteria, including *Salmonella*, originating from faecal contamination of the slaughtered animals, or from the slaughterhouse and/or cutting plant environment, remains difficult to detect, and is inherent to slaughter procedures (Belluco et al., 2015). However, it is one of the major requirements to comply to European legislation (Commission Regulation 2073/2005) which stipulates that contamination of pig carcasses with *Salmonella* should be monitored before chilling. Monitoring can be performed at two different steps at slaughter, with different perspectives concerning the interpretation of the outcome: carcasses are sampled before chilling in order to evaluate the hygiene of the slaughter procedure (Commission Regulation 2073/2005), and sampling is performed after chilling but before cutting to assess the amount of contaminated pig carcasses entering the food chain (EFSA, 2011). Several studies have already assessed the recovery of *Salmonella* on pig carcasses, and all reported a significant decrease of contaminated carcasses after chilling (Arguello et al., 2012; Botteldoorn et al., 2003; De Busser et al., 2011; Gonzales-Barron et al., 2013; Tamplin et al., 2001). This decrease has been attributed to the direct impact on the *Salmonella* cells of the cold temperature and drying of the carcass surface due to the cold air flow in the chilling room (Arguello et al., 2012). However, it has not been studied whether the reason of this decrease is linked to a killing of the live *Salmonella* cells, or if other phenomena related to the chilling period, such as a tight attachment of *Salmonella* to the skin, are involved.

The aim of the present study was to assess the impact of both the chilling step and the sampling method on the recovery of *Salmonella* from pig carcasses.

2. Material and methods

2.1. Slaughterhouses

Two pig slaughterhouses, A and B, each with a different commonly applied carcass chilling system, were selected. Slaughterhouse A practiced a fast cooling with an initial chilling step at a temperature between −18 and −20 °C with an air speed of 2 to 8 m/s during 90 min, followed by a stabilization at a temperature from 0 to 2 °C during 30 min. Slaughterhouse B used a conventional cooling system in which the carcasses are chilled in a cold store room at 0 to 4 °C during 16 h without forced

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air flow. After the described chilling phase, in both slaughterhouses, carcasses were ready for further processing.

2.2. Strains

Two frequently isolated *Salmonella* serovars on pork, *S. typhimurium* and *S. derby*, were employed (EFSA and ECDC, 2013; van Hoek et al., 2012). Four *Salmonella* isolates, two of each serovar, originating from pig faeces (De Busser et al., 2011), and characterized at serovar level by the Belgian *Salmonella* National Reference Center following the Kaufmann-White scheme, were used in the present study. The strains have been genotyped using pulsed field gel electrophorese (PFGE) by the Lab of Hygiene and Technology at Ghent University, to ensure inclusion of four different strains. Therefore the PulseNet protocol (Ribot et al., 2006) was applied with the following modification: the running condition was 6 V/cm at 14 °C in 0.5× Tris-Borate-EDTA buffer for 20 h with a ramping time from 2.2 to 54.2.

To prepare the inocula, strains were grown in tryptic soy broth (TSB) (Bio-Rad, Marnes-La-Coquette, France) and incubated for 24 h at 30 °C. After incubation, each broth was diluted in TSB to a final bacterial concentration of approximately 10^3 CFU/ml dilution broth and under cooled conditions transported to the slaughterhouse.

2.3. Inoculation and sampling of pork skin

Both pig slaughterhouses were visited three times. At each visit, 16 pieces of pork skin (10 cm × 20 cm) were collected from the back of different pig carcasses immediately after splitting. Each piece was attached, skin side up to a polypropylene (PP) tablet of approximately the same size, and on each piece, three squares of 25 cm^2 were delineated (Fig. 1). Per *Salmonella* strain (n = 4), four such skin pieces were prepared. Those four skin pieces were inoculated according to the following strategy: two were inoculated with 10 μl (= 10 CFU) of the dilution broth per square, while on the two other skin pieces, 50 μl (= 50 CFU) per square was inoculated. Each inoculum was spread over each 25 cm^2 square using a sterile inoculation loop.

The exact number of *Salmonella* present in the 10 and 50 μl dilution broths was determined by plating each dilution onto Plate Count Agar (PCA) just before use in the experiments, and colonies were counted after 24 h incubation.

Per *Salmonella* strain, one 10 CFU-inoculated pork skin piece and one 50 CFU-inoculated piece were then attached with a carrier strap to different carcasses just before entering the chilling room. The two other pieces were stored for 10 min at environmental temperature, after which they were sampled (= sampling step before chilling). The tablets that entered the cooling were collected in slaughterhouse A after 2 h and in slaughterhouse B after 16 h, at the moment that the carcasses left the chilling room (= sampling step after chilling).

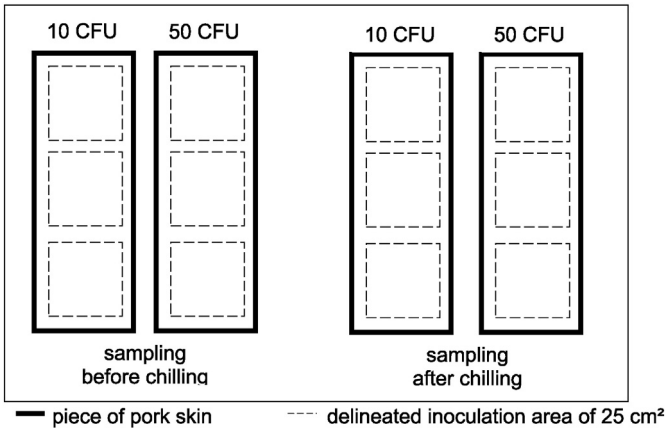

Fig. 1. Procedure for the inoculation of pork skin samples with one *Salmonella* strain.

To assess the impact of sampling, each of the three delineated squares was separately first swabbed with a premoistered (with 10 ml of 0.1% peptone water) cellulose sponge stick (3M™ Sponge-Stick SSL100, 3 M, Diegem, Belgium) (= swabbing method), after which each 25 cm^2 square was cut out and placed in a sterile bag (= destruction method).

The samples were transported to the laboratory under cooled conditions.

2.4. Salmonella isolation

Isolation was performed according to ISO 6579:2002 and ISO 6579:2002 Amd1:2007 (Anonymous, 2007). Therefore, 10 ml buffered peptone water (BPW) (Bio-rad) was added to the swabs, and the skin piece samples were diluted 1 to 10 in (w/v) BPW. All samples were homogenized in a stomacher blender for 1 min and incubated at 37 °C. After 16 to 20 h, of each preenrichment broth: a) 100 μl was added to 10 ml Rappaport Vassiliadis culture with soya (RVS) (Bio-rad), b) 1 ml was transferred to 10 ml Müller Kauffmann tetrathionate novobiocin broth (MKTTn) (Oxoid, Hampshire, UK) and c) three droplets (0.1 ml) were spotted on a modified semisolid Rappaport-Vassiliadis agar (MSRV) plate (Lab M, Lancashire, UK). After incubation of these selective enrichment media for 24 h at 42 °C (37 °C for MKTTn), the MSRV plates were examined for the presence of typical migration zones and a loopful of the edge of the migration zone was streaked on a xylose lysine deoxycholate (XLD) agar plate. A loopful from each RVS and MKTTn enrichment broth was streaked on a XLD agar plate. Following incubation for 24 h at 37 °C, all XLD plates were examined for the presence of typical colonies. Two suspect colonies per XLD plate were selected and confirmed biochemically using triple sugar iron, indol and lysine.

2.5. Genotyping

To distinguish the inoculated *Salmonella* strains used in the present study from those possibly present on the pork skin prior to inoculation, all *Salmonella* isolates were characterized by PFGE and profiles were compared with those of the original profiles of the inoculated strains (data not shown). Profiles were analysed by GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient with 2.1% position tolerance.

2.6. Statistical analysis

Excel software and Stata/MP 12.1 (StataCorp, 2011) were used for all analyses. Differences between slaughterhouses, sampling step (before and after chilling), inoculation amount (10 and 50 CFU), sampling method (swab versus destruction) and enrichment media were identified with a mixed-effects logistic regression applying a P-value of 0.05 for significance.

3. Results

The level of inoculation ranged from 7 to 12 and from 44 to 54 *Salmonella* cells, considering the 10 μl and 50 μl inocula respectively. In total, 288 (two slaughterhouses, each visited three times with 48 samples per visit) samples were inoculated and analysed. *Salmonella* was recovered from 223 samples (Table 1). No other than the inoculated *Salmonella* strains were recovered from the pork skin samples. The amount of inoculated bacteria influenced significantly the recovery rate of the inoculated samples: taking both sampling methods into account, the recovery rate was lower when 10 CFU (n = 93/144) than 50 CFU (n = 130/144) ($P < 0.05$) was inoculated. Furthermore, this lower recovery rate at lower inoculation levels was independent of the sampling step (50 and 62 before chilling and 43 and 68 after chilling, for 10 CFU and 50 CFU respectively) ($P > 0.05$). At the lowest inoculation level (10 CFU), sampling before chilling yielded more positive samples