Influence of pigskin on *Salmonella* contamination of pig carcasses and cutting lines in an Italian slaughterhouse

Silvia Bonardi,[1] Ilaria Bruini,[1] Irene Alpigiani,[1] Alice Vismarra,[1] Elena Barilli,[1] Franco Brindani,[1] Marina Morganti,[2] Paola Bellotti,[2] Luca Bolzoni,[2] Stefano Pongolini[2]

[1]Food Hygiene Unit, Department of Veterinary Science, University of Parma, Parma; [2]Risk Analysis Unit, Institute for Experimental Veterinary Medicine of Lombardy and Emilia-Romagna, Parma, Italy

Abstract

Ninety pig carcasses and twenty one food contact surfaces (FCSs) were tested for *Salmonella* in a slaughterhouse processing *ca.* 380 pigs/h between 2014-2015. Sampling was performed during seven sessions. Four carcass sites of 100 cm² each (back, belly, jowl externally, and the diaphragmatic area internally) were swabbed after evisceration. Meat conveyors and dressing tables were tested swabbing areas of 200 to 400 cm². After pre-enrichment in buffered peptone water, samples were tested by *Salmonella* MDS® assay and the presumptive positives were confirmed by the ISO 6579 method. *Salmonella* isolates were serotyped following the Kauffman-White-Le Minor scheme and genotyped by *Xba*I pulsed field gel electrophoresis. *Salmonella* was isolated from 16/90 [17.8%; confidence interval (CI) 95%=11.2-26.9] carcasses and 4/21 (19.0%; CI 95%=7.7-40.0) FCSs. Four serovars were identified on carcasses. *S. enterica* 4,[5],12:i:- was the most prevalent (43.75%), followed by *S.* Rissen (31.25%), *S.* Derby (12.5%) and *S.* Bovismorbificans (12.5%). Two serovars were found on FCSs, namely *S.* Derby (75%) and *S.* Livingstone (25%). During one sampling session, a failure in carcass dehairing occurred and caused significantly higher prevalence of carcass contamination (60%) than in the remaining sessions. Moreover, in the same session, *Salmonella* prevalence was marginally significantly higher on FCSs than in the remaining sampling days, suggesting that dehairing affects contamination not only on carcasses, but also on the working surfaces.

Introduction

For many years *Salmonella* has been the second most common food-borne pathogen in the European Union after thermotolerant *Campylobacter*. In several food-borne outbreaks pork and pork products have been associated with strong epidemiological evidence to salmonellosis cases in humans; in 2013, for instance, 8.9% of 314 strong-evidence outbreaks were associated with the consumption of pig meat and products thereof. On the contrary, poultry meat and bovine meat were responsible for 5.1 and 1.6% of the strong-evidence salmonellosis outbreaks in the EU, respectively (EFSA and ECDC, 2015).

Pigs are often healthy carriers of different *Salmonella* serovars, with *S.* Typhimurium, *S.* Derby, *S. enterica* 4,[5],12:i:- (known as the monophasic variant of *S.* Typhimurium) and *S.* Rissen as the most prevalent (Arguello *et al.*, 2012; EFSA and ECDC, 2015; Rostagno *et al.,* 2007; van Hoek *et al.*, 2012). These sub-clinically infected pigs may shed *Salmonella* with their faeces and/or keep the microorganisms in the gut, the associated lymph nodes or the tonsils (Fedorka-Cray *et al.*, 1999), thus representing a potential contamination source during slaughtering operations and eventually a threat to human health.

The aim of this study was to investigate the prevalence of *Salmonella* on pig carcasses and the relationship between carcass contamination and cross-contamination of the cutting line in a slaughterhouse located in Northern Italy. Genotyping was performed to trace the distribution of the different strains between carcasses and the slaughter environment.

Materials and Methods

Sampling

From February 2014 to May 2015, 90 pig carcasses and 21 food contact surfaces (FCSs) at cutting section were tested for *Salmonella* in a slaughterhouse of Emilia Romagna region, Northern Italy. The abattoir had a capacity of *ca.* 380 pigs per hour. Pigs at slaughter in this area of the country are minimum nine-month old with an average live weight of 160 kg, as required for the production of Parma ham, traditional salami and other cured pork products, which are *Protected Designation of Origin* (PDO) products. Carcasses belonged to 20 batches of pigs. A batch was defined as a group of pigs coming from a single herd on a given day. Pigs were reared in different herds of Northern Italy and were randomly sampled during 7 visits performed at intervals of 60-70 days. After evisceration three to seven carcasses per batch (average number: 4.5 per batch) were selected. The number of tested carcasses per visit varied among 10, 15 and 20. Four different carcass sites of 100 cm² each (back, belly and jowl externally and the diaphragmatic area

internally) were swabbed before chilling using a sterile sponge moistened with buffered peptone water (BPW) (3M Sponge Stick; 3M Health Care, St. Paul, MN, USA). After sampling, the sponge was placed in a sterile bag and stored at refrigeration temperature.

Three environmental surfaces per visit were sampled for a total of 21 samples, represented by 10 dressing tables and 11 meat conveyors. The working surfaces were tested swabbing areas of different size (200 to 400 cm² *ca.*) using a sterile sponge moistened with BPW (3M Health Care). Sponges were placed in sterile bags and immediately refrigerated. All samples were transferred to the laboratory under refrigeration on the day of collection and tested the same day.

Salmonella detection and typing

A total of 111 samples (90 carcass swabs and 21 environmental swabs) were tested using the molecular detection system assay for *Salmonella* (MDS *Salmonella*; 3M Health Care) based on the isothermal amplification and bioluminescence detection of specific sequences of *Salmonella* DNA. The samples were previously pre-enriched in BPW (3M Health Care) suspending each sponge in 100 mL of the liquid medium. The suspension was manually shaken for 2 min and then left at

room temperature for 2 h for resuscitation. The cultures were incubated at 37±1°C for 18±2 h. Thereafter, 20-µL aliquots of the broth cultures were tested by the MDS *Salmonella* assay following the manufacturer's instructions. Positive results were considered *presumptive positive* to be confirmed by the ISO 6579:2002 reference method (ISO, 2002).

Following the ISO 6579:2002 (ISO, 2002) method, the BPW cultures were inoculated into 10 mL of Mueller-Kauffmann tetrathionate broth (MKTT; Oxoid, Basingstoke, UK) and 10 mL of Rappaport-Vassiliadis soy broth (RVSB; Oxoid), with dilution rations of 1:10 and 1:100, respectively. The MKTT and RVSB cultures were incubated at 37±1°C and 41.5±1°C for 24 h, respectively. After incubation, a 10-µL loop of each culture was streaked onto Xylose Lysine Desoxycholate agar (XLD; Oxoid) and Chromogenic *Salmonella* agar (Oxoid) plates, incubated at 37±1°C for 24 h. Suspect colonies were seeded into Triple Sugar Iron Agar (Biolife, Milan, Italy), Lysine Iron Agar (Oxoid) and Christensen's Urea Agar (Biolife) and incubated at 37±1°C for 20-24 h. Cultures showing reactions typical of *Salmonella* were tested by slide agglutination with an O-omnivalent *Salmonella* serum (Denka Seiken, Tokyo, Japan). Biochemical identification to the genus level was performed by using the API 20 E® microsubstrate system (BioMérieux, Marcy l'Etoile, France).

Salmonella serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (Bio-Rad, Marnes-La Coquette, France; Denka Seiken; Sifin, Berlin, Germany). Definitive identification of *S. enterica* 4,[5],12:i:- and its differentiation from *S.* Typhimurium were obtained phenotypically by repeating phase inversion at least three times without evidencing expression of phase-two flagellar antigen and genotypically with the PCR protocol described by Barco *et al.* (2011).

Salmonella pulsed field gel electrophoresis (PFGE) typing was performed according to standard methods (PulseNet, 2010) with *Xba*I restriction of DNA. Confidence intervals in the observed prevalence were estimated by using the Wilson binomial approximation (Brown *et al.*, 2001). Statistical significance of differences in prevalence between groups was evaluated through Fisher's exact tests.

Results

Salmonella contamination in pig carcasses

Salmonella was isolated from 16 out of 90 [17.8%; confidence interval (CI) 95%=11.2-26.9] carcasses in five out of seven visits. In these five visits, the prevalence of *Salmonella*-positive carcasses varied widely, ranging from 10% (in three visits) to 40 and 60% (in the remaining two visits). The observed differences in the contamination of pig carcasses among visits can be explained with specific conditions observed at the slaughterhouse on the sampling days.

During a sampling session a failure in the dehairing procedure of pig carcasses occurred and a total of nine carcasses out of 15 (60%) ended up as *Salmonella*-positive. The prevalence on carcasses during this sampling session was significantly higher than in the remaining sessions (Fisher's exact test; P=9.3 10⁻⁷). In addition, carcasses coming from three different herds shared *S.* Bovismorbificans and *S. enterica* 4, [5], 12:i:- isolates belonging to the same PFGE genotype, suggesting contaminations between carcasses at the slaughterhouse. Conversely, during another sampling visit, 10 carcasses belonging to pigs coming from two herds were examined and *Salmonella* was isolated from four carcasses out of five belonging to a single herd, while all carcasses from the second herd were *Salmonella*-negative.

Four different *Salmonella* serovars were identified on pig carcasses (Table 1). *S. enterica* 4,[5],12:i:- was the most prevalent serovar (7/16; 43.75%), followed by *S.* Rissen (5/16; 31.25%), *S.* Derby (2/16; 12.5%) and *S.* Bovismorbificans (2/16; 12.5%). *Salmonella* isolates were genotyped by *Xba*I PFGE and different profiles were found among *S. enterica* 4,[5],12:i:-, *S.* Rissen and *S.* Derby serovars (Table 1). Two PFGE profiles were revealed for each serovar detected on pig carcasses.

Salmonella contamination in the slaughterhouse environment

Salmonella was detected in four out of 21 (19.0%; CI 95%=7.7-40.0) FCS samples (Table 1). *Salmonella* was isolated from 3/11 (27.3%) meat conveyors and 1/10 (10%) dressing tables. Two serovars were identified, namely *S.* Derby (3/4; 75.0%) and *S.* Livingstone (1/4; 25.0%). *Xba*I PFGE identified three different pulsotypes

among *S.* Derby isolates. *Salmonella* on FCSs was isolated in three out of seven visits. Interestingly, two of the isolates were recovered during the sampling session when the failure in the dehairing procedure occurred. The prevalence in FCS samples during this sampling session was marginally significantly higher than in the remaining sessions (Fisher's exact test; P=0.0796), suggesting that the dehairing procedure affects the contamination not only on carcasses, but also on the production surfaces in contact with them.

Discussion

The prevalence of carcass contamination by *Salmonella* in an Italian slaughterhouse processing *ca.* 380 pigs per hour was evaluated.

In our study the most common *Salmonella* serovars detected were *S. enterica* 4,[5],12:i:-, *S.* Rissen and *S.* Derby, as observed in previous surveys (Bonardi *et al.*, 2013; Alpigiani *et al.*, 2014). These serovars are commonly isolated from pigs in several EU member states and data reported by EFSA confirm the prevalence of *S.* Derby and *S. enterica* 4,[5],12:i:- in pigs at slaughter (EFSA, 2008; EFSA and ECDC, 2015). Moreover, in 2014, *S. enterica* 4,[5],12:i:- was responsible for more than 45% of the human cases of salmonellosis in Emilia Romagna region, Northern Italy. In that period, *S.* Derby ranked fifth among the serovars detected from affected humans, and *S.* Rissen was also included among the top 10 most common serovars. The detection of *S.* Bovismorficans on pig carcasses is interesting as this serovar caused a large outbreak in Germany due to the consumption of raw minced pork and fermented raw pork sausages (*Zwiebelmettwurst*) (Gilsdorf *et al.*, 2005). In 2013 *S.* Bovismorficans was responsible for 0.6% of all confirmed human cases of salmonellosis reported in the EU member states (EFSA and ECDC, 2015). These epidemiological and clinical data support the role of pigs as carriers of

Table 1. *Salmonella* **serovars and number of pulsed field gel electrophoresis profiles detected on pig carcasses and food contact surfaces at cutting lines in an Italian slaughterhouse.**

Serovar	Carcasses (n)	FCSs (n)	PFGE profiles (n)
S. enterica 4,[5],12:i:-	7 (43.75)	-	2
S. Rissen	5 (31.25)	-	2
S. Derby	2 (12.50)	3 (75.0)	4
S. Bovismorbificans	2 (12.50)	-	1
S. Livingstone	-	1 (25.0)	1
Total	16/90 (17.8)	4/21 (19.1)	10

FCSs, food contact surfaces; PFGE, pulsed field gel electrophoresis. Values in brackets are expressed as percentage.

Salmonella strains transmissible to humans.

A further important finding of the study was the significant increase in carcass contamination following a failure in the dehairing procedure that occurred during one of the sampling visits. Pigs were introduced into the slaughter line not properly dehaired and 60% of the tested carcasses ended up as *Salmonella*-positive, the highest contamination value of the study. This could have been the consequence of persistence of bristles on the pigskin.

Other studies support the role of pigskin in the microbiological contamination of pig carcasses. Rossel *et al.* (2009) observed that carcass contamination was directly linked to skin contamination of live pigs before stunning. Skin contamination rates were related with lairage duration and lairage hygienic conditions. In their study the probability of carcass surface contamination decreased from 59 to 35% whether the skin was contaminated or not. As polishing and singeing of pig carcasses during the slaughter process do not always overcome skin contamination by *Salmonella*, other post-harvest interventions could be suggested, such as a second singeing step after carcass polishing or decontamination of carcasses (De Busser *et al.*, 2013). A second singeing could be particularly important because the polishing step seems to be responsible for 5-15% of all carcass contamination (Berends *et al.*, 1997) due to the establishment of *Salmonella* on brushes and scrapes, which are difficult to clean (Borch *et al.*, 1996). In addition, hot water decontamination at 80°C for 14-16 sec. and hot water decontamination followed by acid lactic are effective in reducing *Salmonella* on pig carcasses (Jensen and Christensen, 2000; Hamilton *et al.*, 2010). Alternative treatments are represented by hand-held steam suction and the combination of steam and ultra-sound (Goldbach and Alban, 2006). Recently, regulation No 2015/1474/EC (European Commission, 2015) allowed food business operators (FBOs) to use recycled hot water to remove surface microbiological contamination from carcasses of domestic ungulates and farmed game under the precondition that the entire meat production chain strictly adheres to good manufacturing practices and hazard analysis critical control point principles. An important suggestion for FBOs can be the maintenance of high hygienic standards at lairage and the shortening of the lairage time, in order to reduce *Salmonella* spread among holding pigs belonging to the same batch (Bonardi *et al.,* 2016).

A noteworthy component of hygiene control systems is the availability of rapid and reliable methods for the detection of *Salmonella* in food and environmental samples. The *Salmonella* MDS assay (3M Health Care) used in this survey was characterised by a rapid response time (24 h) which allowed the FBO to quickly perform extraordinary interventions of cleaning and disinfections at the cutting line. Indeed, *Salmonella* cross-contamination from carcasses to the cutting equipment is considered unavoidable (Berends *et al.*, 1998). In our study, *Salmonella* cross-contamination from carcasses to FCSs at the cutting line was revealed only on one sampling day, when the same *S.* Derby pulsotype was detected both on a carcass and a dressing table. In other sampling sessions, the pulsotypes found on the cutting equipment were not detected on the carcass surfaces. This result was probably linked to the small number of carcasses tested per day (10 to 20) in comparison with the large number of slaughtered pigs (*ca.* 3000 per day), which could transfer a variety of *Salmonella* strains onto the cutting line. Even if we did not find any recurring *Salmonella* strain on FCSs and therefore did not observe any persistent isolate on the cutting line, other authors demonstrated that the isolates found on the cutting equipment might be part of the *resident flora*, as *Salmonella* may persist in biofilms in the slaughterhouse environment (van Hoek *et al.*, 2012; Piras *et al.*, 2015).

Berends *et al.* (1998) demonstrated that when contaminated pig carcasses are routinely brought into cutting lines, interim cleaning and disinfection of surfaces and utensils during breaks and at the end of the working day would most likely prevent not more than about 10% of all cross-contamination that takes place during a working day.

Conclusions

Our study focused on carcass and FCS contamination in a large pig abattoir of Northern Italy, where *ca.* 3000 pigs per day are processed. Carcasses contamination seemed to be influenced by hygiene practice during slaughter. This is the case of the failure in the dehairing of pigs, which was followed by the highest prevalence of *Salmonella*-positive carcasses ever seen in the study. The occasional failure in carcass dehairing demonstrated that also working surfaces were affected by an increment in *Salmonella* contamination. These findings are important because they confirm the role of bristles and pigskin in the final contamination of the carcasses and the slaughter environment. In addition, they stress the importance of hygienic measures during the whole slaughtering process, together with the implementation of any strategy to reduce the prevalence of *Salmonella* on the skin of pigs prior and during slaughter.

S. Derby, *S. enterica* 4,[5],12:i:-, *S.* Rissen and *S.* Bovismorbificans found on carcasses belong to serovars responsible for human foodborne infections linked to pig meat, thus confirming the importance of reducing *Salmonella* contamination at slaughter. Since pork and products thereof are the type of meat most often linked to notified outbreaks of salmonellosis within the European Union, microbiological testing at slaughter represents a useful tool to monitor this food-borne infection.

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