Salmonella prevalence and microbiological contamination of pig carcasses and slaughterhouse environment

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Abstract

In seven EC swine abattoirs *Salmonella* prevalence (ISO 6579/2002) and serotypes of 25 piglets, 61 finishing pigs (lymph nodes, colon content, carcass and liver surface) and slaughterhouse environments (scalding water, surfaces in contact with meat and not in contact with meat) were investigated. Moreover, aerobic colony count [total viable count (TVC); ISO 4833] and *Enterobacteriaceae* (ISO 21528-2) of piglets and finishing pigs' carcasses were evaluated, and the results compared with EU process hygiene criteria (Reg. EC 2073/2005). *Salmonella* was not isolated in any of the piglets samples. Prevalence differed between slaughterhouses (P<0.5), and *Salmonella* was isolated from 39 of 244 samples of finishing slaughtered pigs (15.9%) and from 4 of 45 environmental samples (8.9%). In pig samples, carcasses showed the highest prevalence (18%) followed by colon content (14.8%), lymph nodes (13%) and liver (1.6%). *S.* Anatum was the most prevalent serotype (71.8%), followed by *S.* Derby (33.3%), *S.* Bredeney (5%) and *S.* Holcomb (2.5%). Between environmental samples, *S.* Anatum (50%), *S.* Bredeney and *S.* Derby (25%) were identified. Total viable mean counts (log_{10} CFU/cm^2) of carcass surfaces ranged from 4.6 and 5.7 for piglets, and from 4.6 and 5.9 for finishing pigs, while *Enterobacteriaceae* ranged between 1.1 and 5 for piglets and between 2.1 and 5.3 for finishing pigs. These results were not in compliance with EU performance criteria. Total aerobic viable counts and *Enterobacteriaceae* mean levels of environmental samples appeared critical, particularly referred to surfaces in contact with meat (splitting equipment) and indicated an inadequate application of good manufacturing and hygiene practices during slaughtering and sanitisation.

Introduction

The food safety legislation in the European Union (EU) regulates the food chain and sets down specific rules for food of animal origin. The food businness operators (FBOs) have the primary responsibility of ensuring food safety. At slaughterhouse, the implementation of good hygiene practice and procedures based on hazard analysis and critical control point (HACCP) principles are essential to prevent microbial carcass contamination in order to ensure health protection and meat safety (Lindblad and Berking, 2013). Microbiological data are necessary for the implementation and mainteinance of HACCP-based systems (Zweifel *et al.*, 2005). For microbiological analyses of carcass samples, performance criteria are given in Commission Regulation (EC) No. 2073/2005 that sets out performance criteria for total viable counts (TVC), *Enterobacteriaceae* and *Salmonella* as process hygiene indicators. *Salmonella* detection at slaughterhouse can also be useful data for surveillance plans aimed to monitor the pig production chain (Nauta *et al.*, 2013). According to the Regulation (EC) No. 2160/2003 EU Member States (MS) are required to take effective measures to control *Salmonella* in different species, including pigs, in order to reduce the incidence of human salmonellosis. During the last two decades, pork has been recognised as a common food vehicle for human exposure (Mannion *et al.*, 2012) and many MS (*e.g.*, United Kingdom, Sweden, Germany, and Belgium) have already set out *Salmonella* surveillance and control programmes for pigs and pork (Arguello *et al.*, 2012). However, most MS have not yet implemented such programmes, including Italy. Each MS has to consider whether interventions should be set at farm and/or abattoir level (De Busser *et al.*, 2013) and different strategies could be chosen depending on the country-specific pig industry, the *Salmonella* herd-status and the slaughterhouse structure (Baptista *et al.*, 2010). Anyway, the abattoir remains the most appropriate stage of the food chain for the evaluation of the carriage of *Salmonella* and other zoonotic agents by farm animals (Bonardi *et al.*, 2013). Based on a recent EFSA report, 10.3% of slaughter pigs were found to be infected with *Salmonella* in lymph nodes and 8.3% of the carcasses were contaminated too (EFSA, 2008). Pig carcass contamination can result from the intestinal carriage of *Salmonella* in the pig itself, but also from contact with other contaminated carcasses or surfaces at slaughterhouse (Botteldoorn *et al.*, 2003). Hygiene varies between abattoirs and could have an impact on carcass contamination (McDowell *et al.*, 2007).

Aim of this study was to obtain data about the prevalence and the serotypes of *Salmonella* in piglets and finishing pigs at slaughterhouse. Moreover, the microbiological contamination of piglets and finishing pigs' carcasses was evaluated and the results compared with EU process hygiene criteria.

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Materials and Methods

The study was carried out in seven EC swine abattoirs (sA, sB, sC, sD, sE, sF, sG) in Sardinia. Samples were collected from 25 piglets and 61 finishing pigs during ten sampling days (SD). sA, sB and sF were visited two times (SD1 and SD2). sC, sD, sE and sG were visited once. During the first visit at sA, sB and at sC, specimens were collected from piglets (approximately 15 kg in weight) coming from local farms. During SD2 at sA and sB and at sD, sE, sF and sG, specimens were collected from finishing pigs (100-120 kg in weight). Pigs slaughtered at sA, sB, sD, sE, and sG and during SD1 at sF were from local farms. Pigs slaughtered during SD2 at sF were from Spain.

Salmonella isolation and serotyping

Samples were collected as previously described (Piras *et al.*, 2011). From each pig, after dressing and before chilling, samples of lymph nodes, colon content, carcass surface and liver surface were collected. Overall, 344 samples were collected from piglets and adult pigs (86 lymph-nodes, 86 colon content, 86 carcass surface and 86 liver surface). Moreover, the following samples were collected from slaughterhouse environments: scalding water with a sterile collection tube (10 samples), surfaces in

contact with meat (9 samples of carcass splitting equipment and 9 of dehairing equipment) and surfaces not in contact with meat (9 samples collected from the wall surface of the dirty zone, 9 from the wall surface of the clean zone and 9 from drains) with a sterile sponge. For the isolation of *Salmonella*, the ISO method 6579/2002 (ISO, 2002) modified according to EFSA report on *Risk assessment and mitigation opinion of* Salmonella *in pig production* was used. Presumptive colonies were submitted to phenotypic identification with the API ID 32E system (bioMérieux, Marcy l'Etoile, France). One colony from each positive sample (44 in total) was selected and sent to the laboratories of the *Centro Nazionale di Referenza per le Salmonellosi* in Legnaro (Padua, Italy), serotyped by agglutination tests with specific O and H antisera (Staten Serum Institute, Copenhagen, Denmark) and classified according to the Kauffmann-White scheme.

Process hygiene criteria evaluation

Each carcass was sampled after dressing and before chilling by sponge, according to ISO 17604 (Reg. EC No.2073/2005) at cheek, belly, back and ham sites in order to evaluate the process hygiene criteria stated by Reg. EC No.2073/2005. Therefore, samples were analysed for aerobic colony count (ISO 4833; ISO, 2003) and *Enterobacteriaceae* (ISO 21528-2) and the results expressed in $\log_{10}$ colony forming units/cm^2 (CFU/cm^2). Process hygiene criteria were also evaluated in samples collected from surfaces in contact and not in contact with meat described above. An analysis of variance (ANOVA) using the general linear model (GLM) procedure was performed for all considered variables, and when F-values were significant at the P<0.05 level, mean differences were separated by the least significant differences (LSD).

Results

Salmonella prevalence

Salmonella was not isolated in any of the samples collected from piglets, while it was isolated from 40 of 244 samples of finishing slaughtered pigs (16.4%) and from 4 of 45 environmental samples (8.9%). Table 1 shows *Salmonella* prevalence per slaughterhouse in finishing pigs and environmental samples. *Salmonella* prevalence was different among slaughterhouses (P<0.5). sF showed the highest prevalence in pig samples (46.2%), followed by sA (6.25%) and sG (2.5%). *Salmonella* was not isolated in any of the pig samples collected at sB, sD and sE. With regard to the sampling site, the highest prevalence was observed in carcasses (18%), followed by colon content (14.8%), lymph nodes (13%) and liver (1.6%). At sF, 12 pigs were found to carry *Salmonella* in lymph nodes and/or colon content, and in 9 of these pigs the pathogen was also isolated from the carcass. As regard to environmental samples, *Salmonella* was isolated at sF and sG with an overall prevalence of 11% (3/27) in samples of surfaces not in contact with meat and 5.5% (1/18) in samples of surfaces in contact with meat. At sF, *Salmonella* was isolated in one sample of drain water and in one of the wall surface of the dirty zone. At sG, *Salmonella* was isolated in one sample of drain water and in one sample of carcass splitter equipment.

Salmonella serotypes

A total of 44 *Salmonella* isolates were detected and 4 serotypes identified. Table 2 shows the detail of the *Salmonella* serotypes isolated from the pig and environmental samples per slaughterhouse and SD. *S*. Anatum was the most prevalent serotype in pig samples accounting for 71.8% (28/44) of all isolates. Other *Salmonella* serotypes isolated from the pig samples were *S*. Derby (33.3%, 13/44), *S*. Bredeney (5%, 2/39) and *S*. Holcomb (2.5%, 1/39). At sA, *S*. Derby was isolated from a sample of colon content, while *S*. Holcomb from the liver surface of one pig. At sF, during SD1 and SD2, *Salmonella* was detected in all pig sample type and also in samples of surfaces in contact with meat. All the isolates detected during SD1 were *S*. Anatum, while the isolates detected during SD2 were *S*. Derby. At sG, *S*. Bredeney was detected from a sample of carcass surface and from a sample of drain, while *S*. Anatum from a sample of carcass splitting equipment.

Three different serotypes were identified among 4 isolates detected from the environmental samples: *S*. Anatum (50%, 2/4), *S*. Bredeney and *S*. Derby (25%, 1/4).

Process hygiene criteria evaluation

Table 3 shows TVC and *Enterobacteriaceae* mean levels ($\log_{10}$ CFU/cm^2; mean±sd) of piglets and finishing pigs' carcass surface.

Carcass surface TVC regarding piglets carcass samples sC showed the highest levels (5.74±0.56), while lower mean counts were recorded at sA (5.00, with a prevalence of 10%) and sB (4.62±0.45). In finishing pigs' carcass surface samples higher mean values were recorded in all the slaughterhouses, in comparison to piglets. sB showed the highest levels (6.35±0.12). Mean counts >5 log were registered at sG (5.94±0.39), sD (5.92±0.32), sF (5.87±0.76) and sA (5.27±0.79). Significantly (P<0.5) lower levels were noticed at sE (4.69±0.48).

Carcass surface *Enterobacteriaceae*

sC showed significantly (P<0.1) higher levels (5.09±0.87) in carcass surface samples collected from piglets with respect to the other slaughterhouses. Lower mean counts were registered at sB (1.19±1.31), with a prevalence of 40%. As regard to finishing pigs carcass surface samples, sD showed the highest levels (5.34±0.56), followed by sF (4.85±1.80). Mean counts >3 log were noticed at sG (3.85±1.40), sA (3.80±0.27) and sB (3.57±0.81). The lowest levels were noticed at sE (2.17±1.25).

Table 4 shows TVC and *Enterobacteriaceae* mean levels ($\log_{10}$ CFU/cm^2; mean±sd) of surfaces in contact and surfaces not in contact with meat.

Environment total viable count

As regard to samples collected from contact surfaces with meat sF showed the highest lev-

Table 1. *Salmonella* prevalence in adult pigs and environmental samples per slaughterhouse.

Samples	Abattoirs					
	sA	sB	sD	sE	sF	sG
Pig						
Lymph nodes	- (0/8)	- (0/3)	- (0/10)	- (0/10)	40 (8/20)	(0/10)
Colon content	12.5 (1/8)	- (0/3)	- (0/10)	- (0/10)	40 (8/20)	(0/10)
Carcass	- (0/8)	- (0/3)	- (0/10)	- (0/10)	55 (11/20)	10 (1/10)
Liver	12.5 (1/8)	- (0/3)	- (0/10)	- (0/10)	50 (10/20)	(0/10)
Total	32	12	40	40	80	40
Environmental						
CM	- (0/4)	- (0/2)	- (0/2)	- (0/2)	- (0/4)	50 (1/2)
NCM	- (0/6)	- (0/3)	- (0/3)	- (0/3)	33 (2/6)	33 (1/3)
Scalding water	- (0/1)	- (0/1)	- (0/1)	- (0/1)	-	- (0/1)
Total	11	6	6	6	10	6

-, not detected; CM, surfaces in contact with meat; NCM, surfaces not in contact with meat. The number of positive samples out of the total is reported in brackets.

els (6.31±0.86 log), particularly in samples of dehairing equipment, followed by sB (5.87±0.96 log). The lowest levels were recorded at sA (3.22±2.8 log). Also for surfaces not in contact with meat samples, sF showed the highest levels (>6 log). Slightly lower counts were recorded at sE and sC (5.66±0.71 and 4.76±1.79 log respectively). The others abattoirs showed levels ranging from 2.8 to 4.

Environment *Enterobacteriaceae*

As for samples collected from surfaces in contact with meat, sF showed the highest mean counts (5.80±0.9). Levels of 2.62±2.2 and 3.42±0.98 were registered at sA and sB, respectively. For surfaces in contact with meat samples at sE and sF mean levels >5.5 log were detected. The other abattoirs showed significantly (P<0.1) lower counts, ranging from 1.5 and 2.7.

Discussion

This study showed a large variability in *Salmonella* prevalence among the different slaughterhouses, in accordance with other studies, showing that contamination depends on the slaughterhouse (hygienic parameters and qualification of personnel), the sampling day and the origin and the number of infected pigs delivered during the same day (Botteldoorn *et al.*, 2003). The importance of healthy pigs, carrying human pathogenic *Salmonella* strains, is already well known (Fosse *et al.*, 2009). As said, in our study, at sF *Salmonella* isolates belonging to the same serotype were detected during two different SD in lymph nodes and/or colon content and from the carcass. In addition, the same serotypes were detected from the liver surface and from environmental samples, namely the carcass splitter equipment. These results indicate a presumable cross-contamination of *Salmonella* on carcasses and slaughterhouse environment. However, further analysis (*e.g.* through pulsed field gel electrophoresis) is needed in order to characterise the isolates and better understand the *Salmonella* contamination routes. Our results on *Salmonella* prevalence in colon content samples of finishing pigs (14.8%) were slightly lower than those detected in other studies that showed levels of 21.9 % (Bonardi *et al.*, 2013) and 24.9% (Visscher *et al.*, 2011).

In our study, differences regarding serotypes detected at sF were noticed in relation to the origin (Spain and local) of the finishing pigs. During SD1, all the *Salmonella* isolates were identified as *S*. Anatum, which is quite common in pigs slaughtered in Spain

Table 2. *Salmonella* serotypes distribution per slaughterhouse, sampling day and sample.

Slaughterhouse	SD	Colon content	Lymph node	Carcass	Liver	Surfaces		Total
						NCM	CM	
sA	2	Derby (1)			Holcomb (1)		2	
sF	1	Anatum (6)	Anatum (5)	Anatum (10)	Anatum (5)		Anatum (1)	27
	2	Derby (2)	Derby (3)	Derby (1)	Derby (5)		Derby (1)	12
sG	1			Bredeney (1)		Bredeney (1)	Anatum (1)	2
Total		9	8	12	11	1	3	44

SD, sampling day; NCM, surfaces not in contact with meat; CM, surfaces in contact with meat. The number of isolates is reported in brackets.

Table 3. Total viable and *Enterobacteriaceae* mean counts (log10 CFU cm²; mean±sd) of piglets and finishing pigs carcasses.

Slaughterhouse	Piglets		Finishing pigs	
	TVC	*Enterobacteriaceae*	TVC	*Enterobacteriaceae*
sA	5.00±0 (10)^{B}	-	5.27±0.79^{B}	3.80±0.27^{B}
sB	4.62±0.45^{A}	1.19±1.31 (40)^{B}	6.35±0.12^{A}	3.57±0.81^{ABC}
sC	5.74±0.56^{A}	5.09±0.87^{A}	nd	nd
sD	nd	nd	5.92±0.32^{A}	5.34±0.87^{A}
sE	nd	nd	4.69±0.48^{C}	2.17±1.25 (80)^{C}
sF	nd	nd	5.87±0.76^{A}	4.85±1.80^{AB}
sG	nd	nd	5.94±0.39^{A}	3.85±1.40^{B}

TVC, total viable count; -, not detected; nd, not determined. Prevalence (%), if different from 100, is reported in brackets. $^{A-C}$Means within columns with different letters are significantly different (P<0.01; P<0.1; P<0.5).

Table 4. Total viable and *Enterobacteriaceae* mean counts (log_{10} CFU/cm²; mean±sd) of environmental samples.

Slaughterhouse	TVC		*Enterobacteriaceae*	
	CM	NCM	CM	NCM
sA	3.22±2.8 (66)^{B}	3.33±2.7 (66)^{B}	2.62±2.2(66)^{B}	2.11±3.01(33)^{B}
sB	5.87±0.96 (75)^{AB}	2.82±3.22 (50)^{B}	3.42± 0.98 (75)^{AB}	1,59±2.4 (50)^{B}
sC	4.58±2.98^{AB}	4.76±1.79^{AB}	4±1.41^{AB}	2,48±0.00 (66)^{B}
sD	4.5±1.76^{AB}	4.02±1.82^{AB}	4±0.56^{AB}	2,75±2.63 (66)^{B}
sE	3.50±3.13 (50)^{AB}	5.12±2.17^{AB}	5.65 (66)^{AB}	6.23 (66)^{AB}
sF	6.31±0.86 (66)^{A}	6.12±1.13^{A}	5.80±0.9(66)^{A}	6.29±0.71 (83)^{A}
sG	4.85^{AB}	5.66±0.71^{AB}	3.64±0.79^{AB}	5.97±0.65^{B}

TVC, total viable count; CM, surfaces in contact with meat; NCM, surfaces not in contact with meat. Prevalence (%), if different from 100, is reported in brackets. ^{A,B}Means within columns with different letters are significantly different (P<0.01; P<0.1; P<0.5).

(Arguello *et al.*, 2012, 2013; Hernandez *et al.*, 2013) and in other European countries (De Busser *et al.*, 2011; McDowell *et al.*, 2007). On the contrary, during SD2 all the isolates were identified as *S*. Derby, which is one of the most common serovar detected in slaughtered pigs, in accordance with EFSA report (EFSA, 2013) and a number of studies carried out in Italy, the rest of Europe (Bonardi *et al.*, 2013; De Busser *et al.*, 2011) and also in Sardinia (Piras *et al.*, 2011). Furthermore, *S.* Derby is between the 10 most reported serotypes in confirmed cases of human salmonellosis (EFSA, 2013), whereas *S*. Anatum has not been linked to cases of human salmonellosis.

Salmonella was not isolated in any of the samples collected from piglets. Not many data are available on *Salmonella* prevalence in piglets. In a study carried out by Funk *et al.* (2004) a very low prevalence – between 0.5 and 0.7 in fecal samples – was detected. An investigation conducted at farm showed *Salmonella* prevalence of blood sera samples of piglets/weaners >20%. However, *Salmonella* was detected only in a single case in the pooled faecal samples taken from the same pens (Nowak *et al.*, 2007).

Total viable mean counts (log_{10} CFU/cm^2) of carcass surfaces ranged from 4.6 and 5.7 for piglets, and from 4.6 and 5.9 for finishing pigs. Such levels are higher than those detected by other studies. Bolton *et al.* (2002), in a small-scale slaughterhouse, registered level accounting between 4.5 and 4.7 log. In Switzerland, Zweifel *et al.* (2008) obtained TVC mean counts that averaged out at 3.3 log. *Enterobacteriaceae* mean counts ranged between 1.1 and 5 for piglets, with a prevalence accounting between 40 and 100%. As for finishing pigs are concerned, mean levels ranged between 2.1 and 5.3 log, with a prevalence between 66 and 80%.

Conclusions

The majority of TVC and *Enterobacteriaceae* results were not in compliance with performance criteria of Reg. (EC) 2073/2005. The high carcass contamination levels indicated that a detailed analysis of the slaughtering process, including microbiological data, is necessary, also considering that carcasses may be contaminated despite the absence of visible contamination (Arguello *et al.*, 2013).

Total viable counts and *Enterobacteriaceae* mean levels of environmental samples appeared to be critical, particularly referred to surfaces in contact with meat (splitting equipment). At sF, TVC and *Enterobacteriaceae* counts indicated an inadequate application of good manufacturing and hygiene practices during slaughtering and sanitisation.

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