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The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses

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SUMMARY

This study was part of an international research project entitled SALINPORK (FAIR CT-950400) initiated in 1996. The objectives were to investigate the occurrence of *Salmonella* in pig slaughterhouses and to identify risk factors associated with the contamination of pig carcasses. Data was collected from 12 slaughterhouses in five European countries. Isolates were characterized by serotyping, phage typing and antimicrobial susceptibility. In one country, no *Salmonella* was found. *Salmonella* was isolated from 5·3% of 3485 samples of pork and from 13·8% of 3573 environmental samples from the seven slaughterhouses in the four remaining countries. The statistical analyses (multi-level logistic regression) indicated that the prevalence was significantly higher during the warmer months and that the environmental contamination increased during the day of slaughter. The polishing (OR 3·74, 95% CI 1·43–9·78) and pluck removal (OR 3·63, 95% CI 1·66–7·96) processes were found to contribute significantly to the total carcass contamination, the latter especially if the scalding water also was contaminated. To reduce carcass contamination, it is recommended to ensure sufficiently high temperatures of scalding water (62 °C) and appropriate cleaning and disinfection of the polishing equipment at least once a day in order to reduce the level of carcass contamination and consequently the prevalence of *Salmonella* in pork.

INTRODUCTION

Despite many efforts to prevent and control food-borne salmonellosis during the last two decades, *Salmonella* continues to be one of the leading causes of human gastroenteritis in most European countries [1]. During the 1990s, the importance of pork as a source of human salmonellosis was increasingly recognized [2–4], and several generalized outbreaks implicating pork and pork products as vehicles for *Salmonella* infection have been described [e.g. 5–10]. In the late 1990s, the proportion of human salmonellosis cases attributable to pork and pork products was estimated to be approximately 10% of all cases in Denmark, 15% in The Netherlands and 20% in Germany [11–14]. Pork and pork products are now recognized as one of the most important sources of human salmonellosis in some European countries.

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Pigs infected with *Salmonella* most often carry *Salmonella* bacteria without showing any symptoms of disease [15]. Unlike some of the classical zoonotic diseases, e.g. tuberculosis, salmonellosis cannot be detected by the traditional meat inspection [16]. *Salmonella* bacteria are primarily located in the gastro-intestinal tract from the oral cavity to the rectum of the subclinically infected pigs. During transport and lairage, subclinically infected pigs may shed *Salmonella* and thereby constitute a source of contamination of other pigs kept in the same environment [17–19]. Positive pigs will carry *Salmonella* on the skin, in the faeces or in the mouth, and the contamination or cross-contamination of carcasses is basically a question of redistributing the *Salmonella* bacteria from the positive pigs during the various slaughter processes. The epidemiology of *Salmonella* at the slaughterhouse level is, therefore, primarily due to direct or indirect faecal contamination of live pigs or carcasses [20].

Control of *Salmonella* in pork at the slaughterhouse level involves selection of uncontaminated raw materials, minimizing contamination and cross-contamination, preventing multiplication of *Salmonella* bacteria and introducing procedures of decontamination [21]. By identifying sources and processes of cross-contamination and modifying slaughter procedures according to these findings, the risk of carcass contamination can be reduced.

There exist many opportunities for a pig carcass to become contaminated during the slaughter process. Based on extensive literature review, where the results from many different studies were combined, Berends et al. [22] estimated that 5–15% of all carcass contamination occurs during polishing, 55–90% during evisceration and 5–35% during further processing, i.e. dressing, splitting and meat inspection. The study presented here tried to identify the factors and processing steps that contribute most to the overall contamination of carcasses and the slaughterhouse environment by using the same sampling protocol in 12 slaughterhouses in five European countries. For the statistical analyses, we used logistic regression analysis with random effects, which is a method widely used in veterinary epidemiology, where the occurrence of clustered data is common [23, 24]. It is, however, to our knowledge the first time that this method has been used to identify factors associated with carcass contamination and explore the epidemiology of *Salmonella* contamination at the slaughterhouse level. The results of the analyses are presented as odds ratios (ORs), which may be used for quantitative risk modelling as suggested by Berends et al. [25].

The specific objectives of the study were:

- to estimate the prevalence of *Salmonella* isolated from pig carcasses, livers, tongues, and from the abattoir environment;
- to assess variations in contamination between slaughterhouses, during the study period and during a slaughter day;
- to identify critical slaughter processes for *Salmonella* contamination of pig carcasses;
- to characterize the isolated *Salmonella* strains by epidemiological typing methods (serotyping, phage typing, and antimicrobial resistance testing), and compare the occurrence of the different types between and within slaughterhouses.

The study was part of an international research project entitled '*Salmonella* in Pork' (SALINPORK, FAIR CT-950400). The project was initiated in 1996 as a cooperation between nine institutions from the following six European Union member states: Denmark, Germany, Greece, Sweden, The Netherlands and the United Kingdom [26]. The overall aim of the project was to investigate the epidemiology of *Salmonella* in the pork production chain, and on this basis propose control options at the farm and in slaughterhouses.

METHODS

Slaughterhouse selection

The selection of slaughterhouses was based on the geographical position and practical design of the slaughterhouses, and the companies' willingness to participate. A total of 12 industrial slaughterhouses (in the following designated from A to L) in five European Union member states were selected. By request of one of the participating countries, the country of origin will not be presented by name, but the location of slaughterhouses per country was as follows: Country 1, slaughterhouses A and B; Country 2, C and D; Country 3, E and F; Country 4, G; Country 5, H–L.

The number of slaughter lines used for pig slaughtering ranged from one to four per slaughterhouse. Nine of the slaughterhouses had only one line, and samples were generally collected from the same line throughout the study period. Slaughterhouse E had two integrated lines, where carcasses during some

Table 1. *Distribution of samples into a number of sampling rounds (six in this example) from one day*

Sampling round …	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	Total
Sampling materials				No. of samples			
Tongues + pharynx	0	5	5	5	5	5	25
Liver	0	5	5	5	5	5	25
Carcass	0	10	10	10	10	10	50
Water from scalding tank	5	2	2	2	2	2	15
Polisher	5	2	2	2	2	2	15
Carcass splitter	5	2	2	2	2	2	15
Water outlets representing the following slaughter processes							
(1) Evisceration	1	1	1	1	1	1	6
(2) Pluck removal	1	1	1	1	1	1	6
(3) Carcass splitting	1	1	1	1	1	1	6
(4) Trimming	1	1	1	1	1	1	6
(5) Just before chilling room	1	1	1	1	1	1	6
Hands from personnel taking care of the following slaughter processes							
(1) Bung removal	0	1	1	1	1	1	5
(2) Evisceration	0	1	1	1	1	1	5
(3) Pluck removal	0	1	1	1	1	1	5
(4) Carcass splitting	0	1	1	1	1	1	5
(5) Meat inspection of carcass	0	1	1	1	1	1	5
Total	20	36	36	36	36	36	200

[a] Sampling was carried out before onset of slaughter.
[b] Sampling was carried out with at least half an hour between sampling rounds.

slaughter processes (e.g. scalding, dehairing and bung loosening) were handled on a single line.

In slaughterhouses C and D, the number of pigs slaughtered per day ranged from 200 to 700 (50–100 per hour) depending on the workload. This number was much lower than in the other slaughterhouses. The highest speed of slaughter was in slaughterhouse E where approximately 800 pigs were slaughtered per hour per slaughter line. The speed at the other slaughterhouses ranged from 140 to 500 pigs per hour per slaughter line.

A low number of pigs slaughtered in slaughterhouses C and D, resulted in fewer samples collected per day as compared to the other slaughterhouses. However, the two slaughterhouses were visited twice as many times. A different sampling protocol was also used in these slaughterhouses due to differences in slaughter processes and flow of pigs. The main difference was that the dehairing operations (i.e. scalding, dehairing, singeing and polishing) were absent, because the pigs were skinned. Therefore samples from the skin removal process were collected instead.

Five slaughterhouses (H–L) used vertical scalding of carcasses by steam instead of the traditional vat scalding. In addition, these slaughterhouses routinely used a procedure to prevent faecal contamination during evisceration, e.g. a plastic bag placed around the anus. Such procedures were also in use in slaughterhouses A and B.

Sampling

Participants in the project agreed upon a common protocol for sample collection (Table 1), however, due to the above-mentioned differences between slaughterhouses, some discrepancies in the practised methods were inevitable.

The slaughterhouses were visited between 3 and 13 times during the study period. Each sampling day was divided into a number of sampling rounds with at least half an hour between rounds (Table 1). The first sampling round was done before the onset of slaughter in the cleaned slaughterhouse. The number of rounds per day varied between 2 and 6 depending on the number of pigs slaughtered. Approximately 100 product samples and 100 environmental samples were collected per day. Product samples were defined as swab samples taken from carcasses, livers and tongues, whereas the environmental samples were

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samples of water from outlets and scalding tanks, and swab samples of equipment and the hands of slaughterhouse personnel. The environmental samples represented various slaughter processes and were collected throughout the slaughter line from scalding to blast chilling. In each sampling round, sampling of the environment was done prior to the sampling of products. The number of samples collected per sampling round is presented in Table 1.

Swabs were made of sterilized disposable diapers (Billies, Mölnlycke B.V., Amstelveen, The Netherlands) with no plastics or preservatives. This technique was described by Van den Elzen and Snijders [27] and validated for *Salmonella* isolation by Swanenburg et al. [28]. The swabs were packed separately in sterile plastic bags (Stomacher). Shortly before sampling the swabs were moistened with 50 ml sterile buffered peptone water with 0·1% Tween (BPW–Tween). By turning the plastic bag inside out while holding the swab through the bag, the sampling area was swabbed. After replacing the swab in the plastic bag, another 50 ml BPW–Tween was added. The swabs were then massaged by hand or in a stomacher bag for 2 min if the latter equipment was available at the slaughterhouse. The fluid was squeezed from the swab into the stomacher bag or poured into a sterile test tube. All samples were forwarded to the laboratory in charge of analysis. Swab and water samples were cooled to 4–5 °C and kept at this temperature until processing. Samples arriving at the laboratory on the same day as collected were not necessarily cooled to 4–5 °C.

Livers of the selected pigs were swabbed over the surface on both sides of the liver with one diaper. Tongues were swabbed with one diaper, from the dorsal part of the larynx, over the pharynx until the tip of the tongue. The external surface of the carcasses was swabbed with one diaper per carcass; in one movement from the tarsus until the ear over the back side of one half of the carcass. Thereafter the diaper was turned and the carcass was swabbed from the tarsus over the belly side until the cranial breast-cavity opening. The total area swabbed was around 0·4 m^2. The livers and tongues were swabbed just after removal of the pluck and the carcasses immediately before or after blast chilling.

Water from the scalding tank and water outlets was collected with sterile syringes or pipettes and poured into sterile tubes. Each sample consisted of 25 ml water. In slaughterhouses A and B, the temperature of the scalding water was measured during each sampling round. The samples of water outlets were not collected from inside the outlet, but rather from the water flowing on the floor towards the outlets, so that they represented a specific slaughter process. In case there was too little water, e.g. before onset of slaughter, swab samples of the floor (*c.* 0·1 m^2) were collected instead. From both the carcass splitter and the polisher, a surface area of approximately 0·1 m^2 was swabbed at each sampling. Swab sampling of the hands of slaughterhouse personnel was carried out by 'shaking hands' with the swab or by swabbing the palm of the worker's hand.

Isolation of *Salmonella*

The participants agreed on common Microbial Standard Operating Procedures for isolation of *Salmonella* [26]. Serotyping was performed according to the Kaufmann–White scheme [29] using the routine procedure of each participating laboratory. Non-typable strains or autoagglutinable strains (rough) were verified as *Salmonella enterica* by conventional biochemical analysis. All strains were forwarded to the Danish Veterinary Institute for further characterization.

Phage typing of *S.* Typhimurium was performed according to the 'Colindale scheme' described by Callow [30] and extended by Anderson et al. [31] using typing phages kindly provided from Dr Linda Ward (Central Public Health Laboratory, Colindale, London, UK). Non-typable strains were re-serotyped before they were assigned as NT. Antimicrobial susceptibility testing was performed by agar-diffusion test [32] using NeoSensitabs as described by the supplier (Rosco, Glostrup, Denmark).

Statistical methods

The project coordinator provided all participants with a data entry file and a written guide to be used for entering the results in Epi-Info [33] in a standardized way. The environmental samples were categorized in a new variable (PROCESS), which described the slaughter process that the samples represented, i.e. lairage, scalding, polishing, bung removal, evisceration, pluck removal, carcass splitting, meat inspection, trimming, skinning, or floor just before chilling. Also two other variables describing the sampling method (swab sample or water sample) and the origin of the sample (hand, knife, equipment or outlet water) was defined. These two variables were called METHOD and ORIGIN, respectively.

Table 2. *Schematic presentation of the three logistic regression analyses describing the outcome and explanatory variables*

Analysis no.	Dependent variable	Coding of dependent variable	Independent variables	Independent variables included as	No. of obs.
No. 1. Environmental samples only	Result of the individual sample	0 = negative 1 = positive	Slaughterhouse Season Sampling day Sampling round Slaughter process[a] Origin of sample Sampling method	Fixed Fixed Random Fixed Fixed Fixed Fixed	3200
No. 2. Product samples only	Result of the individual sample	0 = negative 1 = positive	Slaughterhouse Season Sampling day Sampling round Product type[b]	Fixed Fixed Random Fixed Fixed	3485
No. 3. Carcass and environmental samples	Proportion of positive carcasses in a sampling round	No. of positive carcasses/no. of carcasses sampled	Slaughterhouse Season Sampling day Sampling round Result of sampling the slaughter processes[a] in a sampling round (0/1)	Fixed Fixed Random Fixed Fixed	1130 Carcasses 126 Sampling rounds

[a] For example, scalding, polishing, bung removal, evisceration, pluck removal, splitting, meat inspection or trimming.
[b] For example, carcass, liver or tongue.

The data were hierarchically structured, meaning that one would expect the variations between slaughterhouses to be larger than the variation within one slaughterhouse. Further, that the variation between sampling days was larger than the variation within the same day and finally that the variation between sampling rounds was larger than the variation within the same sampling round. In order to control for the expected differences in variation, a multi-level logistic regression model with random effects was developed [24, 34, 35]. The model was used for three separate analyses (Table 2). In all three analyses, the slaughterhouse, the season, and the sampling round were included as fixed effects, whereas the sampling day was included as a random effect.

Contamination of environment and products (*analyses nos. 1 and 2*)

Analysis no. 1 included only environmental samples, whereas analysis no. 2 only included product samples. In both analyses, the result of the individual sample (positive or negative for *Salmonella*) was the dependent variable.

The slaughter-process variable PROCESS and the variables METHOD and ORIGIN were included as explanatory variables in analysis no. 1. Further, in order to test if the *Salmonella* contamination of the environment increased during the slaughter day and/or during the course of slaughter, a trend test including the sampling round and the PROCESS as continuous variables was performed. In analysis no. 2, the product type (carcass, liver or tongue) was included as an explanatory variable (Table 2).

Identification of critical slaughter processes for carcass contamination (*analysis no. 3*)

The specific objective of analysis no. 3 was to identify slaughter processes contributing significantly to the total contamination of pig carcasses. In other words, the purpose was to test if the *Salmonella* contamination found during any of the slaughter processes was associated with the degree of carcass contamination. The epidemiological unit of interest was no longer the individual samples, but the sampling round. Consequently, the dataset was rearranged, so that one observation contained the following information:

Slaughterhouse

- Season
  - Sampling date
    - Sampling round
      - *Salmonella* isolated/not isolated from the various slaughter processes in a sampling round
      - Proportion of contaminated carcasses per sampling round

As illustrated, the proportion of *Salmonella*-positive carcasses in a sampling round was the dependent variable, whereas the explanatory variables were the result of sampling the various slaughter processes, i.e. if one or more positive samples were obtained from a certain slaughter process in a given sampling round, that process was coded 1. Otherwise it was coded 0 (Table 2).

Initially, to get an idea of the association between the proportion of positive carcasses and contaminated slaughter processes, all slaughter-process variables were screened one by one in a basic model including the slaughterhouse, the season, the sampling day and the sampling round. Afterwards, a final multivariate model was built using forward selection with a test for backward elimination [34] until remaining parameter estimates had a significance level of approximately 0·20. This approach was used because the sample size was insufficient to include all variables with a significance level of 0·20 in the basic model. Then two-factor interaction terms between remaining slaughter-process variables were included in the model and backward elimination was continued until a significance level of 0·05 was reached for all included slaughter-process variables and interaction terms.

For all three analyses, SAS 6.12 (the mixed procedure and the macro GLIMMIX) was used for analysis [36].

RESULTS

In five of the participating slaughterhouses (H–L) located in the same country, *Salmonella* was not isolated from any of 1778 product samples and 1610 environmental samples. The results from these slaughterhouses are not included in the following description.

In the other slaughterhouses (A–G), *Salmonella* was isolated from a total of 5·3% of the 3485 product samples ranging from 2·5 to 8·5% between slaughterhouses (Table 3). Of the 1623 examined carcasses, 62 (3·8%) were contaminated with *Salmonella*. The proportion of positive carcasses ranged from 1 to 8% between slaughterhouses. The overall proportion of positive samples of livers and tongues was higher than the proportion of positive carcasses, but there was some variation between slaughterhouses (Table 3).

Salmonella was isolated from 13·8% of the 3576 environmental samples (ranging from 6·3 to 28·3% between slaughterhouses) (Table 3). In all slaughterhouses, *Salmonella* could be isolated from the environment before onset of slaughter (i.e. sampling round 1), but the prevalence was generally higher in samples taken in sampling rounds during slaughter (Fig. 1).

The contamination level of water from the scalding tank was generally low, although, the prevalence in B and E was somewhat higher than in the other slaughterhouses (Table 3). The polishing equipment in slaughterhouse A was more frequently contaminated with *Salmonella* compared to the other slaughterhouses (Table 3). During the study period, three different slaughter lines were sampled in slaughterhouse A and *S.* Ohio was repeatedly isolated from the polishing equipment on two of those lines. Four of five positive carcasses from slaughterhouse A were contaminated with *S.* Ohio. The carcass splitter in slaughterhouse F was more frequently contaminated with *Salmonella* compared to other slaughterhouses (Table 3). *S.* Infantis was isolated from the carcass splitter on all three sampling days. This serotype was also isolated from 8 of 9 positive carcasses in slaughterhouse F.

High levels of contamination were found in samples from water outlets (Table 3), especially in slaughterhouses E and F. The hands of slaughterhouse personnel were only occasionally contaminated with *Salmonella*. There was little variation between slaughterhouses, although no positive samples were recovered from slaughterhouse E (Table 3). Samples of knives were only collected from slaughterhouses C and D. The level of contamination roughly corresponded to the level found on the hands of personnel (Table 3).

The overall distribution of serotypes can be seen in Table 4. *S.* Typhimurium and *S.* Derby were isolated from all slaughterhouses. *S.* Typhimurium was the most frequently occurring serotype in slaughterhouses A, B, E and G. In slaughterhouse G, approximately 70% of the positive samples belonged to this serotype. In slaughterhouses A, B and E, *S.* Typhimurium was isolated from approximately 50% of the positive samples. *S.* Derby was frequently isolated

Table 3. *Occurrence of* Salmonella *in environmental and product samples collected from 7 of 12 examined slaughterhouses. In 5 slaughterhouses from the same country, no* Salmonella *was found*

	Slaughterhouse															
	Country 1				Country 2				Country 3				Country 4			
	A		B		C		D		E		F		G		Total	
Sampling location	*n*	% pos.	*n*	% pos.	*n*	% pos.	*n*	% pos.	*n*	% pos.	*n*	% pos.	*n*	% pos.	*n*	% pos.
									Product samples							
Carcass	299	1·7	300	5·0	197	6·6	176	8·0	164	1·2	187	4·8	300	1·0	1623	3·8
Liver	150	4·0	150	2·7	110	3·6	90	12·2	122	12·3	162	4·3	150	2·7	934	5·5
Tongue	150	9·3	145	9·0	110	5·5	86	5·8	123	13·8	164	6·1	150	5·3	928	7·9
Total	599	4·2	595	5·4	417	5·5	352	8·5	409	8·3	513	5·1	600	2·5	3485	5·3
									Environmental samples							
Scalding tank	90	1·1	90	5·6	—	—	—	—	43	4·7	45	0·0	90	0·0	358	2·2
Polishing equipment	90	35·6	90	2·2	—	—	—	—	46	4·3	45	0·0	90	1·1	361	10·2
Carcass splitter	90	7·8	90	5·6	19	10·5	19	0·0	31	6·5	45	31·1	90	6·7	384	9·4
Other equipment	23	0·0	—	—	—	—	—	—	—	—	—	—	—	—	23	0·0
Water outlets	180	16·7	179	27·4	172	20·9	139	18·0	88	44·3	87	74·7	180	16·7	1025	27·4
Hands	145	2·8	146	4·8	145	5·5	140	5·0	73	0·0	75	6·7	150	0·7	874	3·7
Knives	—	—	—	—	129	3·9	122	1·6	—	—	—	—	—	—	251	2·8
Total	768	17·2	745	14·6	465	11·0	420	8·1	281	16·0	297	28·3	600	6·3	3576	13·8
No. of sampling days	6		6		13		12		3		3		6		49	

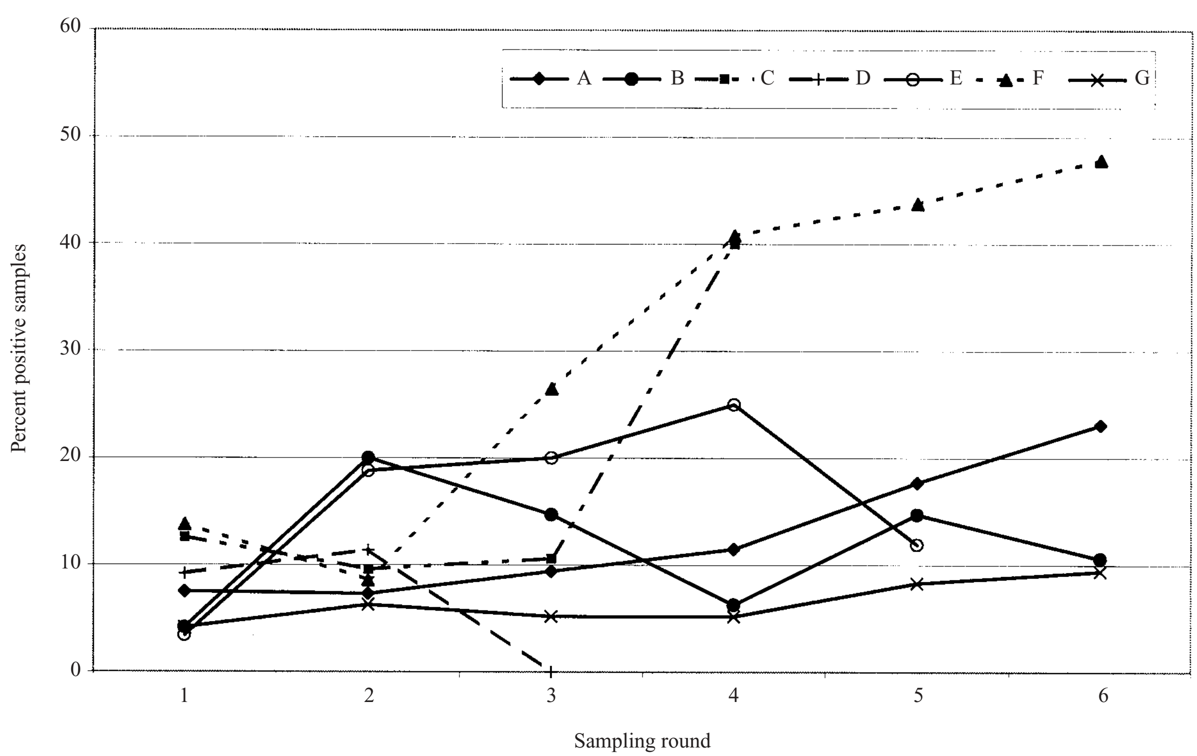

Fig. 1. *Salmonella*-positive environmental samples per sampling round in each of seven European Union slaughterhouses. In five slaughterhouses (H–L), no *Salmonella* was found.

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Table 4. *Occurrence (%) of* Salmonella *serotypes in samples of environment (E) and products (P) in seven slaughterhouses*

	Country 1				Country 2				Country 3				Country 4		
	A		B		C		D		E		F		G		
	E	P	E	P	E	P	E	P	E	P	E	P	E	P	Total
S. Typhimurium	5·2	2·2	6·7	2·7	2·4	0·5	0·5	0·9	8·5	3·2	4·0	1·4	4·8	1·3	4·0
S. Derby	0·3	0·2	3·9	2·0	2·2	2·2	2·9	3·7	0·7	0·2	5·4	0·8	0·7	—	1·7
S. Infantis	0·5	—	—	—	—	—	1·7	1·4	1·1	—	8·8	1·6	—	—	0·8
S. Ohio	4·7	1·0	—	—	—	—	—	—	—	—	—	—	—	—	0·5
S. Panama	—	—	—	—	0·9	—	—	—	2·5	3·2	—	—	0·8	1·2	0·5
S. London	—	—	—	—	2·6	2·6	—	—	1·4	0·2	1·0	—	—	—	0·4
S. Livingstone	0·2	—	0·3	0·7	0·4	—	—	—	3·2	—	1·3	—	—	—	0·3
S. Virchow	—	—	—	—	—	—	—	—	—	—	6·4	—	—	—	0·3
S. 9.12:LV:	0·5	0·5	0·2	—	—	—	—	—	—	—	—	—	—	—	0·3
S. Bredeney	—	—	—	—	0·9	0·2	2·1	0·3	—	—	—	—	—	—	0·2
S. Brandenburg	—	—	—	—	—	—	—	—	0·7	—	1·0	0·4	—	—	0·1
S. Goldcoast	—	—	—	—	—	—	0·7	0·9	—	—	—	—	—	—	0·1
S. Mbandaka	—	—	—	—	—	—	—	0·3	—	—	1·3	0·6	—	—	0·1
Not typable	0·3	0·3	—	—	0·6	—	—	0·6	—	0·5	—	0·2	—	—	0·2
Others	0·3	—	0·3	—	1·1	—	0·2	0·6	0·4	1·0	5·4	0·2	—	—	0·5
Total	12·0	4·2	11·4	5·4	11·0	5·5	8·1	8·5	18·5	8·3	34·7	5·1	6·3	2·5	10·0
No. of samples	618	599	595	595	465	417	420	352	281	409	297	513	600	600	7061
No. of typed isolates	74	25	68	32	51	23	34	30	52[a]	34	103[b]	26	38	15	709

[a] Seven double infections.
[b] Eighteen double infections and 1 triple.

from slaughterhouses B, C, D and F. Some serotypes like *S.* Livingstone, *S.* Infantis and *S.* Panama were also frequently recovered from slaughterhouses from several countries, whereas, e.g. *S.* Bredeney and *S.* Brandenburg were only recovered from slaughterhouses within a single country. Finally, *S.* Ohio, *S.* Virchow and *S.* Goldcoast were only found in a single slaughterhouse and with the exception of *S.* Ohio isolations were made on the same day.

The distribution of serotypes varied between slaughterhouses in different countries, but showed only little variation between slaughterhouses within the same country (Table 4). The diversity was lowest in slaughterhouse G, where only three different serotypes were isolated, and highest in F, where 14 different serotypes were found.

Within each slaughterhouse, there was a good correlation between the serotypes found in the environment and those isolated from the products (livers, tongues and carcasses). In general, no serotypes were isolated from the products without also being recovered from the environment, whereas some serotypes occurring in the environment were not recovered from the product samples (Table 4).

In total, 280 isolates of *S.* Typhimurium were recovered from slaughterhouses A–G. Of these, 269 were phage typed. The most frequently encountered phage type was *S.* Typhimurium phage type (DT) 12, which was isolated from all slaughterhouses except C and D (Table 5). The multi-drug-resistant *S.* Typhimurium DT104 was also among the more prevalent types. This type was isolated from all slaughterhouses except A, B and F. In slaughterhouse A, approximately 50% of the *S.* Typhimurium isolates belonged to phage type U288. This type was not found in any other slaughterhouse. With very few exceptions, the within-slaughterhouse distribution of phage types showed that phage types found in samples of products were also found in samples of the environment on the same day. For a relatively large proportion of the *S.* Typhimurium isolates (26·8%), the phage type could not be determined.

Of the 709 isolates of *Salmonella*, 228 (32·2%) were tested for antimicrobial resistance. A total of 113 (49·6%) isolates were resistant to one or more antimicrobials. Fifty-five (24·1%) isolates were multi-drug resistant, which is defined as resistant to at least four antimicrobial compounds (Table 6). The

Table 5. *Distribution (%) of* S. *Typhimurium phage types in isolates from environmental (E) and product (P) samples in seven slaughterhouses*

	Country 1				Country 2				Country 3				Country 4		
	A		B		C		D		E		F		G		
	E	P	E	P	E	P	E	P	E	P	E	P	E	P	Total
U288	40·6	66·7	—	—	—	—	—	—	—	—	—	—	—	—	16·7
104	—	—	—	—	—	50·0	100	100	12·5	69·2	—	—	20·7	12·5	9·3
12	3·1	—	72·5	56·3	—	—	—	—	25·0	—	14·3	—	27·6	50·0	29·7
17	—	—	5·0	18·8	—	—	—	—	4·2	—	—	—	—	—	3·0
86	6·3	—	—	—	—	—	—	—	—	—	42·9	—	—	—	1·9
120	—	—	2·5	6·3	—	—	—	—	—	—	—	—	27·6	37·5	4·8
193	—	—	10·0	12·5	9·1	—	—	—	20·8	—	—	—	—	—	4·5
208	—	—	—	—	45·5	—	—	—	—	—	—	—	—	—	1·9
Other	—	—	2·5	—	—	—	—	—	8·3	—	—	—	3·4	—	1·5
Not typable	50·0	33·3	7·5	6·3	45·5	50·0	—	—	29·2	30·8	42·9	100	20·7	—	26·8
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Number typed	32	12	40	16	11	2	2	3	24	13	7	2	29	8	269

Table 6. *Occurrence (%) of resistance of individual antimicrobial compounds, antimicrobial resistance and multi-drug resistance among* Salmonella *isolates from seven slaughterhouses*

	Country 1		Country 2		Country 3		Country 4	
	A	B	C	D	E	F	G	Total
Ampicillin	2·7	—	23·5	42·9	40·4	5·9	56·1	25·4
Chloramphenicol	2·7	—	2·9	42·9	40·4	—	29·3	17·1
Spectinomycin	—	—	2·9	42·9	40·4	—	31·7	17·1
Streptomycin	—	—	14·7	57·1	40·4	—	68·3	26·3
Sulphonamide	2·7	—	55·9	57·1	42·6	29·4	70·7	36·0
Tetracycline	2·7	—	88·2	64·3	48·9	29·4	85·4	45·2
Trimethoprim +	—	—	41·2	—	—	29·4	2·4	9·2
Gentamycin	—	—	2·9	—	—	—	—	0·4
Colistin	8·1	—	2·9	—	4·3	11·8	—	3·5
Resistant (%)	16·2	0	88·2	64·3	53·2	29·4	92·7	49·6
Multi-resistant (%)	0	0	20·6	42·9	40·4	11·8	51·2	24·1
S. Typhimurium DT104 (%)	0	0	2·9	35·7	25·5	0	17·1	11·0
Number typed	37	38	34	14	47	17	41	228

variation between slaughterhouses was large ranging from 0% resistant isolates in slaughterhouse B to 97·2% in G. Also the proportion of multi-drug-resistant isolates varied between slaughterhouses. In slaughterhouse G, 51·2% of the isolates were multi-drug resistant, whereas no such isolates were found in A and B (Table 6). The most frequent combination of resistance (resistance pattern) was observed for the following six compounds: ampicillin, chloramphenicol, spectinomycin, streptomycin, sulphonamide and tetracycline. This pattern occurred in 16·2% of the typed isolates and was characteristic for the isolates of *S.* Typhimurium DT104 (92%), but it was also commonly seen among isolates of *S.* Typhimurium DT12 (39·3%). Looking at the individual compounds, resistance to tetracycline and sulphonamide was most frequently observed (Table 6). Isolates resistant to these compounds were isolated from all slaughterhouses except B.

Results of the statistical analysis

The prevalence of *Salmonella* in slaughter pigs in the country, where slaughterhouses H–L were located, was known to be extremely low and different from the prevalence in the other countries. The fact that *Salmonella* was not isolated from these slaughterhouses was therefore mainly considered to be a consequence of a very low input of *Salmonella* rather than a reflection of the hygienic performances of the slaughterhouses. Consequently, we decided not to include the results from these slaughterhouses in any of the statistical analyses.

Salmonella *contamination of the environment* (*analysis no. 1*)

The first logistic regression analysis included results from 3200 environmental samples collected in slaughterhouses A–G. The result of the analysis showed that the difference in the environmental contamination level between the seven slaughterhouses was not statistically significant, however, the confidence intervals were quite wide and the ORs for slaughterhouses E and F were higher than for the other slaughterhouses (Table 7). There was a pronounced seasonal variation, where the probability of finding a positive environmental sample was more than seven times higher during the summer months and approximately 3·5 times higher during the spring compared to the autumn (Table 7). Compared to the first sampling round, the contamination level of the slaughterhouse environment steadily increased during the day. At the end of a slaughter day (sampling round 6), the probability of recovering a positive environmental sample was almost four times as high compared to the first sampling round (Fig. 1, Table 7). Also, the result of the trend test showed an overall significant increase in the environmental contamination level during the slaughter day (Table 7).

There were also differences between the contamination levels at the various slaughter processes, but the result of the trend test indicated that there was no overall increasing or decreasing trend in the contamination level during the course of slaughter (Table 7, Fig. 2) Compared to the last sampling point (the floor just before the blast chilling), the probability of recovering a positive sample was lowest when sampling from the scalding process and highest when samples were taken from the trimming process. The other processes were roughly categorized into two groups. In the first group, including samples from the polishing, pluck removal, splitting and evisceration processes, the probability of recovering *Salmonella* was approximately 2·5 times as high compared to the samples from the last sampling point. In the second group, encompassing the bung removal, skinning and meat inspection processes, the same probability was between 1·5 and 2 times as high (Table 7).

Finally, from the analysis it was found necessary to include the origin of the samples and the sampling method in the model, since the ranking of the processes according to the probability of finding *Salmonella* was dependent on both of these variables. As can be seen from Table 7, samples of hands, knives and equipment were less frequently found contaminated than samples of outlets, and there was no difference in the contamination level between samples of hands and knives. In general, samples of water resulted in more *Salmonella* isolations than swab samples.

Salmonella *contamination of products* (*analysis no. 2*)

Analysis no. 2 included the results from 3485 product samples collected in slaughterhouses A–G. There was no statistical significant difference in the occurrence of *Salmonella* in the products between slaughterhouses or between sampling rounds. The contamination level was higher in the summer months. Compared to autumn, the probability of finding *Salmonella* on the products was more than 11 times higher in the summer period (Table 8).

The probability of finding *Salmonella* on the carcass was 2·5 times less likely than finding *Salmonella* on the tongue and 1·5 times less likely than finding *Salmonella* on the liver (Table 8). Compared to the liver samples, the probability of finding *Salmonella* on the tongue was approximately 1·5 times higher [$\exp(0{\cdot}95-0{\cdot}45)=1{\cdot}56$].

Identification of slaughter processes associated with carcass contamination (*analysis no. 3*)

In slaughterhouses C and D, the slaughter processes differed markedly as the pigs were skinned. Consequently, the results from these slaughterhouses were excluded from the analysis. Further, seven sampling rounds from slaughterhouse E were excluded because of missing observations. In total, the analysis included 1130 carcass samples collected during 23 sampling days and 126 sampling rounds in slaughterhouses A, B, E, F and G.

The initial screening of the slaughter-process variables in the basic model suggested that the proportion

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Table 7. *Results of the logistic regression analysis no. 1. Results from slaughterhouses H–L excluded*

	OR	95% CI Low	95% CI High	*P* value
Slaughterhouse				
A	1·89[a]	0·45	7·92	0·387
B	1·89[a]	0·45	7·86	0·389
C	2·91[a]	0·75	11·26	0·129
D	1·95[a]	0·48	7·92	0·358
E	5·03[a]	0·88	28·77	0·077
F	3·94[a]	0·67	23·11	0·137
G	1[a]	—	—	
Season				
Winter (Dec.–Feb.)	2·19[ac]	0·79	6·06	0·132
Spring (Mar.–May)	3·49[ab]	1·02	11·97	0·047
Summer (June–Aug.)	7·36[b]	2·66	20·40	0·000
Autumn (Sep.–Nov.)	1[c]	—	—	
Sampling round				
1	1	—	—	
2	1·74[a]	1·19	2·53	0·004
3	2·14[ab]	1·40	3·28	0·000
4	2·10[ab]	1·34	3·30	0·001
5	2·92[bc]	1·85	4·61	0·000
6	3·97[c]	2·51	6·27	0·000
Result of trend test	1·26	1·16	1·37	0·000
Process				
Scalding	0·27	0·10	0·72	0·008
Polishing	2·64[b]	1·36	5·11	0·004
Bung removal	1·82[ab]	0·56	5·93	0·318
Evisceration	2·46[b]	1·46	4·17	0·000
Pluck removal	2·68[b]	1·55	4·63	0·000
Splitting	2·64[b]	1·55	4·51	0·000
Meat inspection	1·56[ab]	0·51	4·72	0·432
Trimming	3·35[b]	1·96	5·75	0·000
Skinning	1·78[ab]	0·90	3·53	0·096
Before chilling	1[a]	—	—	
Result of trend test	1·00	0·95	1·05	0·969
Origin of sample				
Hand of personnel	0·12[a]	0·07	0·20	0·000
Knife	0·07[a]	0·03	0·16	0·000
Equipment	0·40	0·22	0·72	0·002
Outlet	1	—	—	
Method				
Swab sample	0·40	0·24	0·64	0·000
Water sample	1	—	—	

[a, b, c] Indicates that there is no statistical difference between the levels of a variable assigned the same letter, e.g. [a] under the variable sampling round indicates, that there is no statistical difference between the level of contamination between sampling rounds 2, 3 and 4.

of positive carcasses was related to the isolation of *Salmonella* from several slaughter processes. In particular, isolation of *Salmonella* from the polishing, trimming, scalding and pluck removal operations seemed to be associated with an increased risk of carcass contamination (Table 9).

In the final multiple regression model, probability of recovering *Salmonella* from a carcass was found to

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Table 8. *Result of the logistic regression analysis no. 2 for contamination of products with* Salmonella. *Results from slaughterhouses H–L excluded*

	OR	95% CI Low	95% CI High	*P* value
Season				
Winter	2·58[ab]	0·49	13·46	0·2607
Spring	2·04[ab]	0·26	16·06	0·4999
Summer	11·70[a]	2·26	60·48	0·0034
Autumn	1·00[b]	—	—	—
Location				
Liver	1·57	1·11	2·22	0·0117
Tongue	2·57	1·86	3·56	0·0001
Carcass	1	—	—	—
Test of fixed effects	D.F.	χ^2		*P* value
Slaughterhouse	38	6·68		0·6700
Sampling round	3431	1·78		0·1290

[a,b] Indicates that there is no statistical difference between the levels of a variable assigned the same letter.

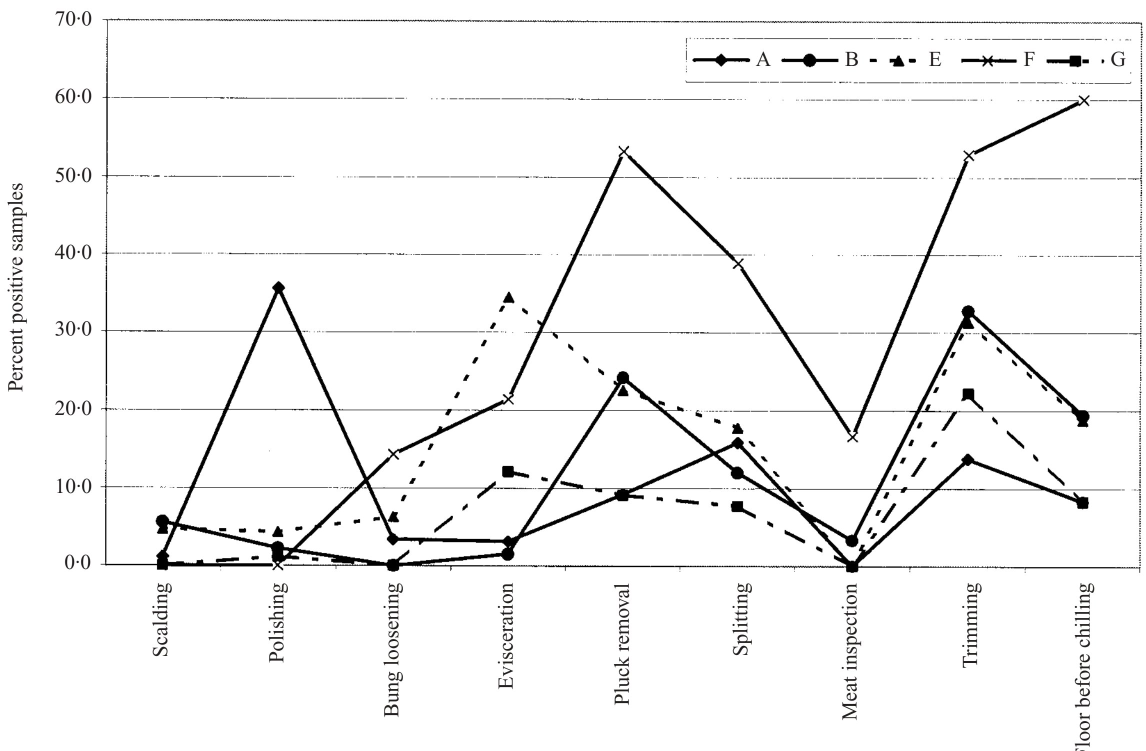

Fig. 2. Isolation of *Salmonella* from nine different slaughter processes in five European Union slaughterhouses. Due to different slaughter procedures, slaughterhouses C and D were not included in this graph. In five slaughterhouses (H–L), no *Salmonella* was found.

be positively associated with the isolation of *Salmonella* from the polishing equipment (OR 3·74, 95% CI 1·43–9·78; Table 10). Further, the finding of *Salmonella* during the pluck removal procedure was found to increase the probability of finding *Salmonella* on the carcass. This association was shown to be modified by the finding of *Salmonella* in water taken from the scalding process. In other words, an interaction

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Table 9. *Marginal odds ratios of carcass contamination between positive and negative processes, controlled for the effect of slaughterhouse, sampling day, season and sampling round*

		95% CI			Proportion of positive carcasses	
Process	OR	Low	High	*P* value	*Salmonella* not isolated from the process	*Salmonella* isolated from the process
Scalding	2·44	1·12	5·31	0·0253	16/1025	11/105
Polishing	5·04	2·01	12·62	0·0006	15/888	12/242
Bung removal	0·29	0·07	1·24	0·0945	26/1104	1/26
Evisceration	0·92	0·35	2·39	0·8663	23/943	4/187
Pluck removal	2·21	1·04	4·71	0·0404	10/795	17/335
Splitting	1·58	0·83	3·00	0·1675	9/660	18/470
Meat inspection	3·63	0·78	16·85	0·0999	25/1120	2/10
Trimming	2·73	1·18	6·32	0·0196	10/797	17/333

Table 10. *Result of the logistic regression analysis no. 3 of slaughter processes contributing to the total carcass contamination. Results from slaughterhouses C and D, and H–L excluded*

		95% CI		
	OR	Low	High	*P* value
Season[a]				
Summer	11·94[b]	1·14	125·46	0·0391
Winter	4·22[bc]	0·46	38·66	0·2001
Autumn	1·00[c]	—	—	—
Sampling round	1·05	0·85	1·30	0·6658
Polishing				
Positive	3·74	1·43	9·78	0·0074
Negative	1·00	—	—	—
Scalding × Pluck				
Pospos	3·63	1·66	7·96	0·0011
Other	1·00	—	—	—
Test for fixed effects	D.F.	χ^2		*P* value
Slaughterhouse	17	1·07		0·40

[a] After exclusion of data from slaughterhouses C and D, no slaughterhouses were sampled in the spring.

[b,c] Indicates that there is no statistical difference between the levels of a variable assigned the same letter.

between the finding of *Salmonella* during scalding and pluck removal was observed. When samples from both the scalding and the pluck removal process were positive, the probability of finding a contaminated carcass was more than 3·5 times higher (OR 3·63; 95% CI 1·66–7·96) than if neither or just one of the processes yielded one or more positive samples in a sampling round. Finally, the model showed that the level of carcass contamination was significantly higher, almost 12 times, in the summer months compared to the autumn, and that there was no effect of the slaughterhouse or sampling round (Table 10).

DISCUSSION

Compared to the seasonal and day-to-day variation within the slaughterhouses, there was little difference in the proportion of *Salmonella*-positive samples between the seven slaughterhouses of four European Union countries. However, these slaughterhouses differed significantly from five slaughterhouses in a fifth European Union country, where no *Salmonella* was found. The results support the suggestion that *Salmonella* infections in slaughter pigs in this country occur infrequently.

All three statistical models demonstrated a pronounced seasonal variation, where the *Salmonella* isolation rates were higher during the warm months. Increased ambient temperature could lead to a potential multiplication of the pathogen in the environment, which eventually may contribute to increased infection of pigs as well as contamination of carcasses. Also proliferation of bacteria present in the abattoir environment may increase during high ambient temperatures, and thereby result in more carcasses being contaminated. Although, most slaughterhouses have a temperature-regulation system installed, the cooling capacity is often exceeded during hot weather. Finally, the rise in ambient temperatures is believed to increase the stress levels of the pigs resulting in an excessive shedding of *Salmonella* in the environment, which may lead to more infected pigs and contaminated carcasses [17, 37–39]. The seasonal variation observed in this study was probably caused by a combination of the above-mentioned factors, but the relative contribution of each factor is not known.

In this study, we did not attempt to estimate the impact of the day-to-day variation, but included the day of sampling as a random effect in the statistical models. However, by looking at the descriptive results, it was obvious, that a pronounced day-to-day variation existed. This variation could not readily be explained, but differences in the *Salmonella* status of the herds delivering pigs for slaughter on the various sampling days most likely contributed to this variation. Also sporadically occurring breaches in the regular slaughter hygiene, e.g. laceration of the gut during evisceration, are likely to influence the overall contamination level of a single day's production [22]. Morgan et al. [40] also found that the *Salmonella* contamination level varied significantly from one sampling day to the next and concluded on this basis, that the hygienic performance of an abattoir cannot be assessed on a single visit. We believe that our study supports this conclusion.

Statistical analysis no. 1 showed that the environmental *Salmonella* contamination increased during a slaughter day, suggesting a build-up of bacterial load in the environment during working hours. This increase could not be demonstrated for the product samples and the importance of this finding is therefore difficult to assess. However, Yu et al. [41] did find that carcasses passing through a contaminated polisher contained a higher bacterial count in the afternoon than in the morning. This indicates that if bacterial build-up takes place within or on equipment in close contact with the carcass, an increased product contamination during the working hours may occur, although our study did not support this conclusion.

Some slaughterhouses had indications of a residential flora ('isolation of the same serotypes and phage types on different sampling days'), which would indicate insufficient cleaning practices, however the finding can also be explained by a constant influx of the same serotype and phage types from the herds delivering pigs to the slaughterhouse. When parts of the slaughter line are not completely cleaned and disinfected, *Salmonella* strains can reside on the slaughter equipment and in the drain water [22]. Sørensen et al. [42] have described two such cases of persistent environmental contamination, where *S.* Infantis was found harbouring in the exhaustion channel above the carcass splitter in one slaughterhouse and the dehairing equipment in another slaughterhouse. In both cases, it proved extremely difficult to locate and eliminate the persistent infections. In our study, examples of the presence of a persistent environmental contamination in the slaughter line were the occurrence of *S.* Ohio on the polishing equipment in slaughterhouse A and *S.* Infantis on the carcass splitter in slaughterhouse F. In both slaughterhouses, a high proportion of the positive carcasses was contaminated with these serotypes, suggesting that persistently contaminated equipment is a major source of carcass contamination.

Bacterial populations, including *Salmonella*, present on the pig carcass are reduced considerably by scalding and singeing, but the carcass is likely to be recontaminated during the dehairing and polishing operations [43–45]. Yu et al. [41] also found that 'dirty' polishing equipment contributes to the total level of carcass contamination. In our study, a part of this contribution could be attributed to persistently contaminated polishing equipment in a single slaughterhouse. The rotating flails inside the polisher are rather difficult to sanitize properly, and the persistent contamination with *S.* Ohio in slaughterhouse A indicates, that *Salmonella* can proliferate and cause contamination of pig carcasses over a long period. The persistent contamination with *S.* Infantis in slaughterhouse F, was not identified as a risk factor in analysis no. 3, which might be explained by the relatively few observations from this slaughterhouse.

Salmonella was most often isolated from samples representing the pluck removal and trimming processes, but only the finding of *Salmonella* during pluck removal was found to be associated with a higher risk of carcass contamination and only if the scalding water was also positive for *Salmonella* (Table 10). So although the scalding process did not turn out to be a significant risk factor in itself, the finding of *Salmonella* in the scalding water significantly increased the effect of isolating *Salmonella* from the pluck removal process. This finding may be explained by the following hypothesis: during scalding some water will almost inevitably enter the lungs due to voluntary or involuntary respiration of the recently killed pigs [46–48]. If the water is contaminated with *Salmonella*, the risk of isolating *Salmonella* from the lungs will increase, which consequently will lead to an increased risk of carcass contamination when the pluck is pulled from thoracic cavity. This risk is further increased if the lungs, e.g. due to adhesions following a previous lung inflammation, are ruptured during the extraction. In this situation, contaminated scalding water may leak into the thoracic cavity and aerosols may become widely distributed in the surroundings. In other words, if the occurrence of *Salmonella* in the scalding water can be prevented, the risk of contaminating the carcass during removal of the lungs and associated organs will be reduced. In support of this is the fact that the prevalence of *Salmonella* on carcasses was lowest in slaughterhouse G, where no *Salmonella* was isolated from the scalding tank during the course of the study.

Usually, scalding reduces the number of *Salmonella* spp. on the carcass surface. However, if the water temperature drops below the recommended 62 °C and/or the amount of organic material is sufficient to protect the bacteria against the heat, the risk of bacteria surviving this process is increased [49]. The temperature of the scalding water was measured during each sampling round in slaughterhouses A and B. The prevalence of *Salmonella* in the scalding water was highest in slaughterhouse B, where the temperature varied between 60 and 60·9 °C. When *Salmonella* was found, the temperature was 60·3 °C or lower. In slaughterhouse A, the temperature varied between 61 and 62 °C and *Salmonella* was only isolated from the scalding water on one occasion. In a study by Davies et al. [50], the proportion of positive carcasses after scalding was found to be higher when the temperature was below 61 °C compared to a scalding temperature of 61–62 °C. Together, these observations suggest that the survival of *Salmonella* in the scalding tank increases when the water temperature falls below 61 °C. In order to ensure a constantly high temperature (62 °C), continuous monitoring of the temperature in the scalding tank is necessary.

Inappropriate evisceration techniques are also considered to be associated with a significantly higher risk of isolating Enterobacteriaceae from the carcass [22, 51]. We did not identify the bung loosening or the evisceration as risk factors for carcass contamination. This could be due to the fact that in two of the slaughterhouses (A and B), preventive measures, e.g. sealing of the rectum with a plastic bag in order to prevent faecal contamination were in use. These kinds of techniques have been shown to reduce the level of carcass contamination significantly [52–54]. In slaughterhouses E–G, where no such preventive measures were taken, the level of contamination during evisceration was higher (Table 3). Slaughterhouses without special precautions to reduce faecal contamination will probably benefit from establishing such techniques.

One of the striking results of the antimicrobial resistance testing was that the occurrence of resistant *Salmonella enterica* was significantly lower in slaughterhouses A and B. Resistant isolates were only recovered from slaughterhouse A and there were no multi-drug-resistant isolates among these (Table 6). The observed differences may reflect patterns of use of antimicrobials in slaughter-pig herds in the different countries. In order to observe country anonymity, this issue can not be discussed further.

In conclusion, *Salmonella* was not isolated from any of five slaughterhouses in one of the participating countries. From the seven slaughterhouses in the four remaining countries, *Salmonella* was isolated from 5·3% of 3485 product samples (ranging from 2·5 to 8·5%), and from 13·8% of 3573 environmental samples (ranging from 6·3 to 28·3%). Overall, *S.* Typhimurium (40% of isolates) and *S.* Derby (17%) were the most prevalent serotypes. Among *S.* Typhimurium isolates, phage type (DT) 12 (29·7%) and DT104 (9·3%) were most commonly found. Of 228 isolates tested for antimicrobial susceptibility, 113 (49·6%) were resistant to at least one antimicrobial, whereas 55 (24·1%) were resistant to four or more antimicrobials (multi-drug resistant). The statistical analysis of the data indicated that the occurrence of *Salmonella* in the abattoir environment increases during the day and that, in particular, two slaughter processes (polishing and pluck removal) contribute significantly to the total carcass contamination. The latter was especially true if the scalding water was also contaminated. The finding of consistently contaminated equipment in two slaughterhouses and the fact, that *Salmonella* was isolated from 7·9% of samples of the abattoir environment before onset of slaughter, further indicates, that the practised cleaning procedures are insufficient in preventing *Salmonella* from becoming established in the environment. Sufficiently high temperatures of the scalding water (62 °C) and appropriate cleaning and disinfecting of the abattoir equipment at least once a day, but preferably during each break, is therefore recommended. Other methods of scalding, e.g. vertical scalding by steam as already done in some slaughterhouses may also be considered. Finally, the overall occurrence of *Salmonella* was shown to be influenced by the season. The higher contamination level observed in the warm months may partly be explained by increased *Salmonella* excretion by infected pigs and partly by an increased proliferation of bacteria in the environment. The relative importance of these two factors requires further study.

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