



Original Article

## Salmonella screening in pork slaughter flowchart

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### ABSTRACT

The aim of this study was to trace the sources of *Salmonella* contamination during the pig slaughter flowchart. Ten lots of pigs sent for slaughter were followed (four animals per lot), and two weeks before slaughter they were selected based on the presence or absence of *Salmonella* in their farm stalls. Stool samples were collected after stunning, and from the surface swabs of the carcass in different parts of the flowchart. Samples were also collected immediately after the animals left the dehairing machine, after opening the abdominal cavity, before the carcass entered the cooling chamber, and from jowl samples. The water samples used in the scalding tank were collected before commencing the slaughter process and after the passage of the animals. For the comparison of band patterns, the isolates were analyzed by rep-PCR. The percentage of isolation was 35.3% after stunning, 17.6% immediately after the animals left the dehairing machine, 17.6% after evisceration, 23.5% before entering the cooling chamber and 5.8% from the jowls. The serotypes obtained were: Senftenberg, Idikan, Typhimurium, Heidelberg, Minnesota, Panama and *Salmonella* group O:4,5. By rep-PCR analysis, it was found that *Salmonella* strains that reached the slaughterhouse in carrier pigs may not be eliminated during processing, thereby making its isolation from the carcasses possible. It was also observed that the strains introduced by animals can infect others in different stages of the slaughter flowchart, thus resulting in cross-contamination.

### INTRODUCTION

Despite its importance to consumers, pork products may serve as carriers for pathogens (FRANCO; LANDGRAF, 2003). Foodborne diseases are caused by the consumption of food contaminated with a specific infectious microbe or its toxin (BRASIL, 2001). This kind of food poisoning represents a worldwide public health concern. Salmonellosis (an illness caused by bacteria of the genus *Salmonella*), is one of the most frequent foodborne diseases and is contracted by the ingestion of contaminated animal products, such as eggs, meat and milk (CDC, 2012; WHO, 2013).

It is of great importance to evaluate the microbiological quality of pig products because of its relevance as a

common way of transmitting foodborne diseases (BRASIL, 2001; FRANCO; LANDGRAF, 2003). The performance of *Salmonella* screening along the pig production chain enabled us to determine if the contamination detected at the farm reached the slaughter and meat processing stages. In addition, the origin of the microorganism was evaluated by determining if the pork products were infected at the farm or in the slaughterhouse, by cross-contamination with contaminated tools and equipments, or through other animals or human carriers.

Therefore, by hypothesizing that *Salmonella* carrier pigs can keep the microorganism viable until the final product, and also be a source of cross-contamination

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within the facility, this study aimed to track down *Salmonella* in the pig slaughter flowchart.

## MATERIALS AND METHODS

In this study, 10 lots of pigs for slaughter from a full cycle farm in the Brazilian state of Rio Grande do Sul were evaluated. The lots were selected after fecal samples were analyzed for *Salmonella*, two weeks before the animals were sent to the slaughterhouse. Sample collection was performed by walking randomly inside the stalls with disposable shoes covers. Later, a swab was rubbed on the shoes covers to obtain the samples. After collecting three samples per stall, the material was immediately transported to the laboratory in Cary Blair medium (Himedia, Mumbai, India), set inside coolers with ice.

Four animals from each selected lot were monitored during slaughter and processed at a legally established abattoir, registered and inspected by the Division of Animal Products Inspection of the Secretariat of Agriculture, Livestock and Agribusiness of Rio Grande do Sul. Fecal samples were collected during the slaughter, by inserting a swab into the animal's rectum right after stunning. Likewise, specimens were also taken from four different points of the flowchart: immediately after the animals left the dehairing machine, after opening the abdominal cavity, just before carcasses entered into the cooling chamber, and jowl tissues were obtained from the same animals.

Samples were obtained by swab frictions in a 100 cm<sup>2</sup> area bounded with a sterile stainless steel model on the skin surface (the exit of the dehairing machine and the entrance of the cooling chamber), the inner surface of the carcass (during the abdominal cavity opening), and jowl. Moreover, water samples were collected from the scalding tank, before starting the slaughter of each lot, and just before the passage of the animals. Thereafter, the samples were placed in sterile glass vials in an approximate quantity of 30 mL. This study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Pelotas, and identified by the CEEA 3313-2015 code.

For *Salmonella* analysis, swabs were put into test tubes with 10 mL of Buffered Peptone Water (BPW Acumedia). The material was incubated for pre-enrichment purpose and further procedures were performed to detect *Salmonella*, as recommended by the U.S. Food and Drug Administration (FDA) (ANDREWS; ANDREW; HAMMACK, 2014). Swabs were dipped into each water sample from the scalding tank, and tested according to the description above.

For serotype identification, *Salmonella* isolates were sent to the Department of Bacteriology of the Enterobacteria Laboratory, Oswaldo Cruz Foundation (Fiocruz, Manguinhos, Rio de Janeiro).

The DNA isolates were extracted according to the method of Sambrook; Russell (2001). Briefly, the pellet obtained by centrifugation of 1mL of BHI culture was resuspended in 100µL of STES buffer [0.2M Tris-HCl, 0.5M NaCl, 0.1% SDS (m/v), 0.01M EDTA, pH 7,6]. Also, 50µL of glass beads and 100µL of phenol-chloroform were added. After a 1 min homogenization, the mixture was centrifuged at 13,000g for 5min. The supernatant was collected and precipitated in 2 volumes of absolute ethanol and 0.1 volume of 5M NaCl at -70°C for 30 min. Another centrifugation was performed at 13,000g for 20min, the supernatant was discarded and the pellet washed with 70% ethanol. After elution with 40µL of elution buffer (10mM Tris-HCl, 1mM EDTA, pH 7.4), 1µL of RNase (10µg/µL) was added and the extracted DNA was stored at -20°C.

The molecular profiles of the isolates were determined by rep-PCR according to Rasschaert et al. (2005), using the primer (GTG)<sub>5</sub> (VERSALOVIC, 1994). Briefly, the conditions of rep-PCR were as follows: 2.5µL DNA, 2µL of 5'-GTGGTGGTGGTGGTGG-3' oligonucleotide, 12.5µL Master Mix (Qiagen, Germany) and 8µL water to complete the reaction volume. The amplification cycles were performed as follows: 1 cycle at 94°C for 5min, 30 subsequent cycles at 95°C for 30s, 45°C for 1min and 60°C for 5min, and finally 1 cycle at 60°C for 16min. To visualize the band patterns of the different regions amplified in the genome, the PCR products were stained with GelRed and electrophoresis was performed using a 2% agarose gel.

The rep-PCR patterns were interpreted according to the classification criteria suggested by Tenover et al. (1995). Using the classification in four forms: indistinguishable (no different band), closely related (2-3 distinct bands), possibly related (4-6 distinct bands) and different (more than 7 different bands).

## RESULTS AND DISCUSSION

Of the 17 collected isolates, the highest percentage (35.3%) originated from samples picked by inserting the swab into the animal's rectum, after stunning. The results showed that not all the animals of a *Salmonella*-positive lot carry the microorganism. However, the presence of carriers in negative lots showed that, although the sample collection method at stalls was as representative as possible, the group results should not be individually applied (Table 1).

Table 1 – Presence of *Salmonella* in the farm and in the pig slaughter flowchart.

Lots	Initial Water	Rectum <sup>a</sup>	After Dehairing Machine	After Evisceration	Entrance of Cooling Chamber	Jowl	Final Water
<b>Positives<sup>b</sup></b>							
1	-	--+-	---+	+---	+---	----	-
2	-	+---	--+-	----	----	----+	-
3	-	----	----	----	----	----	-
4	-	----	----	----	----	----	-
<b>Negatives</b>							
1	-	-+--	---+	--+-	----	----	-
2	-	----	----	----	----+	----	-
3	-	----	----	----	----+	----	-
4	-	----	----	----	----	----	-
5	-	-+--	----	----	----	----	-
6	-	--++	----	--+-	----+	----	-

<sup>a</sup>In the columns with four symbols (+ or -), each corresponding to a pig. The order of animals is the same in every line. *Salmonella* absence (-); *Salmonella* presence (+).

<sup>b</sup>Positive: lots with the presence of *Salmonella* in fecal samples obtained in the stalls. Negative: lots with no *Salmonella* in these samples.

In the other collection points, such as after removing animals from the dehairing machine, after evisceration, before entering the cooling chamber, and jowl sampling, the outcomes were 17.6, 17.6, 23.5, and 5.8%, respectively. Busser et al. (2011), in Belgium, monitored 226 pigs during slaughter, and obtained *Salmonella* isolates from 109 animals (48.2%) in at least one collecting moment. In this study, 17 isolates (7.7%) were obtained from 220 samples, and isolated from 14 different animals. This lower percentage may be related to differences in sanitary conditions among abattoirs, and the rigor in the application and inspection of self-control programs.

No isolates were obtained from the scald water tank. In fact, *Salmonella* viability is not expected at the water scalding tank. By evaluating the water scalding tank in two abattoirs in the Netherlands, Swanenburg et al. (2001) obtained two isolates in one of them. Likewise, Piras et al. (2011) obtained one isolate in Sardinia, Italy, when evaluating water from the scalding tank of five abattoirs. Other studies that analyzed *Salmonella* (BONARDI et al., 2013; BUSSER et al., 2011; PEARCE et al., 2004) could not isolate this microorganism. According to Roberts et al. (2005), the temperature of the scald water tank must be above 60°C to avoid the contamination of carcasses. Brazilian law stipulates that the water temperature of the scalding tank should range between 62 to 72°C (BRASIL, 1995).

Three (17.64%) isolates were obtained after withdrawing animals from the dehairing machine. This result represents a higher percentage as compared with the one (1.25%) achieved by Hernandez et al. (2013) in Spain, from the 80 samples analyzed. The presence of *Salmonella* in carcasses after scalding may be as a result of lacking a proper setting of time, or temperature, during this step. In this case, carcasses must remain in the scalding tank for two to five minutes (BRASIL, 1995). Another possible cause is the presence of dirt on the skin

surface. It could serve as protection for *Salmonella* during immersions at high water temperatures.

During the experiment, *Salmonella* was isolated from 3 of the 17 (17.6%) samples obtained after evisceration, a high-risk step for fecal contamination. However, Ducas; Silva (2011) found no positive outcome for this microorganism in the 18 carcass samples collected after evisceration in an abattoir with Federal Inspection conducted in Minas Gerais state, Brazil. Moreover, Pearce et al. (2004), in Europe, retrieved 7% of isolates at the same step. Facility conditions and employee training may have a great influence on these results, considering the high manipulation of viscera by operators at that phase.

In this study, 5.8% of all isolates were gathered from jowl samples. This is an important health issue since the jowl, a raw material for sausage production, has also been reported as a possible *Salmonella* transmitter to humans (CABRAL et al., 2014; MÜRMAN et al., 2009).

In addition, 23.5% of the resulting isolates in this research were acquired from carcasses before entering the cooling process. Several studies have been conducted to evaluate the presence of *Salmonella* in carcass prior to refrigeration, with mixed results. In a study conducted by Colla et al. (2014), in Rio Grande do Sul, 32.5% (39/120) of carcass samples had *Salmonella*. In Spain, Arguello et al. (2011) isolated *Salmonella* in 39.7% (356/896) of carcasses assessed before passing to the cooling step. Bonardi et al. (2013) conducted an investigation in Italy, and isolated *Salmonella* in 10.9% (49/451) of the analyzed carcass samples. Bonardi et al. (2003), in Italy, obtained 6% (9/150) of isolates. Ducas; Silva (2011), in Minas Gerais, could not isolate *Salmonella* from 18 carcasses. These different findings are probably caused by both the peculiar sanitary conditions of each studied abattoir, and the greater or

lesser prevalence of the microorganism at the porcine farms.

Concerning *Salmonella* serotypes, 7 of them were found: Senftenberg, Idikan, Typhimurium, Heidelberg, Minnesota, Panama, and O:4,5. Of the 4 isolates obtained from the farm animals, 2 serotypes were Senftenberg, 1 Ikidan, and 1 Minnesota. At the abattoir, the isolates were 8 Senftenberg, 2 Typhimurium, 1 Heidelberg, 1 Minnesota, 1 Panama, and 1 O:4,5. Bessa; Costa; Cardoso (2004), in Rio Grande do Sul, identified 26 different serotypes, of which Panama, Heidelberg, Senftenberg, and Typhimurium are the most prevalent. However, Tavechio et al. (2002) studied the foodborne disease outbreaks caused by *Salmonella* in São Paulo, from 1996 to 2000, and found the serotype Senftenberg as the most commonly involved. This is a worrisome health issue because the serotype Senftenberg recorded a significant prevalence in the present work. In the state of Mato Grosso, Silva et al. (2009) identified the serotypes Derby, London, Give, and Typhimurium, of which the latter is the only isolate obtained in this study. In Paraná, Pandini et al. (2015) detected Heidelberg Mbandaka and Newport as the most common serotypes in aviaries. These differences confirm a regional variation in serotype distribution and prevalence.

The isolates were analyzed by the rep-PCR technique. A comparison of the banding patterns enabled assessment of the persistence of strains in the flow chart, and assisted in identifying cases of cross contamination.

By monitoring animals from positive lots, the following results were obtained: in lot 1, the isolate obtained from the farm was indistinguishable when compared to those obtained from the rectum of animals 3 and 4. In the same way, the isolate of lot 2 obtained from the farm was indistinguishable from the isolate obtained from the rectum of animal 3. The isolate from lot 3 of the farm was classified as closely related to the one found in animal 1 at the abattoir, and indistinguishable from the isolate obtained from animal 4. These findings confirm animal contamination through the same strain isolated from lot samples picked at the farm. In lots 4 and 5, isolates were not retrieved during the slaughter flowchart. This indicates that these animals, despite coming from a lot with positive cases of *Salmonella*, neither carried the microorganism nor suffered from cross contamination with other pigs.

In addition, lot 2 displayed isolates in animals 1, 3 and 4. In animal 1, the isolates were obtained after the removal of the dehairing machine, after evisceration, and before gaining entrance into the refrigeration chamber. In animal 3, the isolate was only from the rectum, and not from the other stages. This suggests that the hygienic-sanitary procedures were effective. Based on comparison, all isolates from animal 1, were classified as indistinguishable from each other. All isolates from

different animals of the same lot were classified as indistinguishable when contrasted among themselves. These results certify that the remaining contamination at the abating process emanated from the farm.

In at least one of the evaluated sites, lot 3 presented isolates in three animals during slaughter. The absence of any other isolate from animal 1, only of the rectum, indicates the possible efficiency of the implemented hygiene measures in avoiding contamination. The isolate obtained from animal 3 has been considered closely related to the one from the farm. Both animals were *Salmonella* negatives, but later got infected by the same strain found in the farm and in the other two animals of the monitored lot. This data revealed that the microorganism did not only remain during the slaughter, but that there was also a cross-contamination during the processing.

At the farm, the tracking of animals from the lots that tested negative for *Salmonella* revealed that during the slaughter of animals from lot 1, both the isolate obtained from the rectum of animal 2, and the isolate obtained after eviscerating animal 3, were classified as indistinguishable. On the other hand, the isolate of animal 4 chosen after leaving the dehairing machine was considered closely related to them. In the absence of isolates from the farm and having isolates only from the animal rectum, as recorded in lots 3 and 5, it was possible to infer that the feces of these animals have not been part of the lot material sampled at the stalls, or possibly contaminated at the farm after collection. The presence of equality and/or similarity between the isolates derived from animals at different stages, suggests cross-contamination in the slaughterhouse through appliances, or by contact with other carcasses or handlers, considering that these animals (3 and 4) have gone through the same steps after animal 2.

Only one isolate was determined in the abattoir while analyzing lot 2, before entering the cooling chamber. The absence of isolates in the other stages, shows that the carcass contamination supposedly happened during the flowchart, because such isolates just appeared at the end of the slaughter line, after intense manipulation.

In lot 3, an isolate was obtained from animal 4 before entering the cooling chamber, and classified as indistinguishable from others. Since the animal belonged to a farm stall that tested negative to *Salmonella* and also remained negative until the slaughter, the infection was assumed to have occurred during processing.

The isolate of lot 5 emanated from the rectum of animal 2. In this case, the hygienic-sanitary procedures were effective in preventing carcass contamination at the following stages.

Finally, in lot 6, two animals reached the abattoir positive for *Salmonella*. This microorganism was isolated in subsequent steps of the slaughter process. Moreover, all isolate results were indistinguishable. This can be linked to a persistent contagion during processing. The information addressed in this particular lot proves that the contamination from carrier animals at the abattoir can stay viable during the slaughter flowchart, under inefficient hygienic-sanitary procedures.

### CONCLUSIONS

This study proved that *Salmonella* strains reaching the abattoir by means of carrier pigs may not be eliminated during processing. The isolation of bacteria from the carcasses and jowl at the abattoir correlates with the ones from the farm, and confirm this fact. In addition, the strains introduced by animals can infect others in different stages of the slaughtering flowchart by cross-contamination. Therefore, strict care in the execution of hygienic and sanitary procedures during the slaughter and sanitary control of animals in the farms, are highly recommended to ensure healthy food for consumers.

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