PAB
Pesquisa Agropecuária Brasileira

Food Technology/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Salmonella enterica and enterobacteria in pig carcasses processed on different slaughter days

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Received
December 21, 2021

Accepted
January 26, 2022

How to cite
RIZZOTTO, D.; MONTES, J.H.; KICH, J.D.; PERIPOLLI, V.; BIANCHI, I.; OLIVEIRA JÚNIOR, J.M. de; DUVAL, E.H.; SCHWEGLER, E.; MOREIRA, F. *Salmonella enterica* and enterobacteria in pig carcasses processed on different slaughter days. **Pesquisa Agropecuária Brasileira**, v.57, e02813, 2022. DOI: https://doi.org/10.1590/S1678-3921.pab2022.v57.02813.

Abstract – The objective of this work was to evaluate the contamination by *Salmonella* sp. and enterobacteria in pig carcasses from the first and last batches slaughtered in a same week, at different stages of the slaughtering line. Samples were collected from the first and last batches slaughtered on Monday and Friday of each week, respectively, during five weeks, totaling ten batches. From each batch, ten carcasses were collected in eight stages of the slaughter line: bleeding, scalding, singeing/evisceration, inspection, spinal cord removal, final washing, blast chilling, and after cooling. A total of 800 samples were analyzed for *Salmonella* sp. and enterobacteria quantification. The last batch of the week showed twice the chances of the pig carcasses being contaminated with *Salmonella* sp. and, consequently, a greater amount of enterobacteria (1.00 $\log_{10}$ CFU per square centimeter) than the first batch (0.88 $\log_{10}$ CFC per square centimeter). A higher count of enterobacteria was also observed in the stages of bleeding (2.37 $\log_{10}$ CFU per square centimeter) and scalding (2.36 $\log_{10}$ CFU per square centimeter). The last batches slaughtered in the week show a greater contamination than the first ones, and there is a greater contamination of carcasses by *Salmonella* sp. and enterobacteria in the initial stages of pig slaughter, i.e., at bleeding and scalding.

Index terms: foodborne illnesses, fridge, pig batch.

Salmonella enterica e enterobactérias em carcaças suínas processadas em diferentes dias de abate

Resumo – O objetivo deste trabalho foi avaliar a contaminação por *Salmonella* sp. e enterobactérias em carcaças suínas do primeiro e do último lote abatido na mesma semana, em diferentes etapas da linha de abate. Foram coletadas amostras do primeiro e do último lote abatido na segunda e na sexta-feira de cada semana, respectivamente, durante cinco semanas, o que totalizou dez lotes. De cada lote, foram coletadas dez carcaças, em oito etapas da linha de abate: sangria, escaldagem, chamuscador/evisceração, inspeção, retirada da medula espinhal, lavagem final, choque térmico e refrigeração. Um total de 800 amostras foi analisado para quantificação de *Salmonella* sp. e enterobactérias. O último lote da semana apresentou duas vezes mais chances de as carcaças estarem contaminadas com *Salmonella* sp. e, consequentemente, maior quantidade de enterobactérias (1,00 $\log_{10}$ UFC por centímetro quadrado) que o primeiro lote (0,88 $\log_{10}$ UFC por centímetro quadrado). Uma quantidade maior de enterobactérias também foi observada nas etapas de sangria (2,37 $\log_{10}$ UFC por centímetro quadrado) e escaldagem (2,36 $\log_{10}$ UFC por centímetro quadrado). Os últimos lotes abatidos na semana

apresentam contaminação maior do que os primeiros, e há maior contaminação das carcaças suínas por *Salmonella* sp. e enterobactérias nas etapas iniciais do abate de suínos, isto é, na sangria e na escaldagem.

Termos para indexação: doenças transmitidas por alimentos, frigorífico, lote de suíno.

Introduction

Several countries in Latin America are implementing national epidemiological surveillance systems for foodborne illnesses (FBIs). In Brazil, most FBIs are caused by bacteria, among which *Samonella* sp. is one of the most important (Brasil, 2018b).

Samonella sp. is a pathogen with a great zoonotic potential, as salmonellosis is one of the most commonly reported foodborne diseases worldwide. Outbreaks in humans are caused by the consumption of products derived from pigs, which represent an important source of contamination (Pires et al., 2014). This shows the need of assessing the hygiene conditions during the pig slaughtering process, which can be done using several groups of bacteria – such as, for example, *Escherichia coli* and *Salmonella* sp., both members of the Enterobacteriaceae family – as a quality standard (Milios et al., 2014).

A study carried out in Southern Brazil showed a high prevalence of *Salmonella* sp. in pigs at pre-slaughter, with values ranging from 8.7 to 24% in pre-cooled carcasses (Paim et al., 2019). In an exploratory research carried out by Ministério da Agricultura, Pecuária e Abastecimento (MAPA) on the presence of *Salmonella* sp. in pig carcasses in Brazil, *Salmonella* sp. was found in 10.3% (81 out of 788) of the carcass samples collected before cooling and in 5.5% (42 out of 756) of the samples collected after cooling (Anuário…, 2016).

The surface of carcasses can carry different types and quantities of microorganisms, making the slaughter stages critical points in the process to prevent, reduce, or eliminate microbiological hazards (Choi et al., 2013). Some preventive actions to control this problem include the implementation of food safety programs, such as the Hazard Analysis and Critical Control Point, during the entire process of slaughter and meat processing, with monitoring through microbiological analysis (Fajardo-Guerrero et al., 2019).

Brazil is considered one of the main food producers worldwide (ABPA, 2021). For the increase in the consumption and rise in the market of pork or its by-products, it is essential that the final product is within the current hygienic and sanitary standards and does not cause risks to consumer health, which requires minimizing, as much as possible, the presence of microorganisms that cause FBIs (Paim et al., 2019).

The objective of this work was to evaluate the contamination by *Salmonella* sp. and enterobacteria in pig carcasses from the first and last batches slaughtered in a same week, at different stages of the slaughtering line.

Materials and Methods

The experiment was carried out in a federally-inspected pig slaughterhouse, which is located in the state of Santa Catarina, Brazil, and where the average slaughter is of 4,630 pigs per day. The first and last batches slaughtered in a same week – on Monday and Friday, respectively – were sampled for a period of five consecutive weeks, from May to June 2018, totaling ten batches.

Upon their arrival at the slaughterhouse, the pigs were unloaded with the aid of mobile ramps and directed to the pens where they were separated by batch. There, they were cleaned with water jets and left to rest from 4 to 5 hours. After the animals were removed from the pens, these were cleaned with water and disinfectant for the arrival of the next batch.

The pigs were taken through corridors to the beginning of the slaughter line, being electrically stunned at the end. At this stage, the pigs whose carcasses would be sampled were identified by a cut in their ear. After bled on the mat, the pigs were hung on hooks of the conveyor for their blood to leak out. Then, the slaughtered pigs were released into a scalding tank at an average temperature of 62ºC and hung again on the conveyor for their carcasses to be scorched by flames in a continuous tunnel. After being scorched, the carcasses were cleaned (remaining hair was removed) and washed with water jets.

In the clean slaughter area, the rectum of the animals was occluded with an appropriate pistol and the carcasses were eviscerated for the removal of giblets and the head. Since the heads were removed, the initial identification of the carcasses for sampling

was substituted by plastic tapes tied to the double chin. After this procedure, the carcass was divided into two parts (half carcasses) using an electric saw – sterilized in boiling water between one carcass cut and the other –, with the half carcasses still hanging from the same hook. Afterwards, the carcasses were inspected and taken on the transport rails for spinal cord removal and then washing with water jets. After all these procedures, the carcasses were placed in a cooling chamber at -7°C.

The evaluated samples were collected from ten carcasses from each batch, always from the first carcass of the batch, skipping four carcasses, and then from the next carcass. The same carcass was sampled after each stage of the entire slaughter line, i.e., bleeding, scalding, singeing/evisceration, inspection, spinal cord removal, final washing, blast chilling, and after cooling. The samples were specifically collected from the carcasses after thermal shock, in average 1 hour after the shower, and from the left carcass, at the cooling point, at -8°C, in average 12 hours later.

For enterobacteria quantification, 800 samples were collected by rubbing three 132 cm^2 areas of the belly, loin, and ham surfaces, totaling an area of 396 cm^2 per sample. A marker was used to indicate the pre-established areas for sample collection on the carcass. For *Salmonella* sp. collection, the entire area of each carcass was frictionized, forming a pool used to detect the presence or absence of the bacteria. Two sterile 3M sponges (3M United States, Saint Paul, MN, USA) – one for *Salmonella* sp. and the other for enterobacteria –, hydrated with 10 mL of 1.0% peptone water, free from biocides, were used for each carcass at each collection point. After sample collection, the sponges were placed in plastic bags and stored under refrigeration in isothermal boxes.

The samples were analyzed for the presence of *Salmonella* sp. and of enterobacteria quantified in colony forming units (CFU) in the microbiology sector of the slaughterhouse laboratory.

For enterobacteria, the ISO 21528-2:2017 method (ISO, 2017a) of plate count was used. For this 1.0% peptone water was added to the sample, homogenizing and removing the 1.0 mL aliquot. This volume was placed in a petri dish containing 15 mL of violet red bile agar. After agar solidification, the plates were incubated in the inverted position, at a temperature of 36±1°C, for 18 to 24 hours, and then CFU were counted.

For the analysis of *Salmonella* sp. in the samples, the MDS II 3M detection assay kit (3M United States, Saint Paul, MN, USA) was used, following the loop-mediated isothermal DNA amplification molecular technique, based on the isothermal amplification of specific DNA sequences and described by Domesle et al. (2017).

After screening, the positive samples underwent a confirmatory analysis using method ISO 6579-1:2017 (ISO, 2017b). First, the sample was enriched through a specific medium and then through a selective one. Afterwards, the samples were spread on agar plates to isolate the colonies. After the isolation of the characteristic colonies, biochemical tests were carried out and an antiserum was used for confirmation. If the sample presented a positive result, the isolates were serotyped on a plate with antisera using the microagglutination technique, as described by White-Kauffmann-Le (2007).

The data were tabulated in an Excel spreadsheet where the amount of *Salmonella* per stage and per day of slaughter was counted, transformed from mL to cm^2, and expressed as log_{10} CFU per square centimeter. The normality of the enterobacteria data was verified with the Shapiro-Wilk (PROC UNIVARIATE) test. For the analysis of *Salmonella*, the logistic regression test and odds ratio (PROC LOGISTIC) were used. Enterobacteria were quantified by the Wilcoxon and Kruskall-Wallis (PROC NPAR1WAY) tests, and means were compared by the Dwass-Steel-Critchlow-Fligner test. Differences were considered significant at 5% probability. All analyzes were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

The contamination of pig carcasses, both by *Salmonella* sp. and by enterobacteria, was frequent but decreased significantly throughout the stages of pig slaughter and processing of the carcasses (Table 1).

A greater contamination by enterobacteria was observed in the last batches of the week, slaughtered on Fridays (1.00 log_{10} CFU per square centimeter), than in the first batches slaughtered on Mondays (0.88 log_{10} CFU per square centimeter) ($p<0.05$) (Figure 1). In

addition, higher enterobacteria counts were obtained in the stages of bleeding (2.37 $\log_{10}$ CFU per square centimeter) and scalding (2.36 $\log_{10}$ CFU per square centimeter) ($p>0.05$), which did not differ from each other (Figure 2).

Therefore, significant differences were found in the degree of contamination by enterobacteria in the samples collected in the first and last slaughtered batches of the week. This difference was approximately five-fold greater in the last batch slaughtered within the same week, i.e., bacterial counts were significantly higher in the last batches than in the first ones.

The presence of *Salmonella* sp. was detected in 123 of the 800 samples collected and analyzed throughout the study. Comparing the evaluated days of the week by logistic regression, the carcasses of the last batch of the week were twice as likely by the odds ratio to be contaminated with *Salmonella* sp. than those of the first batch of the week.

These data agree with the findings of Corbellini et al. (2016), who evaluated two sets of samples collected from pigs, considering sampling days. These authors reported more positive estimates for samples collected on Tuesday than on Monday.

Bleeding and scalding were the stages with the highest number of carcasses contaminated by *Salmonella* sp., with 57 and 60 contaminated samples out of 100. A large decrease was observed after the stages of singeing/evisceration (3/100), inspection (2/100), and final wash (1/100) (Figure 3).

During the pig raising process, several factors can cause contamination by *Salmonella* sp., making it difficult to completely eliminate this pathogen before

Table 1. Characterization of pig carcasses regarding the amount of enterobacteria and the number and serovars of *Salmonella* sp. in the sampling points along the slaughter line during five weeks in a federally-inspected slaughterhouse in the state of Santa Catarina, Brazil.

Colletion point (slaughtering stage)	Average of enterobacteria ($\log_{10}$ CFU per cm^{-2})	Number of carcasses positive for *Salmonella* sp. on Monday and Friday	Serovar
Bleeding	11,380.31	57	Derby Monophasic (1,4, [5], 12: i :-) Infantis Schwarzengrund Infantis Braenderup Heidelberg Agona
Scalding	5,258.49	60	Schwarzengrund Rissen Panama Infantis Javiana Anatum Ohio Livingstone Typhimurium Bredeney Monophasic (1,4, [5], 12: i :-) Panama Derby
Singeing /evisceration	31.47	3	Infantis
Inspection	111.12	2	Infantis
Spinal cord removal	135.69	0	-
Final wash	653.83	1	Monophasic (1,4, [5], 12: i :-)
Blast chilling	1.46	0	-
After cooling	0.01	0	-

the animals reach the slaughterhouses. Moreover, the volume of animals slaughtered throughout the day in the same slaughter line and without proper sanitation increases the chances of cross-contamination.

In the slaughtering line, cross-contamination can occur between carcasses due to handling by employees and direct contact with equipment and utensils. For this reason, proper hygiene is essential – as the greater the number of animals slaughtered per day, the greater the risk of contamination – and also avoiding the contact of the carcass with intestinal contents (Paim et al., 2019).

The reliability of data referring to *Salmonella* sp. was also verified, specifically the odds ratio of contamination along the slaughter line through the receiver operating characteristic curve. The results showed 92% reliability considering the variation of the curve – the further away from the central line, the greater the reliability of the data found. When evaluating the sampled points along the slaughter line, after scalding and bleeding, the probability of finding *Salmonella* sp. was of 60 and 55%, respectively.

The carcasses of all slaughtered batches were positive for *Salmonella* sp., and the presence of the pathogen was observed in 57 carcasses in the first stage of slaughter (Figure 3). According to Corbellini et al. (2016), pig batches delivered with a high level of this microorganism may result in a higher than average contamination of carcasses by *Salmonella* sp. on slaughter day. The data obtained in the present study confirm those of Paim et al. (2019), who found that all ten batches sampled after bleeding were positive for the bacteria, suggesting that the pigs already arrived contaminated at the slaughter line.

In Brazil, most farmed pigs are positive for *Salmonella* sp., as shown by Silva et al. (2012),

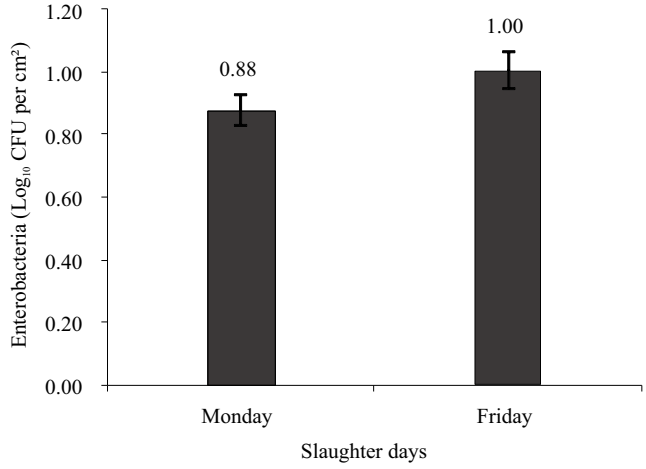

Figure 1. Quantification of enterobacteria (log_{10} CFU per cm^2) in pig carcasses according to slaugther days (Monday and Friday) in the same week during five weeks in a federally-inspected slaughterhouse in the state of Santa Catarina, Brazil.

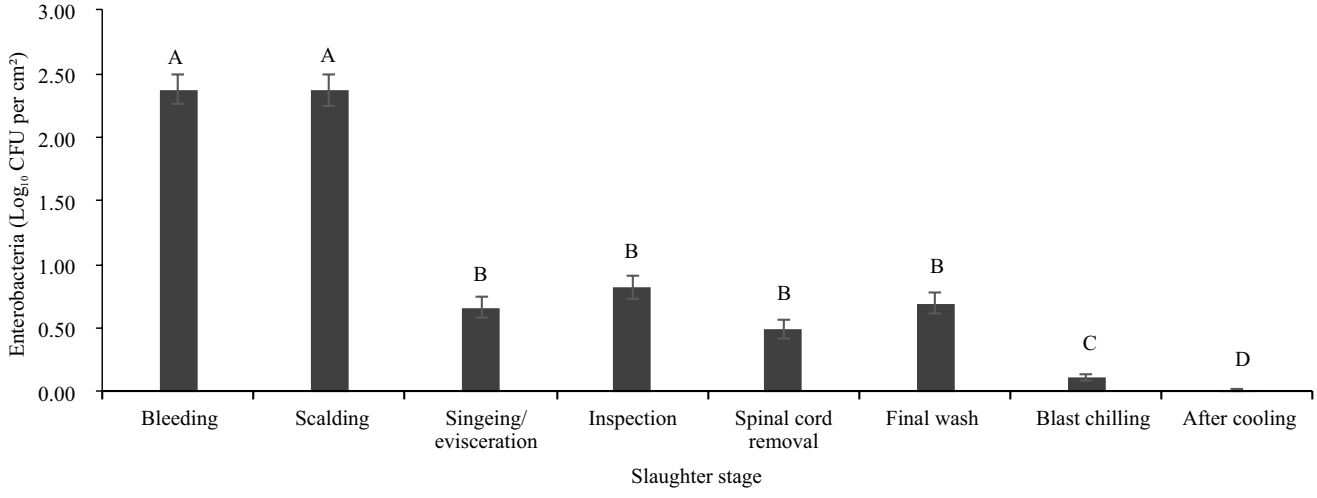

Figure 2. Quantification of enterobacteria (log_{10} CFU per cm^2) in pig carcasses in different stages of the slaughter line during five weeks in a federally-inspected slaughterhouse in the state of Santa Catarina, Brazil. Equal letters, on the bars, do not differ by the Dwass-Steel-Critchlow-Fligner test, at 5% probability.

who found, through ELISA's technique, 80.6% seroprevalence in batches at finishing and 23.8% of cecal contents positive for the bacteria. In another study carried out in the province of Córdoba, Argentina, Vico et al. (2020) observed a prevalence of 41.5% *Salmonella*, strongly associated with infections of this microorganism in the pre-slaughter period (distance between the farm to the slaughterhouse and lairage time), with a possible dissemination to other animals exposed to the same environment. Baer et al. (2013) point out that *Salmonella* spp. have the ability of resisting in an environment for long periods of time, which can cause asymptomatic infections; the infected carrier animals can be a source of contamination for the healthy population of the herd, including in slaughterhouses.

Dang-Xuan et al. (2017) reported a prevalence of 45% *Salmonella* sp. in fresh pork meat in Vietnam and a 17.7% probability of human beings contracting the disease due to the consumption of boiled pork. These results are indicative of how important it is to collect data on the contamination of pork carcass and meat by *Salmonella* sp., which is prevalent in several parts of the world and may cause serious health problems to humans.

Contamination by enterobacteria did not differ significantly ($p>0.05$) at carcass inspection, final washing, singeing/evisceration, and spinal cord removal, with 0.81, 0.69, 0.65, and 0.49 log_{10} CFU per square centimeter, respectively. However, a significant reduction in contamination was observed in the stage of blast chilling, followed by that of after cooling, with values of 0.11 and 0.00 log_{10} CFU per square centimeter ($p<0.05$), respectively (Figure 3).

After the scalding process, there was no significant reduction in the enterobacterial contamination of pig carcasses. This was the stage with the greatest contamination by *Salmonella* sp., followed by bleeding. All sampled batches (60 carcasses) were positive for this bacteria. It is noteworthy that these two stages of slaughter take place in the dirty area of the slaughterhouse.

In 80% of the batches evaluated during scalding, the serovars of *Salmonella* sp. were different from those found in the bleeding stage, showing a possible cross-contamination between batches and that scalding is a critical stage for the occurrence of such contamination (Table 1). The water inside the scalding tank comes into contact with the organic matter released from the carcasses and can become highly contaminated, although water temperature during this process must be between 62 and 72°C (Brasil, 2018b). If the scalding water is at a temperature below 60°C or has a large amount of organic matter, microorganisms may survive and there may be cross-contamination between carcasses. Zeng et al. (2021) concluded that the recycled water used in the dehairing machine

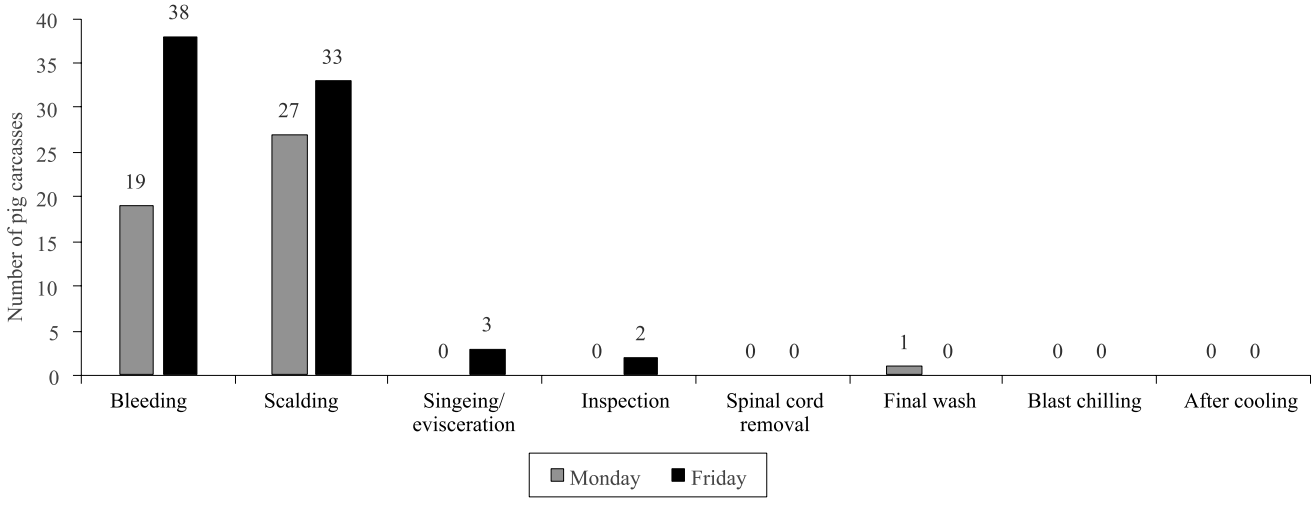

Figure 3. Number of pig carcasses positive for *Salmonella* sp. at different stages of the slaughter line, with samples collected on Monday and Friday of the same week during five weeks, in a federally-inspected slaughterhouse in the state of Santa Catarina, Brazil.

was the main source of carcass contamination in the slaughterhouse.

However, the alkalinization of the scalding water can contribute to the elimination of *Salmonella* sp. (Inagaki et al., 2018), which made irrelevant the accumulation of organic matter in the water at 62°C in the present study. Therefore, scalding can reduce bacteria in the carcasses (Buncic & Sofos, 2012) if these are clean when entering the slaughter line.

Another critical stage in pig slaughtering is evisceration, which involves the total removal of animal viscera, increasing the chances of contaminating not only the carcass but also the entire slaughter line due to the risk of perforation and leakage of contents during belly opening (Swart et al., 2016). However, in the present study, a low level of contamination by enterobacteria and *Salmonella* sp. was observed at this stage, since only three carcasses from the same batch were positive for the bacteria. Furthermore, the serotype found in the carcasses in the previous stages was the same as in singeing/evisceration, which does not even indicate cross-contamination between them.

Similar results were found by Zeng et al. (2021), who concluded that evisceration did not seem to be critical since the percentage of contamination of the carcasses was similar before (16.7%) and after (18.3%) this slaughter stage. Cardoso & Silva (2015) suggested that the main cause of reduction in contamination after evisceration was the scorching of the carcasses, in direct contact with the flames, at high temperatures that can exceed 700°C, which almost completely eliminate microorganisms.

The last batch of the week was positive for *Salmonella* sp. at the slaughter point after the inspection stage, right after the carcass was split into two. Carcass division is a critical step in terms of microbial contamination in the slaughter line, and the disinfection of the saw is recommended after processing each carcass (Cardoso & Silva, 2015). Despite this result, this stage showed a low contamination both by enterobacteria and *Salmonella* sp., as only two samples were positive for *Salmonella* sp., whose serovars were the same found in the previous stages.

The final wash can also be considered a critical control point, even though the obtained result does not differ from those found in singeing/evisceration, inspection, and spinal cord removal. At that stage, there still was contamination by enterobacteria and two samples in the last batch of the week were positive for *Salmonella* sp., with similar values to those of 1.25 and 3.9%, respectively, reported by Hernández et al. (2013) and Mannion et al. (2012). The action of water on the carcasses during final washing can contribute to the spread of bacteria on the surface of the carcasses and between them.

In the present study, the presence of *Salmonella* sp. was verified in the washing step in one of the evaluated batches, that is, in a single day. However, it is not possible to conclude there was cross-contamination between batches since a sample from a carcass from the first batch was contaminated and the same serotype was found in the scalding step in this same carcass. This result is indicative that there was a reduction and not the elimination of the bacteria throughout the slaughter stages.

Samples collected after thermal shock and after cooling showed different levels of contamination by enterobacteria, but not by *Salmonella* sp. This may be related to the use of controlled temperature, which reduces the microbiological load of the slaughtered carcasses, besides decreasing their pH. In the carcass blast chilling and cooling stages, the negative temperature – which must be below -8 and -7°C, respectively (Brasil, 2018b) – is one of the great contributors to the reduction of that microorganism. Arguello et al. (2012) found that the low temperature and reduction in water activity were two important factors to help reduce the concentration of *Salmonella* sp. in the cooling chamber due to a continuous air flow.

In the classification of *Salmonella* sp., 15 different serovars were found. The most frequent, in a decreasing order, were: 31 monophasic Typhimurium (1,4, [5], 12: i:-), 25 Infantis, 21 Derby, 20 Typhimurium, and 6 Schwarzengrund (Figure 4). Of the serotyped samples, a total of 39.7% were identified as Typhimurium and its variants.

The monophasic variant *Salmonella* Typhimurium, which has swine as one of its main hosts, causes salmonellosis in humans who consume contaminated pork meat (Gonzales-Barron et al., 2012; Crump et al., 2015). Campos et al. (2019) mainly attributes the high percentage of the serovar Typhimurium clinically affecting humans to an intensified production chain and increased production of pigs and pork meat on an international scale.

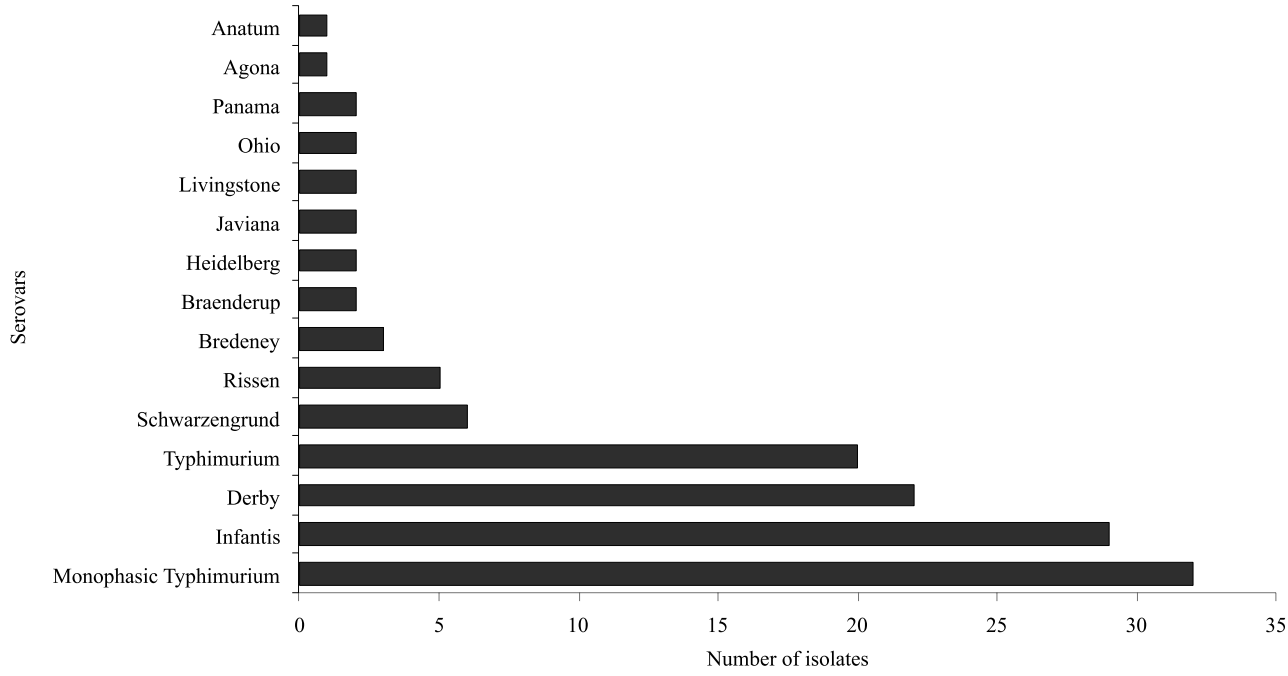

Figure 4. Percentage of *Salmonella* sp. serovars in pig carcasses, whose samples were collected on Monday and Friday during five weeks throughout the slaughter process in a federally-inspected slaughterhouse in the state of Santa Catarina, Brazil.

Another 13 other serovars were also found, of which the most relevant, in a decreasing order, were: 25 Infants, 21 Derby, and 6 Schwarzengrund. When swabbing pig carcasses along the slaughter line, Paim et al. (2019) also observed the presence of several serovars of *Salmonella* sp., among them Typhimurium, Infantis, and Derby.

Considering the obtained results and Instrução Normativa Nº 60 of Agência Nacional de Vigilância Sanitária (Anvisa, 2019) and MAPA (Brasil, 2018a), in the present study, the pig slaughtering process followed, respectively, the microbiological standards for ready-to-eat foods for human consumption and the regulations for microbiological control in pig and cattle slaughterhouses.

Conclusions

1. The pig carcasses of the last batches of the week, slaughtered on Fridays, show a greater contamination by *Salmonella* sp. and enterobacteria than those of the first batches, slaughtered on Mondays.

2. There is a greater contamination of pig carcasses with both *Salmonella* sp. and enterobacteria in the initial stages of pig slaughter – bleeding and scalding –, regardless of the day of the week.

3. All actions taken along the slaughter line are effective in reducing the microbial load on the carcasses at the end of processing, making them suitable for commercialization.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the scientific initiation scholarship granted to the second author.

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