VETERINÁRIA NOTÍCIAS

DOI: 10.14393/VTN-v25n2-2019-45525

Article

Reduction of *Salmonella* spp. levels in swine carcass at the slaughterhouse, using hot water bath at 80°c

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ABSTRACT

Porcine salmonellosis is of important economic and food safety importance, as it is a cause of food infections in humans, and is present in large scale in finishing pigs due to lymph node latency and rearing conditions. The objective of this study was to evaluate the reduction in *Salmonella* spp. in slaughter swine carcasses, after slaughtering under a bath with water at 80 ° C. Ninety swine carcasses were evaluated after slaughter at four harvesting points (leg, loin, belly and double chin), before and after bathing with water at 80 °C, in 720 samples, with quantitative analysis by the number method more probable. In slaughtering 43.33% (39/90) of the animals were positive before hot water application represented by 62 positive samples. After the intervention, 88.71% (55/62) of the positive samples zeroed the counts, in seven samples there was no reduction and in 11 samples, there was positivity in previously negative animals. The typifications of all positives were *Salmonella* Typhimurium. The samples with the greatest reduction in the count were double chin and belly samples with a concentration of 330 NMP / g that subsequently zeroed. Treatment with hot water bath in the carcasses was efficient, with significant difference of positivity before and after the intervention. There were cases of cross contamination after intervention in animals that remained positive and animals negative. Intervention by bathing the carcasses after gutting with 80 ° C water reduces the *Salmonella* spp. count and is economically viable.

Keywords: Count; hot wather; *Salmonella;* Swine carcasses

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Received: october, 2018; Accepted: September, 2019

Introduction

Salmonella spp. it is a bacterium of the *Enterobacteriaceae* family, involved in numerous cases of foodborne infection in humans, especially infections arising from the consumption of swine products (CDC, 2018). In Brazil, salmonellosis until 2017 led the cases of foodborne infections registered, with 38.2% of cases. From 2000 to 2013, over 1500 cases were reported (BRASIL, 2018). In most cases, swine are asymptomatic carriers; however, they can intermittently excrete the bacteria due to cross contamination or animal positivity, and carry the pathogen for fresh meat and or by products (CASTAGNA et al., 2004).

The faecal-oral route is considered the main route of transmission, and the bacterium quickly settles in the host, expressing virulence genes to overcome these barriers (HURD et al., 2001; OCHOA; RODRIGUEZ, 2005; SCHWARTZ et al., 2006). The elaborate set of genes can confer the ability of infection in a host diversity, in which the bacteria colonize the enterocytes, surpassing the gastrointestinal barrier. The animal may develop gastroenteritis or the bacterium may reach secondary lymphoid cells and tissues and develop septicaemia and death or become asymptomatic carrier, important in the spread and infection of others (OHL; MILLER, 2001; RHEN; DORMAN, 2005; GRIFFITH; SCHARTZ; MEYERHOLZ, 2006; KARASOVA et al., 2009).

Salmonella in swine is found especially in lymph nodes and digestive tract, making faeces and mesenteric lymph nodes important sources of carcass contamination in the slaughterhouse (BESSA; COSTA; CARDOSO, 2004; CASTAGNA et al., 2004; ROSTAGNO; HURD; McKEAN, 2009). Many animals reach slaughter with high levels of positivity and few studies describe the decontamination effect of swine carcasses. Carcasses from batches with high contamination undergo intervention with hot water in the slaughterhouse as an extra measure for decontamination before carcass

cooling and associated with the other HACCP and Hazard Analysis interventions, resulting in decontamination efficiency (MUSING et al., 1997; BERENDS et al. 1997; HURD et al, 2002; CHRISTENSEN, 2004).

The objective of this study was to evaluate the reduction in *Salmonella* spp. in swine carcasses before and after they are bathed with water at 80 ° C.

Material and methods

Samples were collected in September 2014 in a refrigerator under Federal Inspection, located in the west of Santa Catarina, capable of slaughtering 5,000 pigs per day. Ninety swine carcasses were randomly sampled after evisceration on three slaughter days, with 30 animals per day before and after intervention with a hot water bath at 80 °C, which is the normal temperature of use for refrigerator water which has been properly treated and tested for framing as drinking water. Bathing is a step that occurs after evisceration before the refrigeration step. Thus, the collections were performed soon after evisceration and after bathing. Each carcass was sampled at four different points, leg, loin, belly and double chin, according to Circular No. 130/2007 / CGPE / DIPOA (BRAZIL, 2007), in a total of 360 samples before and 360 samples after the intervention with the hot water. The duration of the hot water bath followed the normal flow of carcasses passage through the nomad until storage, being on average 1.5 minutes. During this period, all carcasses were bathed in an arc shower over their entire surface with constant pressure and a constant temperature of 80 ° C. The points were sampled using sterile wipes soaked in physiological solution, in 100 cm² of the carcass, delimited by a sterile plastic mold, totalling 400 cm² of sampled area for the whole carcass. After collection, the tissues were placed back in their original sterile packaging, identified and immediately sent to the laboratory for analysis.

The experiment was approved by the Animal Experimentation Ethics Committee of the State University of Santa Catarina (UDESC), under number 1.58.13.

Samples from the refrigerator were processed following the Most Probable Number (MPN) quantitation technique, adapted from that described by Blodgett and Garthright (1998). Each of the 720 samples collected were pre-enriched in 1:10 2% buffered peptone water, incubated in a bacterial aerobic greenhouse at 37 °C / 18 to 24 hours. After this period each sample was transferred to six tubes of Rappaport Vassiliardis selective enrichment broth containing 9 mL of broth, and in the first five tubes added 1mL of the sample (10^{-2} dilution), and after homogenization of the fifth tube was passed 1mL from this to the sixth tube (10^{-3} dilution). The tube series was incubated at 42 °C / 18 to 24 hours. After incubation, all Rappaport Vassiliardis tubes were seeded on XLT4 selective agar and incubated at 37 °C/18 to 24 hours. Suspected *Salmonella* colonies were inoculated in biochemical tests, according to Silva (2007), and then submitted to poly O antiserum, in serology, to confirm the presence of *Salmonella* spp.. When confirmed the *Salmonella* spp. colonies, they were counted by most probable number (MPN) by the combination of positive tubes, as described by Blodgett and Garthright (1998). In the *Salmonella* NMP used, indices are based on the combination of tube positivity and represent fixed values based on growth average and dilutions made. The index used is that fixed for the combination of tubes in the dilution series where plaque bacterial growth is present.

Positive samples were subjected to polymerase chain reaction (PCR) typing by the microarray method, which allows simultaneous evaluation of the expression of thousands of genes in different tissues in a given organism, at different stages of development or environmental conditions (WATTIAU et al., 2008a; WATTIAU et al., 2008b).

Statistical analysis was performed by chi-square test (X^2) with a significance level of 5% and log reduction of bacterial counts.

Results

Of the 90 carcasses surveyed by the quantitative MPN technique, 43.33% (39/90) were positive for *Salmonella* spp., in at least one of the sampled points, before intervention with hot water. Of the 360 samples taken before the hot water intervention, the number of positives was 62 samples (17.22%). Of these, 88.71% (55/62 samples) were negative after the 80° C hot water bath application, and 11.29% (7/62) samples had lower growth after the intervention. However, this reduction in counting was less than three logs, representing 11% of samples where intervention with hot water did not result in effectiveness. There were 17.74% (11/62) of the samples that were negative before the intervention and positive after the hot water bath at 80 ° C.

Table 1 shows the positivity results of the samples, according to the carcass sampling location, before and after the 80° C water bath. It can be observed that before bathing, belly and double chin showed the largest number of positive samples (39% at each point), followed by leg and loin (11% at each point). After bathing, most positive samples were double chin (71.43%) and loin and belly (14.28% at each point). All ham samples, positive before bathing, were negative after the procedure.

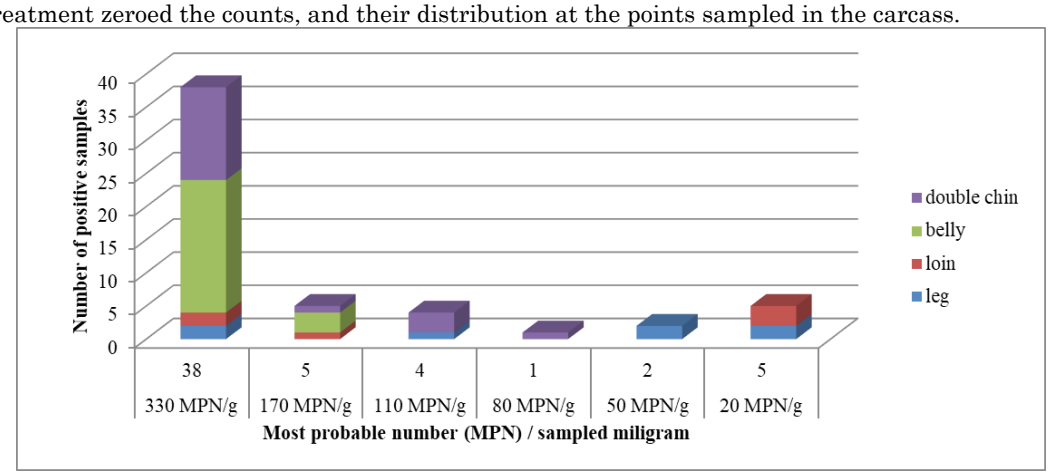
Table 1 - Absolute number and percentages of *Salmonella* positive samples before and after water bath at 80 ° C, segregated by sampled area.

	Before bath at 80 ° C		After bath at 80 ° C	
	Positives	%	Positives	%
Leg	7 [a]	11	0[b]	0
Loin	7 [a]	11	1[b]	14,28
Belly	24 [a]	39	1[b]	14,28
Double chin	24 [a]	39	5[b]	71,43
Total	62	100	7	99,99

[a,b] Different letters on the same line indicate statistical difference (p> 0.001).

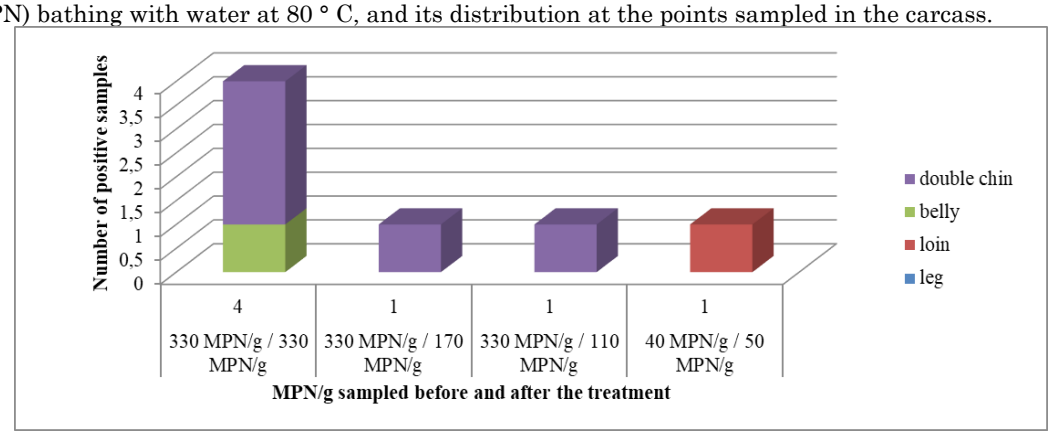
Of the 55 samples that after treatment with hot water negated *Salmonella* spp. counts, 69% (38/55) had counts of 330 MPN/ g before intervention, distributed at different collection points, which are illustrated in Figure 1. The other pathogen counts (170 MPN / g, 110 MPN / g, 80 MPN / g, 50 MPN / g and 20 MPN / g) together amounted to 30.9% (17/55) which are also shown in Figure 1.

Figure 1 - *Salmonella* count in MPN / g of positive samples before bathing with water at 80 ° C, which after treatment zeroed the counts, and their distribution at the points sampled in the carcass.



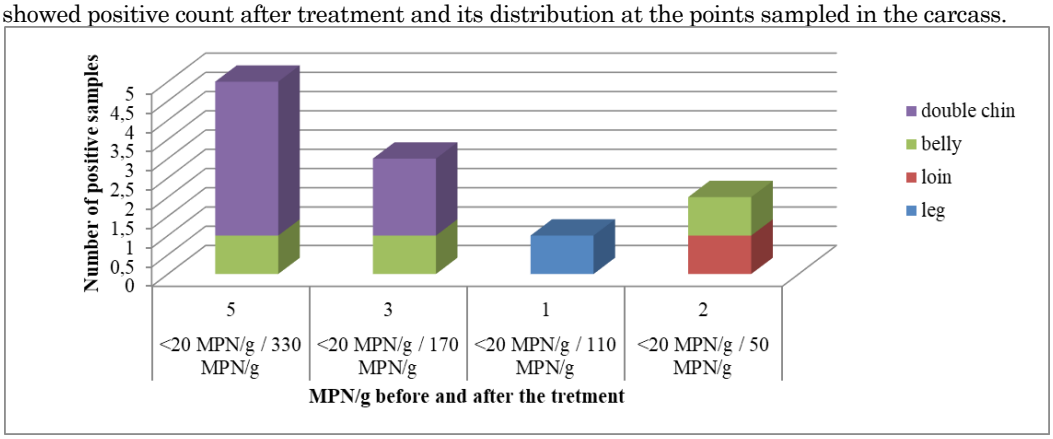
Seven samples (12.72%) remained positive after treatment at different counts and sampling locations. Four samples (7.72%) had rates of 330 MPN / g before intervention and maintained 330 MPN / g after treatment. The 330 MPN / g sample significantly reduced to 170 MPN / g and a 330 MPN / g sample reduced to 110 MPN / g. Still a sample of 40 MPN / g went to 50 MPN / g. These data as well as the sampled locations where this count was taken are illustrated in Figure 2.

Figure 2 - *Salmonella* count in MPN / g of samples that remained positive before and after (a MPN / b MPN) bathing with water at 80 ° C, and its distribution at the points sampled in the carcass.



There were 11 samples (17.74%) that did not present counts at harvest before treatment and were positive after the intervention. Counts and collection locations are shown in Figure 3.

Figure 3 - *Salmonella* count in MPN / g of samples that were negative before bathing with water at 80°C, but showed positive count after treatment and its distribution at the points sampled in the carcass.



The 80 samples positive for *Salmonella*, 62 before (77.5%) and 18 after (22.5%) of the treatment, were typified by microarray resulting from the identification of *Salmonella* Typhimurium serovar only.

Discussion

The positivity for *Salmonella* spp. found in the 90 carcasses analysed was 43%, differing from Pisseti et al. (2012) who in a similar

study found 27.4% of the animals positive for slaughter, and Castagna et al. (2004) found 79% in Rio Grande do Sul, and closer to the findings of Hoek et al. (2012), who found 35.9% positivity in a swine slaughterhouse in the Netherlands.

The number of positive as well as the highest pathogen counts were detected in the double chin and belly, respectively, a fact attributed to the position of the carcass in the area with fluid flow from the lymph node cut directed to these sites. The use of hot water proved to be very efficient exactly in these places where, in cases of higher counts (330 MPN / g), they eliminated 100% of *Salmonella* spp. in 53% of the belly points and 37% of the positive double chin before the intervention (Figure 2). Castagna et al (2004) observed carcass contamination by lymph nodes, especially mesenteric ones.

This work proves that the use of hot water as a carcass bath was effective to reduce contamination by *Salmonella* spp. by 89%. The information matches the findings of Goldbach and Alban (2006) who found 72% efficiency in hot water decontamination in Danish slaughterhouses. These same authors report in their findings that hot water decontamination is effective without compromising the quality of the final product. Also Hamilton et al. (2010) observed that hot water use reduced the surface prevalence of Escherichia coli from 92.9% to 9.8% in a slaughterhouse in Australia. However, for Morild et al. (2011), performing experimental inoculation of *Salmonella* Typhimurium on muscle and skin of swine, observed increase in pathogen count after application of water at 80 ° C and lactic acid.

The use of hot water, although it promotes an extra expense in the refrigerator due to energy consumption, is already an input present in the production line, and the benefit of its use in decontamination justifies the use and increase of charges against its efficiency.

Intervention becomes a cheap and effective alternative, taking into account contamination reduction efficiency and the risk of having *Salmonella* in the finished product. Goldbach and Alban (2006) also report the advantages of using hot water when describing the cost-benefit of pathogen reduction interventions in Denmark.

The 11 samples that were not positive before the intervention and developed after the use of hot water show us the existence of cross contamination in the area after slaughter, by contact with positive carcasses or operator handling. These contamination can be conveyed by operator gloves, contact of positive carcasses and decontaminated carcasses, or by the production processes (hooks and structure). Highlight for the double chin that was the place with more than half of the findings in this mode. Berends et al. (1997) state that 70% of the contamination at the end of the slaughter line comes from a carrier animal.

Samples for shipment to the laboratory were packaged in sterile containers sealed in boxes, negative controls between the sample group were used in the process to signal contamination, in addition to ISO 17025 accredited traceability to the laboratory reinforcing the idea of no cross contamination in the diagnosis. This set of information directs us to reinforce the concept of Good Production Practices (BPP), as well as the use of interventions, because it is possible that much of the problem is the subsequent recontamination to decontamination alternatives. For this, care after using the 80 ° C water bath should be directed to operators and facilities. Measures such as changing gloves, washing and disinfecting facilities, biofilm removal, stringent HACCP oversight can help improve the performance of interventions, including hot water decontamination, so that applying the technique is not just a cost increase.

Goldbach and Alban (2006) state that the temperature of 80 ° C affects the bacterial structure of *Salmonella*, as it is well above the ideal temperature for growth of the pathogen (which is 40 ° C to 42 °C). However,

it can also be hypothesized that part of the success of the technique is due to the mechanical removal of water flow in the finished carcass, a point that did not participate in the variables of this study.

Regardless of whether the reduction synergy is linked to physical removal and water temperature, the study gives us an alternative viability of positive carcasses, and an executable way to reduce the surface contamination pressure of the product. This intervention meets the findings of Hamilton et al. (2010), who attribute success to this intervention and indicate as a good way to improve the conditions of the final product.

Conclusion

Bathing the carcasses after evisceration with water at 80 ° C reduces the *Salmonella* spp. The intervention is economically viable and is accepted by Brazilian law.

Thanks

The laboratory staff for the help in the experiment, and the fridge for the permission to use the structure and resources.

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Banho das carcaças com água a 80°c reduz índices de S*almonella* spp. em carcaças de suínos

RESUMO

A salmonelose suína tem relevante importância econômica e na segurança alimentar, pois além de ser causadora de infecções alimentares em humanos, está presente em larga escala nos suínos de terminação, devido à latência em linfonodos e as condições de criação. O objetivo deste trabalho foi avaliar a redução na contagem de *Salmonella* spp. em carcaças de suínos no abate após as mesmas serem submetidas a banho com água a 80°C. Foram avaliadas 90 carcaças de suínos após abate em quatro pontos de colheita

(pernil, lombo, barriga e papada), antes e após a aplicação de banho com água a 80°C, num total de 720 amostras, com análise quantitativa pelo método do número mais provável. No abatedouro 43,33% (39/90) dos animais foram positivos antes da aplicação da água quente representado por 62 amostras positivas. Após a intervenção 88,71% (55/62) das amostras positivas zeraram as contagens, em sete amostras não houve redução e em 11 amostras houve positividade em animais anteriormente negativos. As tipificações de todos os positivos foram *Salmonella* Typhimurium. As amostras com maior redução na contagem foram amostras de papada e barriga com concentração de índices de 330 NMP/g que posteriormente zeraram. O tratamento com banho de água quente nas carcaças foi eficiente, com diferença significativa de positividade antes e após a intervenção. Houve casos de contaminação cruzada após a intervenção em animais que permaneceram positivos e animais negativos. A intervenção feita com o banho das carcaças suínas após evisceração, com água a 80°C, reduz a contagem de *Salmonella* spp. e é viável economicamente.
Palavras-chave: Controle microbiológico, Salmonelose, Tratamento térmico.

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