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Brief Original Article

Detection and quantification of *Salmonella* spp. in poultry slaughterhouses of southern Brazil

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Abstract

Introduction: *Salmonella* is a major cause of foodborne illness throughout the world. The use of quantitative techniques is important for assessing the risk and determining the capacity of each step of the slaughtering process to decrease or increase bacterial contamination. We aimed to detect and to quantify the presence of *Salmonella* in Brazilian processing plants by real-time quantitative polymerase chain reaction (qPCR).

Methodology: A total of 139 poultry slaughterhouses samples were collected in order to detect to and quantify *Salmonella* by qPCR.

Results: Almost all collection points (3/18), except water from pre-chiller tank, carcasses after pre-chiller, and carcasses frozen at -12ºC for 60 days, and 49% (68/139) of samples were positive for *Salmonella*. Quantification means varied equally among all of the tested sources, and we could not establish any pattern of variation. A large proportion (52.6%) of cloacal swabs was *Salmonella*-positive. Also, contamination in transport cages was increased after the cleaning process, indicating that the process was ineffective. The overall prevalence in samples obtained during the slaughtering process was 48.9%, and on the whole rinsed carcasses, this proportion was 50%. The detection of *Salmonella* in frozen carcasses, even after long periods of storage, indicates that the carcasses are a potential source of infection for consumers.

Conclusions: We found that contamination levels remain similar throughout the slaughtering. qPCR proved to be an efficient method for the detection of *Salmonella*.

Key words: qPCR; Salmonella; slaughterhouse; poultry.

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Introduction

Salmonella is a major cause of foodborne illness throughout the world [1,2]. According to the Brazilian Ministry of Health, *Salmonella* is responsible for more than 30% of foodborne diseases in Brazil [3]. The Center for Disease Control and Prevention (CDC) reports that the most common foods products associated with salmonellosis outbreaks are poultry and its derived products, accounting for up to 47% of all infections [4]. Brazil is a leading supplier of the world's poultry meat, and the Rio Grande do Sul is one of the major producing states [5].

The presence of *Salmonella* in the gastrointestinal tract of healthy poultry is suggested as the main risk factor for human infection [6] since it can cause contamination of the carcasses during slaughtering, thus spreading the microorganism to the final consumers [7]. *Salmonella* control in poultry slaughterhouses is based on its detection. The use of quantitative techniques is important in assessing the risk to determine the capacity of each step of the slaughtering process to decrease or increase bacterial contamination [8].

The Brazilian Ministry of Agriculture, Livestock and Food Supply recommends conventional bacteriological tests for *Salmonella* spp. detection [9]. Moreover, previous studies have shown that the miniaturized most-probable-number (mMPN) method is efficient for the identification and quantification of *Salmonella* on poultry meat [8,10]. However, these methods are time-consuming and laborious. Furthermore, the heterogeneous distribution of *Salmonella* and natural contamination are difficulties encountered for the correct quantification of samples [8]. Therefore, new pathogen-quantification methodologies have been developed, including real-

time quantification PCR (qPCR). The qPCR method is highly selective and accurate for detection of *Salmonella* in food [11].

In this context, this study aimed to detect and quantify the presence of *Salmonella* spp. in Brazilian processing plants by a real-time quantitative polymerase chain reaction (qPCR).

Methodology

Samples selection

Four broiler slaughterhouses under the federal inspection system from the state of Rio Grande do Sul were previously sampled [8,12]. Sampling included cloacal swabs at the reception of the broilers at the slaughterhouses, swabs collected from transport cages (before and after the cleaning and disinfection process) and carcasses collected during the slaughter process (before and after scalding, after plucking, after initial and final washing, after evisceration, before and after pre-chiller, after chiller, frozen at -12ºC (24 hours, 30 and 60 days), and water (from scalding, pre-chiller and chiller tanks).

The collected carcasses were stored in individual sterile plastic bags and identified with seals. In the laboratory, each sample was rinsed with 400 mL of buffered peptone water (Difco Laboratories, Detroit, MI, USA) and homogenized for 30 seconds. Samples were stored in 1% Buffered Peptone Water and aliquoted immediately after processing, being frozen at -18° C until the procedures of DNA extraction and real-time PCR. At the end of the sampling period, five batches were selected, totaling 139 samples.

Bacterial isolation

Conventional microbiology and miniaturized most-probable-number (mMPN) were previously performed [8,12] to detect and to quantify *Salmonella* spp. in collected samples.

A standard curve for quantification of DNA by real-time PCR

The standard curve was performed with the reference strain of *S*. Typhimurium ATCC 14028. Colonies cultivated on plate count agar (PCA) (Oxoid, Basingstoke, UK) were inoculated in brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated at 37°C overnight under agitation at 200 rpm. The optical density (OD 600 nm) was measured in a nanospectrophotometer (Implen, Westlake Village, CA, USA), and the material was centrifuged at 13,000 rpm for 5 minutes. The supernatant was discarded, and the pellet was suspended in PBS. The OD 600 nm was adjusted to 1.0, and 10× serial dilutions were made in PBS. Then, each dilution was plated on PCA and incubated at 37°C for 24 hours to confirm cell viability. The DNA was extracted using a Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. From the extracted DNA, a 3 µL aliquot was separated into microtubes containing 27 µL of RNase-free water for serial dilutions from 10^{-1} to 10^{-10}. A total of 1 µL of each dilution was transferred to 10 microtubes in duplicate with 9 µL of ultrapure water and 10 µL of reaction mix. The negative control was prepared in a microtube containing 10 µL of reaction mix and 10 µL of ultra-pure water, and the positive control consisted of 10 µL of reaction mix and 10 µL of positive control DNA.

Real-time quantitative PCR (qPCR) analyses

The DNA was extracted using a mericon DNA Bacteria Kit (Qiagen, Manchester, UK) according to the manufacturer's protocol. Amplification of *Salmonella* strains was performed with the mericon *Salmonella* spp. Kit (Qiagen, Manchester, UK) designed for the target-specific detection of *Salmonella* species DNA in food. The reactions included a total volume of 20 µL containing 10 µL of reconstituted mericon assay master mix and 10 µL of genomic DNA. Amplification was carried out at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 10 seconds using a Rotor-Gene real-time PCR system (Qiagen, Manchester, UK). All reaction conditions were performed according to the manufacturer's protocol. The interpretation of the results followed the recommendation of the reaction kit's manufacturer; all of the samples that presented cycle threshold (Ct) values were considered positive since the internal control showed a Ct between 17 and 37 [13].

Statistical analyses

The data obtained were subjected to statistical analyses using PASW statistical software (IBM; Armonk, NY, USA). A Oneway ANOVA test was used to compare *Salmonella* detection in samples collected throughout processing. Significance was defined as $p < 0.05$.

Results

Of the 139 collected samples, 49% (68/139) were positive for Salmonella spp. by qPCR method, 10.8% (15/139) by conventional microbiology, and 8.6% (12/139) by mMPN. qPCR was significantly ($p < 0.05$)

higher than detection by conventional microbiology and by mMPN.

From the calibration curve for qPCR, we detected a calibration line with a correlation coefficient (R^2) of 0.9977. The results for qPCR quantification, according to the source of isolation, are described in Table 1. *Salmonella* spp. were not detected in any sample of water from the pre-chiller tank, carcasses after pre-chiller or carcasses frozen at -12ºC for 60 days.

There is no significant difference ($p > 0.05$) in quantification means among all sources. 52.6% of samples from cloacal swab were positive. The total proportion of transport cages contaminated with *Salmonella* presented a nonsignificant ($p > 0.05$) decrease, from 55.5% to 44.4%, after the washing and disinfection process. The overall prevalence of *Salmonella* in samples collected during the slaughtering process was 48.9%. Considering only the rinsed carcasses, it was 50%.

Discussion

Despite the growing concern about emerging pathogens in recent years, *Salmonella* spp. remains one of the most common agents of foodborne diseases in several countries [2]. The presence of *Salmonella* on poultry represents a risk for the slaughtering process since it can be transferred onto preparation surfaces and can contaminate meat [14]. The processing plant environment represents adverse conditions to *Salmonella* survival, but it can resist and can be detected in all stages of the slaughtering process [15]. Also, *Salmonella* strains can grow in foods stored at low (2–4°C) and high (54°C) temperatures [16].

The high frequency of poultry-associated salmonellosis in humans requires rapid, reliable, and low-cost methods to detect *Salmonella* in poultry production chain. This monitoring might be significant in preventing *Salmonella* infection [17]. Conventional methods have been traditionally used to enumerate target bacteria in food. However, these methods have some limitations and require considerable time and labor [17]. In this context, development of real-time quantification PCR has overcome conventional methods. In this study, qPCR shows a more effective detection of *Salmonella* in food, and it was higher than detection by conventional microbiology and by mMPN that were previously performed [8,12]. Previous studies have already shown that qPCR is more effective than conventional bacteriological methods for the detection of *Salmonella* spp. [18]. However, it is important to highlight that non-viable cells can be detected by qPCR, which does not occur in traditional methods of culture and isolation that require viable cells for quantification [19].

High positivity in cloacal swab indicates that the poultry arrives contaminated at the slaughterhouse. There is evidence for contamination of equipment on the slaughter line and subsequent cross-contamination to non-infected chicken [20]. This information highlights the need and the importance of controlling *Salmonella* in the primary production. The total proportion of transport cages contaminated with *Salmonella* decreased. However, the amount of

Table 1. Quantification (mean log UFC/mL) of *Salmonella* spp. during poultry slaughtering process.

Source of isolation	Quantification (mean log UFC/mL)
cloacal swab	1.60
swabs of transport cages (before cleaning)	2.77
swabs of transport cages (after cleaning)	2.96
carcasses before scalding	3.04
carcasses after scalding	2.58
carcasses after plucking	1.16
carcasses after the initial washing	3.64
carcasses after evisceration	2.69
carcasses before pre-chiller	1.74
carcasses after chiller	1.48
carcasses at final washing	1.36
carcasses frozen at −12°C (24h)	1.97
carcasses frozen at −12°C (30d)	2.40
water from scalding	1.48
water from chiller	1.36

Salmonella on positive cages was higher, demonstrating that this process was ineffective. Previous studies have shown that transport cages can potentially act as a source of broiler exposure to *Salmonella* as they are transported into the food processing plants [21].

The prevalence in the carcasses is much higher to that in similar studies conducted in Colombia [22], Costa Rica [23], Canada [24], and the European Union [1]. Although, according to Ramirez-Hernandez *et al*. [22], variability among facilities and regions is significant even in countries with high *Salmonella* prevalence. It is important to highlight that we detected *Salmonella* in all stages of the process, except carcasses after pre-chiller. Carcass contamination during slaughtering process has already been described, including in Brazilian plants [15,25,26]. Although, carcass contamination varied along the slaughtering process and we could not establish any pattern of variation.

In the present study, we verified a reduction in carcass contamination after the scalding process. The temperature of the scalding water varies between 50°C and 70°C and it is intended to open the skin pores to facilitate subsequent plucking, but it might also act in reducing the microbial load [27]. On the other hand, the plucking machine is a possible source of *Salmonella* contamination [28]. However, in our study, this could not be proven since carcasses presented lower contamination after this step. Carcasses after initial washing presented the highest contamination, followed by carcasses before scalding and carcasses after evisceration. Previous studies suggest that the processes of evisceration and spray washing represent a risk of *Salmonella* cross-contamination or recontamination in broilers during slaughtering processing [23].

In our study, we detected *Salmonella* DNA on frozen carcasses in long periods (30 days) of storage. This data is important from the public health point-of-view because, if these bacteria are viable, frozen carcasses sold in retail markets represent a risk for consumers' health.

The presence of *Salmonella* on chicken carcasses probably resulted in water contamination, since we detected the microorganism in the water tank from scalding and from the chiller. Contamination of the chiller tank has been described previously [29,30]. Water contamination is a problem during the slaughtering process since it might facilitate cross-contamination to noninfected carcasses. The carcass washing system, used as a final shower in the evisceration line, is recommended by *The Codex Alimentarius* to reduce the contamination of pathogenic microorganisms, including *Salmonella* [31]. This washing system is adopted by poultry processing plants in the United States, Canada and European Union [32-34]. Recently, Brazilian legislation has allowed this procedure in order to achieve governmental guidelines, which determine zero tolerance for fecal contamination in carcasses that enter into the cooling system [33,35]. Preliminary studies evaluating carcass washing system effectiveness in Brazilian poultry slaughterhouses demonstrated that this procedure avoids the trimming of some carcasses, but does not eliminate the risk of *Salmonella* contamination in chicken meat [33]. However, more studies are necessary to determine the importance of the carcasses washing system in Brazilian food-producing plants.

Conclusion

Our results indicated that contamination levels remained similar throughout the slaughtering process. The detection of *Salmonella* spp. on frozen carcasses, even in long periods of storage, indicates that the carcasses are a potential source of infection for consumers. qPCR proved to be more efficient than conventional bacteriological methods for the detection of Salmonella spp. However, further studies with qPCR assays that allow the differentiation of viable and dead cells are now needed.

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