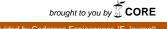FULL ARTICLE

Occurrence of *Salmonella* sp. in poultry carcasses evaluated from the retail trade between 2007 and 2013 in Paraná state, Brazil

Ocorrência de Salmonella sp. em carcaças de frango avaliadas a partir do comércio varejista entre 2007 e 2013 no estado do Paraná, Brasil

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ABSTRACT

Poultry meat is often involved as a vehicle for microorganisms that cause food-borne diseases. *Salmonella* sp. is a major pathogen involved in outbreaks around the world. Based on its importance, the objective of this study was to determine the occurrence of *Salmonella* sp. in frozen and chilled poultry carcasses slaughtered and marketed in the western region of Paraná state, Brazil. A total of 340 samples were collected between January 2007 and April 2013, with 66 (19.41%) carcasses positive for *Salmonella* sp. It can be concluded from the results obtained that the occurrence of *Salmonella* sp. in poultry carcasses remains high, even though it is a product marketed refrigerated or frozen, methods considered appropriate for food preservation. A lower occurrence result was expected due to the 14-year implementation of the national pathogen reduction program established by the Ministry of Agriculture, Livestock and Supply aimed at a gradual reduction in the occurrence of *Salmonella* sp. in these products through constant monitoring of carcasses immediately after slaughter.

Keywords: Chilled carcass. Frozen carcass. Poultry slaughter. Occurrence. PRP.

RESUMO

A carne de aves é um alimento que frequentemente encontra-se envolvido como veículo de micro-organismos causadores de enfermidades, sendo *Salmonella* sp. um desses agentes comumente envolvidos nessas enfermidades em todo o mundo. Com isso, o objetivo deste estudo foi determinar a ocorrência de *Salmonella* sp. em carcaças de frango congeladas e refrigeradas, abatidas e comercializadas na região oeste do estado do Paraná. Um total de 340 amostras foram coletadas entre janeiro de 2007 e abril de 2013. Das amostras analisadas, 66 (19,41%) das carcaças foram positivas para *Salmonella* sp. De acordo com os resultados obtidos pode-se concluir que a ocorrência de *Salmonella* sp. em carcaças de frangos permanece alta, mesmo sendo produtos comercializados refrigerados ou congelados, métodos considerados adequados para conservação de alimentos. Um resultado de menor ocorrência era esperado devido à implementação de 14 anos do Programa Nacional de Redução de Patógenos estabelecido pelo Ministério da Agricultura, Pecuária e Abastecimento visando uma redução gradual na ocorrência de *Salmonella* sp. nestes produtos através de monitoramento constante de carcaças imediatamente após o abate.

Palavras-chave: Carcaças resfriadas. Carcaças congeladas. Abate de aves. Ocorrência. PRP.

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Introduction

Brazil holds a prominent position in poultry production, being the largest exporter and the third largest producer of poultry meat in the world. In 2016, the country was

responsible for the slaughter of 5.86 billion birds, with Paraná state the main national producer, with 1.83 billion birds slaughtered (Associação Brasileira de Proteína Animal, 2016; Instituto Brasileiro de Geografia e Estatística, 2016). The recognition reached by the country in the poultry market stems from continuous advances in production methods, genetic improvement, plant health management and control of slaughter processes, including the monitoring and control of *Salmonella* sp.

Despite constant effort of the poultry industry to ensure the safety of the food produced, the presence of *Salmonella* sp. is still significant, representing one of the industry's biggest concerns. The contamination of poultry meat by *Salmonella* sp. can occur at different steps of the production chain. Some operations, such as scalding, plucking and evisceration, are considered critical because they move a great biological load, playing a fundamental role in the microbial contamination of carcasses and their derivatives (Boni et al., 2011; Von Ruckert et al., 2009). The control of *Salmonella* sp. is a challenge faced not only by Brazil. Studies carried out in several countries indicate a high prevalence of the agent in poultry meat and in slaughter environments, demonstrating the relevance of this pathogen in the world poultry scene (Álvarez-Fernández et al., 2012; Donado-Godoy et al., 2012; Hue et al., 2011; Medeiros et al., 2011; Sakaridis et al., 2011; Zhu et al., 2014).

Considering the epidemiological importance *Salmonella* sp. has in poultry, since 2003, the Ministry of Agriculture, Livestock and Food Supply (MAPA) has been monitoring the presence of the pathogen in carcasses immediately after slaughter through the "Pathogen Reduction Program" (PRP). It was established by the Normative Instruction (IN) 70 (Brasil, 2003a) and replaced in 2016 by IN 20, with the aim of ensuring a gradual reduction of the occurrence of *Salmonella* sp. through constant monitoring of sampling cycles, and to establish an adequate level of consumer protection. According to the legislation, in industries with slaughter volume exceeding 100,000 birds / day, it establishes acceptable limits of 12 positive samples for *Salmonella* sp. in a sampling cycle of 51 carcasses (Brasil, 2016). Through this monitoring, it is possible to identify the industries with high occurrence and to make impact actions with the objective of ensuring acceptable limits of contamination.

After more than a decade since monitoring by the PRP began, a reduction of the occurrence of pathogens in poultry carcasses slaughtered are expected in Brazil. However, the PRP occurrence data, as an official monitoring, are not available to the scientific community and the general population, making it impossible to know if the program is actually effective in reducing *Salmonella* sp. in poultry carcasses and if there has been a reduction of the population exposure by this pathogen.

Thus, the monitoring of PRP occurrence and effectiveness data has been done indirectly by research laboratories of higher education institutions through analysis of products exposed to the sale, since this is the data are available for consultation. Based on this, the objective of this study was to compile the occurrence data of *Salmonella* sp. in a 7-year period (between 2007 and 2013) of chilled and frozen carcass samples exposed in the retail trade in the state of Paraná, indirectly monitoring the effectiveness of the PRP.

Materials and Methods

Between January 2007 and April 2013, 340 poultry carcass samples were analyzed, all of them commercialized in the western region of state of Paraná, in the cities of Assis Chateaubriand, Toledo, Marechal Cândido Rondon and Palotina. Whole carcasses, frozen (200) or refrigerated (140), from different brands and lots were purchased in grocery stores and supermarkets randomly selected, the way they were exposed to the consumers. At the moment of the acquisition to select the samples, the following instructions were required: the samples were with their packages intact, which were within the validity period and identified by the Federal Inspection Service (SIF), the agency of MAPA responsible for fiscal action in slaughterhouses and establishments of processing animal products.

The carcasses were placed inside a thermal box containing recyclable ice and transported to the Laboratory of Inspection and Control of Quality of Food and Water, Department of Veterinary Sciences, Federal University of Paraná, Palotina. All samples were submitted for the isolation of *Salmonella* sp. in 25g, according to the methodology recommended by Normative Instruction 62 of MAPA, with modifications (Brasil, 2003b).

For the pre-enrichment stage, 25g of each carcass (portions of skin and muscle from the chest, wings, neck and cloaca region) were collected and packed in a sterile plastic bag, and then 225mL of buffered peptone water 1% was added. The samples were homogenized for 1 min in stomacher (Seward, England) and incubated at 35-37°C for 18-24 h in a bacteriological oven.

For the selective enrichment, tetrathionate (TT) broth with 0.01% novobiocin, Rapapport-Vassiliadis (RV) broth and

selenite-cystine (SC) broth were used. From the pre-enriched samples, aliquots of 1mL, 0.1mL and 1mL were transferred to the TT, RV and SC broth, respectively. All broths were incubated in a water bath at 42 ± 0.2°C for 24 h.

From each of the selective enrichment broths, one loop was streaked onto selective plates containing xylose lysine deoxycholate, green-brilliant red phenol and bismuth sulfite. All plates were incubated at 35 °C for 24 h. After incubation, 3-5 colonies typical of *Salmonella* sp. of each plate were transferred to tubes containing triple iron agar and lysine iron agar, both incubated at 35°C for 24 h.

The isolates showing a characteristic reaction for *Salmonella* sp. were subjected to the serum agglutination test in the lamina, using flagellar (H) and somatic (O) polyvalent antiserum. Cultures with positive reactions (agglutination) were submitted to the biochemical characterization of urea, indole, methyl red, Voges-Proskauer, citrate and malonate.

The data were submitted to Kruskal-Wallis non-parametric analysis (PROC NPAR1WAY), in which the effect of year of sampling was tested at the 5% level of significance. Means were compared by the Tukey-Kramer test (PROC MEANS). The analyses were performed in the program Statistical Analysis System, version 9.0.

Results and Discussion

Of the total carcasses analyzed between 2007 and 2013 (n = 340), in 66 (19.41%), the presence of *Salmonella* sp. was confirmed. The number of carcasses sampled per year and the results are shown in Table 1, where an occurrence pattern is observed increasing between the years, from 11.67% in 2007 to 35% in 2013.

Based on the results, we realized the occurrence pattern was increasing between the years. However, this scenario was reversed in other countries when we observed some studies where the authors pointed to a decrease in the prevalence of *Salmonella* sp. over the years (Álvarez-Fernández et al., 2012; Dan et al., 2015). This

reduction, evidenced in some countries, may be due to the adoption of a set of mandatory measures developed to control and reduce *Salmonella* sp., such as tracking and surveillance of breeders and hatcheries; vaccination of herds against some serotypes, and introduction of HACCP – Hazard Analysis Systems and Critical Control Points in processing plants (Álvarez-Fernández et al., 2012).

Although Brazil also adopts the same measures previously mentioned, and all samples were processed in establishments inspected by Federal Inspection Service, the results of this research evidenced, in addition to the increase over the years, the detection of a high percentage of *Salmonella* sp. on carcasses. It is important to emphasize that a slaughterhouse certified by Federal Inspection Service must ensure the implementation and monitoring of quality control protocols according to the HACCP program. In addition, this federal certification allows these establishments to export their products.

While official data obtained by the PRP were not made available periodically for review by the general population, since the implementation of the program, there was only one official disclosure through a Technical Note issued in 2010. This Technical Note reports a prevalence of *Salmonella* sp. of 6.4%, obtained through the analysis of 128,293 poultry carcasses between 2003 and 2008 (Brasil, 2010). This is a significantly lower percentage when compared to the results obtained in this study (Table 1), even when considering only the data obtained in the same period that involved the official analyses (2007 and 2008).

One factor that may explain the high positivity index found is that the samples analyzed in this study were obtained directly from the retail market. This is different from PRP, where the samples obtained for official analysis should be obtained from the slaughter, collected immediately after the drip (Brasil, 2016). According to Possebon et al. (2012), there has been a tendency of the samples obtained from the retail to present a greater positivity than the carcasses

Table 1 – Contamination of poultry carcass samples by *Salmonella* sp. analyzed between 2007 and 2013 - Palotina, 2017

Year	Total Sample (n)	Contamination			
		Sample (n)		Frequency(%)	
		Negative	Positive	Negative	Positive
2007	60	53	7	88.33[a]	11.67[b]
2008	120	103	17	85.83[a]	14.17[b]
2009	60	49	11	81.67[ab]	18.33[ab]
2012	60	43	17	71.67[ab]	28.33[ab]
2013	40	26	14	65.00[b]	35.00[a]
Mean				80.59	19.41
P value[I]			0.0084		

[I]Probability value for Kruskal-Wallis non-parametric test; Averages followed by different lowercase letters in the same column differ by the Tukey test (P < 0,05).

collected soon after the precooling stage. This difference was observed by Fletcher (2006), who detected a 21.6% increase in the positivity of samples collected in retail when compared to the industry.

Therefore, the time the samples are collected to perform the detection of *Salmonella* sp. can influence the results of the analyses. The industry applies several treatments during slaughter that could injure the microbial cells present in the carcasses, such as the use of successive rinses of the whole carcass with chlorinated water, the use of antimicrobial agents and the presence of chlorine residual in the samples, thus hindering the microbial isolation (Fletcher, 2006). Still, some countries are permitted to use some substances with antimicrobial potential directly in the carcass, a practice not allowed in Brazil, according to current legislation (Brasil, 1998).

In addition, in retail, for preservation of the product, the carcasses are kept refrigerated or frozen. However, some microorganisms can produce defense mechanisms in response to the temperature oscillation. Among these mechanisms, the most effective are the expression of specialized proteins (which increase the resistance to thermal shock), the change in membrane structure (change in saturation of fatty acids to protect against low temperatures) and the repair of genetic material (Everis, 2001). All these factors, associated or individually, have already verified the resistance of *Salmonella* sp. at a temperature of -20°C for 26 weeks (Dominguez & Schaffner, 2009; Strawn & Danyluk, 2010).

Another factor that may influence the evaluation of the effectiveness of the PRP in Brazil concerns the methodology used in this research. We aimed to investigate the presence or absence of microorganisms of the *Salmonella* genus, without differentiation of the serotypes. Nevertheless, the measures implemented by the National Poultry Health Program (PNSA) and the PRP had some specific serotypes of this pathogen, such as Gallinarum, Pullorum, Enteritidis and Typhimurium. Some studies have shown that after the implementation of these control measures established by these programs the decline of these serotypes occurred (Kottwitz et al., 2013; Voss-Rech et al., 2015; Mion et al., 2016). However, as a reflection of a competitive exclusion, the emergence and reemergence of new serotypes may explain the reason the occurrence of *Salmonella* sp. still remains high in the poultry chain (Ribeiro et al., 2007; Rall et al., 2009; Yamatogi et al., 2012; Fernandes et al., 2016), corroborating with the high occurrence observed in this work.

This scenario was verified in Brazil in the early 2000s when the main serotype described in chicken meat samples was *S.* Enteritidis, with percentages reaching up to 84% when compared to the other serotypes (Costa et al., 1997; Santos et al., 2000; Kanashiro et al., 2005; Ribeiro et al., 2007). This serotype was prominent in the country after the Gallinarum and Pullorum serotypes were controlled (Rabsch et al., 2001). However, data compiled by Kottwitz et al. (2013) show that, in the following years, there was a decline of *S.* Enteritidis in biological samples of chicken, which fell from 77% in 2006 to 9% in 2010, demonstrating the control of this serotype.

This behavior is probably a reflection of the implementation of the National Control Programs of *Salmonella* sp., especially of the PNSA. This program determined the slaughter of matrices with *S.* Gallinarum and *S.* Pullorum and the vaccination of batches of breeding birds with inactivated vaccines against *S.* Enteritidis. This immunoprophylaxis started in 2003 and may have helped reduce the prevalence of this serotype in broilers (Brasil, 2003c). With *S.* Gallinarum, *S.* Pullorum and *S.* Enteritidis under control, new serotypes may find favorable conditions for their maintenance in the poultry breeding environment.

Several methodologies have been developed and used, which, in addition to phenotypic serotyping, become important supports to epidemiological data. However, comprehensive occurrence studies are important tools for overall identification of *Salmonella* sp. contamination in poultry products. This makes it possible to evaluate the need to update and improve existing health programs, since this microorganism is still the main agent associated with foodborne illnesses in Brazil. The control of *Salmonella* sp. is essential for the entire production chain and for guaranteeing an innocuous food.

Conclusion

It was possible to verify that the occurrence of *Salmonella* sp. has increased over the years in samples obtained in the retail trade even after more than a decade of implementation of the PRP in poultry slaughter, established by MAPA. Even considering the reasons that are able to justify the higher occurrence in samples collected from retail trade than those from slaughterhouse, it is important to verify the possibility of crossing the data obtained in commercial samples with those of official PRP data. Thus, it is possible to evaluate the impact of this program with the reduction of *Salmonella* sp. to the consumer and, consequently, to the reduction of the risk of the occurrence of salmonellosis carried by this type of food.

In addition, further studies should use other methodologies, such as phenotypic serotyping, to evaluate the effectiveness of the PRP once it determines controls for some specific serotypes in farms. Based on these kinds of tests, it would at least be possible to evaluate if the occurrence of serotypes is dwindling. However, if the global occurrence doesn't decrease significantly, it may be due to new serotypes finding favorable conditions for their maintenance in the poultry breeding environment. Thus, it is important to evaluate the need to expand the control measures programs for other serotypes along with the serotypes included in the PNSA.

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Conflict of Interest

The authors state that they have no conflicts of interest to declare.

Ethics Statement

Not applicable.

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