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Occurrence of *Salmonella* spp. in the broiler production chain

[*Ocorrência de* Salmonella *spp. na cadeia de produção de carne de frango*]

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ABSTRACT

Brazilian chicken meat is exported to more than 150 countries and consumed by consumer markets that demand high quality and food safety, thus, requiring very strict control of pathogens present in food to guarantee these rigorous safety standards. This study evaluates the reports from the *Salmonella* spp. Control and Monitoring Program of the Brazilian Ministry of Agriculture, Livestock and Food Supply of seven slaughterhouses inspected by the Federal Inspection Service from the western region of Paraná state, Brazil, from March 2017 to February 2019. The broiler litter swab and carcass analyses revealed a *Salmonella* spp. positivity ratio of 5.9% (19/319) and 23.5% (75/319), respectively. The concomitant presence of *Salmonella* spp. in the broiler litter swab and chicken carcasses occurred in 58% of the positive samples. The most frequently isolated serovar in the carcasses was *Salmonella* Heidelberg (85.3%) followed by *Salmonella* spp. (10.6%). During slaughter, carcass positivity to *Salmonella* spp. was significantly different (p=0.047) between the first (19.6%) and the second (29.4%) shifts. The results alert for the possibility of carcass contamination during slaughtering and, therefore, more stringent hygiene measures between shifts must be implemented to mitigate carcass contamination.

Keywords: salmonellosis, contamination, *Salmonella* Heidelberg, slaughterhouse, broiler litter

RESUMO

A carne de frango brasileira é consumida em mais de 150 países, em mercados exigentes com a qualidade e a produção de alimentos seguros, o que justifica o controle de patógenos nesse alimento, a fim de assegurar tais requisitos. O presente estudo analisou dados constantes dos relatórios do Programa de Controle e Monitoramento da Salmonella *spp. do Ministério da Agricultura, Pecuária e Abastecimento do Brasil (MAPA), realizado em sete unidades avícolas e frigoríficas da região oeste do estado do Paraná, com Serviço de Inspeção Federal, no período entre março 2017 e fevereiro de 2019. A análise dos dados revelou a presença de* Salmonella *spp. no suabe de cama de frango em 5,9% dos lotes analisados e em 23,5% das carcaças oriundas desses lotes. A presença concomitante de* Salmonella *spp. no suabe de cama e nas carcaças de frango do lote ocorreu em 58% das amostras positivas. O sorovar mais frequentemente isolado nas carcaças foi* Salmonella *Heidelberg (85,3%), seguido de* Salmonella *spp. (10,6%). Durante o abate, observou-se diferença significativa (P=0,047) na positividade das carcaças para* Salmonella *spp. entre o primeiro (19,6%) e o segundo turno (29,4%). Os resultados indicam a possibilidade de contaminação das carcaças durante o abate, portanto a adoção de medidas mais rigorosas de higienização entre os turnos deve ser implementada a fim de mitigar a contaminação das carcaças.*

Palavras-chave: salmonelose, contaminação, Salmonella *Heidelberg, abatedouro, suabe de cama*

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INTRODUCTION

Currently, Brazil is the third-largest producer of chicken meat and the largest world exporter. Of the total production, 31% is exported and 69% is consumed in the domestic market, considering a per capita Brazilian consumption of 45.27kg/ hab. (Relatório…, 2021). In recent years, chicken meat has been the main source of animal protein on the Brazilian export agenda (Ferreira and Vieira Filho, 2019), being consumed in more than 150 countries. Thus, it is highly important to control pathogens such as *S.* Enteritidis and *S.* Typhimurium while *Salmonella* spp. in raw chicken meat is strictly controlled since salmonellosis is the main cause of gastroenteritis in humans (World…, 2018).

The Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento, MAPA) of Brazil has been regulating, monitoring, and inspecting all links in the production chain of the broiler and turkey complex to reduce the contamination by *Salmonella* spp. during meat production. In 2016, MAPA expanded and improved its actions regarding the National Pathogen Control Program (NPCP) and established the *Salmonella* spp. Control and Monitoring Program that oversees the broiler and turkey slaughtering establishments, among others, registered in the Federal Inspection Service (SIF). These measures aim to reduce the prevalence of *Salmonella* spp. and to establish an adequate level of consumer protection (Brasil, 2016).

The western region of the state of Paraná, considered one of the largest producing and exporting regions in Brazil, is home to a large chicken meat production complex. There are seven companies with an Integrated Broiler Production System that have their own slaughterhouses with a total installed slaughtering capacity of more than 2 million broilers daily. In 2021, 22% of Brazilian broiler chicken exports originated from the western region of Paraná, with gross sales of US$ 2.35 billion (Paraná, 2021).

Studies performed in the state of Paraná have shown that the presence of *Salmonella* spp. is quite common in the carcasses and poultry litter swabs (Pandini *et al*., 2015; Silva, 2019). An increase of carcasses contamination at the end of their processing in slaughterhouses has also been observed (Lopes *et al*., 2007).

This study aims to analyze the presence of *Salmonella* spp. on carcasses collected in slaughterhouses and on broiler litter swabs of commercial poultry establishments where the broilers were housed, in the western region of the state of Paraná, Brazil, the relationship between them as well as with the variables slaughtering shifts and/or time of sampling.

MATERIAL AND METHODS

The study area in the western region of Paraná comprised seven companies that operate in an Integrated System to produce broilers, each with its own refrigeration unit, and a total installed slaughtering capacity of two million broilers per day. The 6,315 poultry production units have one or more poultry housing sheds (Relatório…, 2017).

The secondary data present in the reports on the Control and Monitoring Program of *Salmonella* spp. refer to 319 samples of swabs from the trailer and shoe covers used by the sample collectors of commercial poultry (broilers and turkeys) establishments, as well as 319 chicken carcasses that were collected in the slaughterhouses and evaluated. The data provided by MAPA refers to the period between March 2017 and February 2019. The data regarding carcass origin, slaughtering shift, as well as sampling date and time, were also analyzed. Company location data was kept confidential.

This research was not submitted to the Ethics Committee on the Use of Animals because it used secondary data of the reports on the National Control and Monitoring Program of *Salmonella* spp. from poultry routinely slaughtered in a humanitarian way, following the current regulations.

The entire poultry production complex was considered positive when *Salmonella* spp. was detected in the poultry litter, even if it occurred only in one of the sheds in the complex.

The analyzed establishments were 1 M (=50,001 to 100,000 chickens/hens), 1 G (=100,001 to 200,000), and 5 GG (>200,001), classified

according to daily slaughtering volume (Brasil, 2016). A Confidentiality and Reliability term was signed with MAPA to preserve and protect those involved due to the great sanitary relevance and economic importance of salmonellosis in the Brazilian poultry production chain. Thus, a system of identification letters was created (B-R-A-Z-I-L-S) for each participant so the analyses were performed without identifying the producer/local/company.

The data evaluated between March 2017 and February 2019 were divided into two periods, the 1st period, between March 2017 and February 2018, and the 2nd period, between March 2018 and February 2019. The interval between carcass samplings was two weeks for six slaughtering units (B, R, A, Z, I, and S) and three weeks for one slaughtering unit (L).

Employees/Inspectors of the Federal Inspection Service (SIF) collected the whole carcasses randomly on a date, weekday, slaughtering line, and time selected by a draw, immediately after the carcass drip area and before primary packaging (Brasil, 2016). Once sampled, the carcasses properly packaged and identified were sent to the official laboratories chosen by the company, to be analyzed (Brasil, 2020).

Data were tabulated in Excel spreadsheets and percentages were calculated. To evaluate the performance of poultry litter swabs as a predictor of carcass contamination, a confusion matrix analysis was performed using the "caret" package (Kuhn, 2008) of the statistical software R (R Development…, 2020). The confusion matrix was chosen because it helps to visualize and summarize the performance of poultry litter swabs in comparison with carcass evaluation since it returns the number of “True Positive” (TP - the prediction was positive in poultry litter swabs and in the carcass analyses), “True Negative” (TN - the prediction was negative in both), “False Positive” (FP - the prediction was positive in swab and negative in carcass),and “False Negative” (TN - the prediction was negative in swab and positive in carcass). Using these values, it is possible to evaluate prediction accuracy (TP+TP divided by the total tested), the coefficient of agreement (kappa), and the probability of obtaining an accuracy equal to or greater than at random.

To assess the association between positivity for *Salmonella spp*. and the variables, slaughtering shifts and/or sampling time, the analysis of variance (ANOVA) at 5% was performed using scripts in an R environment.

RESULTS AND DISCUSSION

Data analysis of poultry litter swabs indicated the presence of *Salmonella* spp. in 5.9% (19/319) of the samples (Table 1). These results are lower than the results reported by Pandini *et al*. (2015) that observed 11.4% positivity for *Salmonella* enterica in 342 samples of poultry litter swabs, between 2010 and 2011, with *Salmonella* Heidelberg being the most frequent serovar in the state of Paraná. Likewise, Silva (2019) also evaluated data from litter swab samples in Paraná, between 2017 and 2018, and observed 32.1% (78/243) of *Salmonella* spp. in poultry units not registered with the Paraná Agricultural Defense Agency (ADAPAR). The lower positivity rate observed in this study can be attributed to the fact that all poultry units are registered and evaluated by the State Veterinary Service (SVE), which may result in better control of *Salmonella* spp. in the analyzed poultry units.

Of the positive samples for the poultry litter swab analysis, 73.7% (14/19) occurred in a single company (I) throughout the period. This result may be related to the serovar *Salmonella* Heidelberg which is hard to control as mentioned by Voss-Rech *et al*. (2017) who studied poultry litter treatments against bacteria and viruses and reported that not all treatments used can effectively control *Salmonella* Heidelberg. This hypothesis could not be proven because serovar typification of poultry litter swab samples is not required yet.

Table 1. Occurrence of *Salmonella* spp. in the carcass and poultry litter swab sampled in establishments in the western region of Paraná, according to the period of analysis (2017-2019)

	1st Period		2nd Period		Total	
	March 2017 to February 2018 % (positive/total)		March 2018 to February 2019 % (positive/total)		% (positive/total)	
Company	Litter swab	Carcass	Litter swab	Carcass	Litter swab	Carcass
B	0 (0/23)	0 (0/23)	0 (0/24)	8.33 (2/24)	0 (0/47)	4.25 (2/47)
R	0 (0/24)	20.83 (5/24)	18.75 (3/16)	18.75 (3/16)	7.50 (3/40)	20.00 (8/40)
A	0 (0/24)	50.00 (12/24)	0 (0/24)	37.50 (9/24)	0 (0/48)	43.75 (21/48)
Z	0 (0/24)	16.67 (4/24)	0 (0/24)	12.50 (3/24)	0 (0/48)	14.58 (7/48)
I	37.50 (9/24)	41.67 (10/24)	20.83(5/24)	50.00 (12/24)	29.17 (14/48)	45.83 (22/48)
L	0 (0/16)	6.25 (1/16)	0 (0/24)	4.17 (1/24)	0 (0/40)	5.00 (2/40)
S	4.17 (1/24)	45.83 (11/24)	4.17 (1/24)	8.33 (2/24)	4.17 (2/48)	27.08 (13/48)
Total	6.29 (10/159)	27.04 (43/159)	5.62 (9/160)	20.00 (32/160)	5.96 (19/319)	23.51 (75/319)

The results show that *Salmonella* spp. was not detected in 76.5% (244/319) of the chicken carcasses while 23.5% (75/319) had *Salmonella* spp. (Table 1). In the first period, 27% of the samples (43/159) were positive and, in the second period, 20% (32/160). It is noteworthy that the number of positive carcasses in the period decreased in five companies (R, A, Z, L and S) and increased in two slaughterhouses (companies B and I). Furthermore, the positive result in slaughterhouse I is probably due to the presence of a serovar that is often resistant to the disinfectants most used in slaughterhouses (Colla *et al*., 2012).

At the national level, the presence of *Salmonella* spp. in carcasses decreased slightly more in the evaluated period: from 18% (466/2,592) in 2017 (Brasil, 2018) to 12.6% (352/2791) in 2018, 15.08% in 2019 (Brasil, 2019) and 12.8% (369/ 2881) in 2020 (Brasil, 2021).

The positivity for *Salmonella* spp. observed in the carcasses (23.5%) in Parana is higher than the 3.9% observed for carcasses of young broilers (28 days of age) in the USA, in 2013 (Progress..., 2014). Likewise, from 2013 to 2017, the European Union reported a positivity of 5.6% for poultry units sampled in 25 member countries (The European…, 2018). In Canada, a survey carried out in 2018 on 4,541 samples of chicken carcasses from various regions revealed a positivity ranging from 17.4% to 34.3% (National..., 2016), drawing attention to the great complexity of controlling *Salmonella* spp., even in developed countries.

The *Salmonella* serovars isolated from carcasses collected in the seven slaughterhouses were typified and the results revealed that *Salmonella* Heidelberg was the predominant serovar, identified in 85.3% (64/75) of the carcasses (Table 2). Furthermore, the serovar was not identified in 10.66% of the samples, being thus generally reported as *Salmonella* spp., while *Salmonella* Typhimurium, *S.* Infantis and *S.* Minnesota occurred in 1.3% of the samples (only one case each). Additionally, *Salmonella* Enteritidis, Pullorum or Gallinarum, important serovars of the national poultry health programs were not identified in any studied samples.

Table 2. Number (n) of chicken carcasses positive for *Salmonella* according to the isolated serovar in the western region of Paraná, between March 2017 and February 2019

Serovar	n	%
Salmonella Heidelberg	64	85.33
Salmonella spp.	8	10.66
Salmonella Infantis	1	1.33
Salmonella Minessota	1	1.33
Salmonella Typhimurium	1	1.33

In the United States and Canada, *Salmonella* Heidelberg is cited as the most isolated serovar in the broiler production chain, thus causing public health problems. This serovar has shown progressive resistance to the most used disinfectants in the Brazilian poultry industry, such as chlorhexidine and quaternary ammonia, a trait that has been transferred to each generation (Colla *et al*., 2012). This factor may be related to the bacteria present in the slaughtering line, showing the need for constant efficiency tests and for alternating the active principles for disinfection. Pandini *et al*. (2015) also demonstrated that *S.* Heidelberg is highly resistant to the antibacterial products used in the animal industry, thus constituting a great risk to public health. Additionally, Borges *et al*. (2018) investigated the ability of *S.* Heidelberg to produce biofilm under laboratory conditions and reported that biofilm formation increased with increasing temperature (from 3ºC to 37ºC). Such an observation causes concern in the food processing industries, as this trait favors pathogen survival in hostile environments such as slaughterhouses. Moreover, the *Salmonella* Control and Monitoring Program detected *S.* Heidelberg in 35.7% (135/370) of the positive samples collected in Brazil in 2020 (Brasil, 2021).

Concomitant contamination with bacteria of the genus *Salmonella* was identified in 57.9% (11/19) of poultry litter swab and carcass samples as shown in Table 3.

This hypothesis is reinforced by the fact that of the carcass samples positive for *Salmonella* spp., 85.3% (64/75) were not positive in the poultry litter swabs (Table 3), indicating that the carcasses may have been contaminated during slaughter and processing. According to Lopes *et al*. (2007), a study conducted by the Food Safety and Inspection Service (FSIS) in the United States showed that 5% of the birds arriving at the slaughterhouse were contaminated by *Salmonella* spp. and, at the end of processing, the contamination of chicken carcasses increased to 36%.

Table 3. The presence of *Salmonella* spp. in samples (n) of poultry litter swabs and chicken carcass in poultry establishments in the western region of Paraná (2017-2019)

Litter swab	Carcass		Total
	Present	Absent	n
Present	11	8	19
Absent	64	236	300
Total	75	244	319

The high slaughtering speed, the use of unregulated equipment, the uneven size of the birds, inadequate pre-cooling and cooling temperatures and poor chlorination allow cross-contamination with enterobacteria, including *Salmonella* spp. Besides these factors, the carcass washing system after evisceration of the broilers can also affect the presence of *Salmonella* spp. (Isolan *et al*., 2019).

Regarding the sampling period of the carcasses for analysis, a total of 193 and 126 were harvested in the first and second periods/shifts of slaughtering, respectively, resulting in a positivity ratio of 19.7% (38/193) and 29.3% (37/126) (Table 4), revealing a statistically significant difference in positivity between shifts/periods (ANOVA, p=0.047).

Considering the periods and the time elapsed from the beginning of the slaughtering shifts to the collection of the carcasses, although not significantly different (p>0.05), the positivity increased in both periods with the increasing slaughtering time, either in the first or the second shifts. These results seem to suggest cross-contamination during the processing in the slaughterhouse (Table 4).

Table 4. Positivity for *Salmonella* spp. of chicken carcasses from western Paraná according to the period after the slaughtering shift had started (2017-2019)

Slaughtering shifts / Periods	Up to 4 hours			After 5 hours			Positive/ total carcass	
	Evaluated carcasses	Positive		Evaluated carcasses	Positive			
		n	%		n	%	n	%
First (March 2017 to February 2018)	99	16	16.2	94	22	23.4	38/193	19.68
Second (March 2018 to February 2019)	59	16	27.1	67	21	31.3	37/126	29.36
Total	158	32	20.2	161	43	26.4	75/319	23.50

The results of the data analysis regarding carcass sampling and slaughtering shifts show that 43.6% (139/319) of the sampling occurred on Mondays and 62.6% (87/139) were collected in the first slaughtering shift, which may have influenced the results (data not shown), thus constituting a sampling bias. Additionally, in slaughterhouse R, 100% of the samples were collected in the first hours of the first shift and, in another company (B), 72.5% of the collections were carried out on Mondays (data not presented), in disagreement with Art. 45 of IN-20/2016. Therefore, greater emphasis should be given to the random drawing conducted for sampling aiming to reduce the trends observed.

CONCLUSIONS

The data showed *Salmonella* spp. contamination of broiler carcasses slaughtered in the evaluated slaughterhouses as well as in the poultry units in the western region of Paraná state, Brazil, in the analyzed period. Furthermore, the positive results were significantly different between work shifts. There was concomitant positivity for carcasses and poultry litter in the poultry houses, and *Salmonella* Heidelberg was the predominant serovar in the carcasses evaluated. The results also indicate the possibility of carcass contamination during slaughter, as well as the resistance of *S.* Heidelberg to products used in the hygiene and disinfection of the facilities. The poultry industry should implement stricter hygiene and disinfection measures between slaughtering shifts to mitigate the contamination of carcasses, such as changing the water in the cooling tanks, paying more attention to the cleaning and sanitizing of the used equipment, alternating cleaning products as well as using those proven to be more effective, besides emphasizing the Good Manufacturing Practices, Standard Operating Hygiene Procedure, and Hazard Analysis and Critical Control Points.

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