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Coutinho Cossi, Marcus Vinícius; Vieira de Almeida, Michelle; Rezende Dias, Mariane; de Arruda
Pinto, Paulo Sérgio; Nero, Luís Augusto

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Inspected and non-inspected chilled chicken carcasses commercialized in Viçosa, MG, Brazil: microbiological parameters and *Salmonella* spp. occurrence

Carcaças de frango refrigeradas inspecionadas e não-inspecionadas comercializadas em Viçosa, MG, Brasil: parâmetros microbiológicos e ocorrência de *Salmonella* spp.

Marcus Vinícius Coutinho Cossi^I Michelle Vieira de Almeida^I Mariane Rezende Dias^{II}
Paulo Sérgio de Arruda Pinto^{III} Luís Augusto Nero^{III*}

ABSTRACT

Sixty samples of chilled chicken carcasses submitted (30) and not submitted (30) to Brazilian inspection services were analyzed to investigate if inspected and non-inspected chilled carcasses represented different food safety risks in the region of Viçosa, MG, Brazil. The mean counts of indicator microorganisms (mesophilic aerobes, *Enterobacteriaceae*, total coliforms and *Escherichia coli*) of samples belonging to the inspected and non-inspected lots did not present significant differences ($P>0.05$). Also, no significant differences ($P>0.05$) were observed for the numbers of *Salmonella* spp. and *E. coli* (higher than $2\log \text{ cfu g}^{-1}$) between samples submitted or not to inspection. Statistical differences were observed between the two sample classes only for the numbers of mesophilic aerobes higher than 4 and $5\log \text{ cfu g}^{-1}$ ($P<0.05$). The obtained results indicated the limitations of microbiological parameters to differentiate inspected and non-inspected chilled chicken carcasses commercialized in the specific studied area.

Key words: inspection, *Salmonella*, *Escherichia coli*, *Enterobacteriaceae*, hygiene indicators.

RESUMO

Sessenta amostras de carcaças de frango refrigeradas fiscalizadas (30) e não fiscalizadas (30) por serviços brasileiros de inspeção foram analisadas para investigar se carcaças refrigeradas inspecionadas ou não apresentam diferentes riscos alimentares na região de Viçosa, MG, Brasil. As médias de contagens de microrganismos indicadores de higiene (aeróbios mesófilos, *Enterobacteriaceae*, coliformes e *Escherichia coli*) de amostras inspecionadas ou não inspecionadas não apresentaram diferenças significativas ($P>0,05$). Também não foram observadas diferenças significativas entre amostras inspecionadas e não

inspecionadas com resultados positivos para *Salmonella* spp. e *E. coli* (contagens acima de $2\log \text{ cfu g}^{-1}$). Diferenças significativas ($P<0,05$) foram observadas somente entre os números de amostras com contagens de aeróbios mesófilos superiores a 4 e $5\log \text{ cfu g}^{-1}$. Os resultados obtidos indicam as limitações dos parâmetros microbiológicos para diferenciar carcaças de frango inspecionadas ou não na região específica onde o estudo foi conduzido.

Palavras-chave: inspeção, *Salmonella*, *Escherichia coli*, *Enterobacteriaceae*, indicadores de higiene.

INTRODUCTION

In 2010, Brazilian chicken production was estimated at 11.4 million tons, what established Brazil as one of the greatest world producer. In addition, Brazil is the world greatest exporter of chicken products, presenting a high internal consumption of these products (UBABEF, 2011). Considering the development of chicken production, consumption and exportation, the necessity for safety and quality have increased, leading to a development of programs for quality and hygienic control (GILL et al., 2006).

Being a product of animal origin, chicken carcasses are naturally susceptible to microbiological contamination from several origins (SAKHARE et al., 1999). Spoilage and pathogenic microorganisms can contaminate chicken meat, indicating inadequate conditions of production and compromising the quality

^IPrograma de Pós-graduação em Medicina Veterinária, Universidade Federal de Viçosa (UFV), Viçosa, MG, Brasil.

^{II}Curso em Medicina Veterinária, UFV, Viçosa, MG, Brasil.

^{III}Departamento de Veterinária, UFV, Campus Universitário, Centro, 36570-000, Viçosa, MG, Brasil. E-mail: nero@ufv.br.

*Autor para correspondência.

and safety of the final product (LUBER, 2009). As an example, *Salmonella* spp. is a food borne pathogen usually associated with these products, and considered an important agent of human diseases associated to food consumption (VANDEPLAS et al., 2010).

The verification of these safety and quality parameters is an official responsibility of government agencies in the country of production. These inspection steps have the goal of monitoring the microbiological contamination, assuring the quality and safety of final products. In Brazil, this inspection is conducted at industry level by the Ministry of Agriculture and Animal Production (MAPA) (BRASIL, 2011b). In commercial points, the Ministry of Health through the National Agency of Sanitary Vigilance (ANVISA) (BRASIL, 2011a), is responsible to verify the storage conditions and some safety parameters of the foods. Despite these control tools, in Brazil is still usual the commercialization and consumption of products of animal origin which were not submitted to inspection. As they are not inspected, there is no guarantee of hygienic procedures during the processing of these products, which can jeopardize their quality and safety.

Considering the relevance of the official inspection service for Public Health and to investigate if inspected and non-inspected chilled chicken carcasses represented different food safety risks, this study had the objective of comparing the microbiological profile of these food commercialized in a specific area located at the Southeastern region of Brazil.

MATERIAL AND METHODS

The study was conducted in the region of Viçosa, a city located at Minas Gerais State, in the Southeastern region of Brazil, between March and September of 2010. This city has about to 80,000 habitants, and the consumption of chicken commercialized directly from small farmers is usual, who trade this product in markets, fairs, deli houses and meat retail stores, without official control during the production, slaughtering and handling. As this production is not properly controlled, it is difficult to determine the exact dimension of this trade, leading the identification of the main commercialization points of this product.

For non-inspected chickens, it was identified six establishments that commercialize chilled carcasses. These establishments were visited in five occasions, and a chilled chicken carcass from each one was collected (total: 30 samples). For inspected chickens, five industries (all from federal inspection service) were

identified as suppliers of chilled chicken carcasses in the region markets; then, six chilled carcasses from each industry (all from distinct lots) were obtained from these markets (total: 30 samples). The numbers of chicken carcasses were defined considering indicative sampling, once there are no estimative of the amount of non-inspected chicken commercialized in Viçosa or other region in Brazil. All 60 chicken samples were collected in their original packaging, and kept under refrigeration at 4°C until the moment of microbiological analysis.

For microbiological groups enumeration, 25g of tissue and skin of each sample were aseptically collected and added to 225mL of 0.1% buffered peptone water (BPW, Oxoid Ltd., Basingstoke, England), homogenized and subjected to ten-fold dilution using BPW (BRASIL, 2003a). Then, two selected dilutions of each sample were plated on Petrifilm™ AC (3M Microbiology, St Paul, MN, USA) for mesophilic aerobes enumeration (MA) (incubated at 35°C by 24h), Petrifilm™ EC (3M Microbiology) for coliforms (TC) and *E. coli* (incubated at 35°C by 24-48h), and Petrifilm™ Enterobacteriaceae (3M Microbiology) for Enterobacteriaceae (EB; Enterobacteriaceae) (incubated at 35°C by 24h). After incubation, colonies were enumerated considering the typical characteristics for each group and plate, being the results expressed as colony forming units per gram (cfu g⁻¹).

For *Salmonella* spp., the samples were subjected to protocol described in IN62 (BRASIL, 2003a), with some modifications. Portions of 25g of tissue and skin of each sample were transferred to 225mL of buffered 1% peptone water (Oxoid), incubated at 37°C for 18h. Then, 1mL of the culture was transferred to 10mL of selenite cystine broth (Oxoid), incubated to 37°C for 24h, and 0.1mL transferred to 10mL of Rappaport Vassiliadis (Oxoid), incubated at 41.5°C for 24h. After incubation, the obtained cultures were streaked on brilliant green phenol red lactose sucrose agar (Oxoid) and xylose lysine deoxycholate agar (Oxoid), and incubated at 37°C for 24h. *Salmonella* typical or suspect colonies were transferred to triple sugar iron (Oxoid) and lysine iron (Oxoid) slants, and incubated at 37°C for 24h. Once typical reactions were observed, in at least one of the slants, the obtained cultures were subjected to serological tests with somatic (O) and flagellar (H) polyvalent antisera (Probac do Brasil SA, São Paulo, SP, Brazil), and polymerase chain reaction (PCR) (ALVAREZ et al., 2004). *Salmonella* Enteritidis ATCC 13076 was used as positive control in all analysis. *Salmonella* confirmed isolates were identified by serology at Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. Finally, the results were expressed as *Salmonella* spp. absence or presence in 25g of sample.

The counts for the hygiene indicator microorganisms were converted to \log_{10} and evaluated according to normal distribution and homogeneity. Then, the results from inspected and non-inspected samples were compared by Analysis of Variance ($P < 0.05$). The samples were also compared considering the presence of *Salmonella* spp. and *E. coli* (counts higher than $2\log_{10}$ cfu g^{-1} , once it is an indicative of poor hygienic practices, according to GILL (1998), and ÁLVAREZ-ASTORGA et al. (2002) by the chi-square test ($P < 0.05$). Finally, the samples were grouped according to the levels of contamination determined by the hygiene indicator microorganisms, and the obtained frequencies were compared by the chi-square test ($P < 0.05$). Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA) was used for analysis.

RESULTS AND DISCUSSION

A descriptive analysis of the obtained counts of hygiene indicator microorganisms (MA, EB, TC, and *E. coli*) from chicken carcasses is presented in table 1. No significant differences were observed between the counts from inspected and non-inspected chicken samples ($P > 0.05$). Compared to similar studies (GILL & BADONI, 2005; GILL et al., 2005; HUTCHISON

et al., 2006), higher counts of hygiene indicator microorganisms were observed in the present study. These results indicate inadequate conditions of production and/or storage at markets, independently of them being submitted or not to official inspection.

The frequency of positive results for *E. coli* (higher than $2\log_{10}$ cfu g^{-1}) is presented in table 2, with no observed significant differences ($P > 0.05$). In a similar study, GILL et al. (2005) verified that 12 of 25 chicken samples presented *E. coli* contamination at counts higher than $2\log_{10}$ cfu g^{-1} . However, other studies have shown higher numbers of chicken carcasses presenting *E. coli* counts higher than $2\log_{10}$ cfu g^{-1} , reaching in some cases 100% of the analyzed samples (GILL & BADONI, 2005; GILL et al., 2006; GHAFIR et al., 2008).

Despite having observed a high frequency of samples with high counts of *E. coli*, *Salmonella* spp. was detected in only two chicken samples: one submitted to official inspection, and the other not submitted (Table 2). The suspect isolates obtained from these samples were confirmed by PCR (products with 204bp, according to ALVAREZ et al., 2004), and after serological tests they were identified as *Salmonella* Enteritidis. The obtained data indicate a smaller frequency of this pathogen in chicken carcasses, when compared to other studies. In a study conducted in

Table 1 - Statistical parameters of hygiene indicator microorganism counts in 60 chicken carcass samples, submitted (30) or not (30) to Brazilian inspection services (data in \log_{10} cfu g^{-1}).

Microbial group/Inspection	Mean	SD	SE	VR	MI	MD	MA
Mesophilic aerobes							
Inspected	5.44	0.94	0.19	0.89	4.00	5.45	7.08
Non-inspected	5.66	0.68	0.12	0.46	4.79	5.44	7.19
ANOVA	$F_{(1,54)} = 0.31, p = 0.314$						
Enterobacteriaceae							
Inspected	4.29	1.12	0.22	1.26	2.00	4.20	6.29
Non-inspected	4.17	0.52	0.10	0.27	3.30	4.17	5.57
ANOVA	$F_{(1,52)} = 0.27, p = 0.605$						
Total coliforms							
Inspected	3.06	0.71	0.14	0.50	2.00	2.99	4.46
Non-inspected	3.13	0.48	0.09	0.23	2.00	2.99	4.11
ANOVA	$F_{(1,54)} = 0.16, p = 0.693$						
<i>Escherichia coli</i>							
Inspected	2.72	0.57	0.12	0.32	2.00	2.72	3.76
Non-inspected	2.61	0.44	0.11	0.20	2.00	2.54	3.45
ANOVA	$F_{(1,38)} = 0.42, p = 0.523$						

ANOVA: Analysis of Variance; F: ANOVA value; P: level of significance; SD: standard deviation; SE: standard error; VR: variance; MI: minimum value; MD: median; MA: maximum value.

Table 2 - Numbers of positive results for *Escherichia coli* and *Salmonella* spp. in chicken carcass samples, submitted or not to Brazilian inspection services.

Samples/Inspection	n	<i>Escherichia coli</i> (at least 2.0log ₁₀ cfu g ⁻¹)	<i>Salmonella</i> spp. (at least 1 <i>Salmonella</i> 25g ⁻¹)
All	60	40	2
Inspected	30	22	1
Non-inspected	30	18	1
χ^2		1.2	0.0
P		0.273	1.000

χ^2 = chi-square test; P = level of significance. For all comparisons, the degree of freedom was 1.

São Paulo state (Brasil), the prevalence of *Salmonella* spp. in chicken meat was 19.1% (TESSARI et al., 2003). In Belgium, GHAFIR et al. (2008) conducted a chronologic study of *Salmonella* spp. in foods of animal origin and verified a variation between 9.5% and 25.6% of positive results for chicken carcasses and meat. In England, *Salmonella* spp. was isolated in 31% of the analyzed chicken carcasses (JØRGENSEN et al., 2002). Considering that the usual recommendation for microbiological safety of *Salmonella* spp. in foods is the absence of the pathogen in 25g of the sample (EC, 2005; LUBER, 2009), the two positive samples would represent a risk to consumers. In Brazil the industries must include in the package of the chicken carcasses an alert of the necessity of cooking properly the product due to the possible presence of foodborne pathogens, such as *Salmonella* spp. (BRASIL, 2001b); in counterpart, the consumers of non-inspected chicken are exposed to these risks without any warning.

Usually, only some rules with standard parameters for the microbiological quality of chicken carcasses and meat are observed. In Brazilian legislation, the only official parameter is for chicken products for human consumption which are available at commercial sites, and only thermotolerant coliforms must be investigated and present counts lower than 4.0log₁₀ cfu/g (BRASIL, 2001a). In Brazil, there is currently one official program for *Salmonella* monitoring in chicken carcasses, aiming to obtain control at the industry level (National Program of Pathogens Reduction) (BRASIL, 2003c). According to this program, the observed frequencies of positive results for *Salmonella* spp. (Table 2) would be considered as tolerable. The European legislation states only that *Salmonella* spp. are to be investigated in avian products to be consumed by humans, and must be absent in 25g of the analyzed sample (EC, 2005). In the US, the sanitary control of chicken is conducted by the industry, with well-defined sampling plans and

specific parameters for EC (2 to 3log₁₀ cfu g⁻¹) and *Salmonella* spp. (absent in 20% of the analysis) (USDA, 2003).

Despite indicating the hygienic conditions of production, the distinct microorganisms groups present in chicken carcasses can also contribute to the spoilage of these products when present at high counts (ÁLVAREZ-ASTORGA et al., 2002; TSOLA et al., 2008). For avian products some guidelines are suggested, such as the limit of 6log₁₀ cfu g⁻¹ of MA and 3log₁₀ cfu g⁻¹ of EC, specifically in Spain. In France, the usual recommendation is that MA contamination must not be higher than 5.7log₁₀ cfu g⁻¹ and EC not higher than 4log₁₀ cfu g⁻¹. A similar situation can be observed in the USA, where some states adopt maximum levels of microbial contamination for chicken carcasses and avian products (ÁLVAREZ-ASTORGA et al., 2002; USDA, 2003). When the MA counts varies between 6 and 7log₁₀ cfu g⁻¹, meat products present a perceptible odor, and counts higher than 8log₁₀ cfu g⁻¹ tend to form lime on the surface, associated with alterations in color and consistency (GILL, 1998). So, these microbial parameters are important references to be followed in order to provide proper quality control for meat products, including avian carcasses.

Considering distinct levels of microbial contamination, the samples were grouped and the observed frequencies compared (Table 3). Significant differences (P<0.05) were observed between samples submitted or not to inspection for MA counts higher than 3, 4, and 5log₁₀ cfu g⁻¹. Eight samples submitted to inspection and eight samples which were not submitted presented MA counts higher than 6log₁₀ cfu g⁻¹, the reference parameter suggested by ÁLVAREZ-ASTORGA et al. (2002). For EB, a significant difference was observed between the samples only for counts higher than 3log₁₀ cfu g⁻¹ (Table 3), with the inspected samples seen at a higher frequency. No significant differences were observed between the samples

Table 3 - Numbers of chicken carcass samples, submitted or not to Brazilian inspection services, presenting distinct levels of contamination by hygiene indicator microorganisms.

Microbial group/Inspection	n	-----Levels of contamination (log ₁₀ cfu g ⁻¹)-----				
		>3.0	>4.0	>5.0	>6.0	>7.0
Mesophilic aerobes						
Inspected	30	26	22	16	8	1
Non inspected	30	30	30	25	8	1
χ ²		4.29	9.23	6.24	0.00	0.00
P		0.038	0.002	0.012	1.000	1.000
Enterobacteriaceae						
Inspected	30	22	13	6	3	0
Non inspected	30	28	18	2	0	0
χ ²		4.32	1.67	2.31	3.16	-
P		0.038	0.196	0.129	0.076	-
Total coliforms						
Inspected	30	13	3	0	0	0
Non inspected	30	15	1	0	0	0
χ ²		0.27	1.07	-	-	-
P		0.605	0.301	-	-	-
<i>Escherichia coli</i>						
Inspected	30	7	0	0	0	0
Non inspected	30	3	0	0	0	0
χ ²		1.92	-	-	-	-
P		0.166	-	-	-	-

n = number of samples; χ^2 = chi-square test; P = level of significance. For all comparisons, the degree of freedom was 1.

submitted or not submitted to inspection considering the levels of contamination by TC and *E. coli* higher than 3log₁₀ cfu g⁻¹ (Table 3). Considering *E. coli*, seven inspected and three non-inspected samples presented counts higher than 3log₁₀ cfu g⁻¹, reference level in USA (USDA, 2003), and suggested by ÁLVAREZ-ASTORGA et al. (2002). No samples presented *E. coli* counts higher than 4log₁₀ cfu g⁻¹ (Table 3).

In general, an expected result would be the higher counts of hygiene indicator microorganisms, and also higher frequencies of positive results for *Salmonella* spp. and *E. coli*, in non-inspected chicken carcasses. However, it must be considered that the usual chicken slaughtering occurs in an industrial scale, which facilitates the microbiological contamination due to the automation and velocity of the process (MATIAS et al., 2010). In counterpart, non-inspected chicken carcasses are usually obtained from small producers that slaughter a little number of birds, minimizing the contamination. Despite the absence of significant differences concerning the microbiological contamination of the analyzed chilled chicken carcasses, it is necessary to emphasize that the inspection consider other aspects to assure the quality

and safety of the products of animal origin for human consumption. Animal sanitation and chemical residues, for instance, are factors that must be properly controlled as they are extremely necessary to guarantee avian production, and are included in other monitoring programs in Brazil (BRASIL, 2003a,b). Additionally, chemical residues pose as potential hazards and also can interfere in microbiological analysis (ANDREE et al., 2010).

CONCLUSION

The obtained results demonstrated that inspected and non-inspected chilled chicken carcasses commercialized in the Viçosa region did not present statistical differences when compared by some microbiological parameters. Only MA (counts higher than 3, 4, and 5log₁₀ cfu g⁻¹) were found more frequently in non-inspected samples than in inspected ones.

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