Microbiological quality of seasoned chicken cuts using *Escherichia coli* and *Salmonella* spp. as quality indicators.

Qualidade microbiológica de cortes de frango temperado utilizando como indicadores a presença de *Escherichia coli* e *Salmonella* spp.

Calidad microbiológica de los cortes de pollo templados utilizando la presencia de *Escherichia coli* y *Salmonella* spp. como indicadores.

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Flávio Barbosa da Silva

ORCID: https://orcid.org/0000-0002-2885-0272 Universidade Federal de Jataí, Brasil E-mail: flavio.b.silva@brf.com **Bruna Ribeiro Arrais** ORCID: https://orcid.org/0000-0002-1530-6606 Universidade Federal de Jataí, Brasil E-mail: brunavet13@gmail.com **Marcos Roberto Alves Ferreira** ORCID: https://orcid.org/0000-0002-6658-7196 Universidade Federal de Pelotas, Brasil E-mail: marcosferreiravet@gmail.com Iderval da Silva Júnior Sobrinho ORCID: https://orcid.org/0000-0002-3213-5319 Universidade Federal de Jataí, Brasil E-mail: idervalsobrinhojr@ufg.br Márcia Dias ORCID: https://orcid.org/0000-0002-1394-6941 Universidade Federal de Jataí, Brasil E-mail: diasmarcia@ufg.br **Cecília Nunes Moreira** ORCID: https://orcid.org/0000-0001-7629-3290 Universidade Federal de Jataí, Brasil E-mail: cecilia_nunes_moreira@ufg.br

Abstract

Enteropathogens such as Salmonella spp. and Escherichia coli are important health challenges in the poultry production chain, because when installed in the production chain, they impair the safety of food supply. The determination of the microbiological quality of chicken meat, especially when marketed in spiced form, is necessary, given that consumer prefer this type of processed protein. This work aims to evaluate the microbiological quality of the meat of seasoned chicken marketed in the municipality of Rio Verde, Goias, using Salmonella spp. and Escherichia coli as target microorganisms, and considering as variables the type of establishment, validity date and presence of municipal inspection seal. From 80 analyzed samples, 30% (24/80) of samples were positive for Salmonella spp. and 55% (44/80) for E. coli. Regarding the type of establishment, it was observed that 27.45% (14/51) of supermarket samples and 34.48% (10/29) of meat store samples were contaminated by Salmonella spp. Considering E. coli, 49.01% (25/51) of supermarket samples and 65.55% (19/29) of meat store samples were positive for this pathogen. 80% (64/80) of the samples had the municipal inspection seal (MIS), and 83.75% (67/80) contained the expiration date on the label. The legislation in its narrative guarantee's protection for the consumer with regard to the presence of pathogenic serotypes of these agents, however, it is worth noting that the effective action of health surveillance, and the constant laboratory investigation of the products are necessary. **Keywords:** Public health; Food security; Health surveillance.

Resumo

Enteropatógenos como *Salmonella* spp e *Escherichia coli* são desafios sanitários importantes na cadeia produtiva de aves, pois uma vez instalados na cadeia de produção impossibilitam a oferta de um alimento seguro. A determinação da qualidade microbiológica da carne de frango em especial quando comercializada na forma temperada se faz necessária sendo grande a preferência dos consumidores para esse tipo de proteína processada. Esse trabalho objetiva avaliar a qualidade microbiológica da carne de frango temperada comercializada no município de Rio Verde, Goiás, utilizando como microrganismos alvos *Salmonella* spp e *Escherichia coli*, e considerando como variáveis o tipo de estabelecimento, a data de validade e presença de selo de inspeção municipal. Das 80 amostras analisadas, observou-se que 30% (24/80) das amostras foram positivas para *Salmonella* spp e 55% (44/80) para *E. coli*. Quanto ao tipo de estabelecimento, notou-se que 27,45% (14/51) das amostras de supermercados e 34,48% (10/29) das amostras de casas de carne estavam contaminadas por *Salmonella* spp., e para o agente *E. coli*, 49,01% (25/51) das amostras de supermercado e 65,55% (19/29) das amostras

de casas de carne foram positivas. 80% (64/80) das amostras apresentavam o selo de inspeção municipal (SIM), e 83,75% (67/80) continham a data de validade no rótulo. A legislação em sua narrativa, garante proteção para o consumidor no que diz respeito a presença de sorotipos patogênicos desses agentes, porém, vale ressaltar que a ação efetiva de vigilância em saúde, e a constante investigação laboratorial dos produtos são necessárias.

Palavras-chave: Saúde pública; Segurança alimentar; Vigilância sanitária.

Resumen

Los enteropatógenos como Salmonella spp. y Escherichia coli son importantes desafíos de salud en la cadena de producción avícola, ya que una vez instalados en la cadena de producción, es imposible ofrecer alimentos seguros. La determinación de la calidad microbiológica de la carne de pollo, especialmente cuando se comercializa en forma condimentada, es necesaria, con gran preferencia del consumidor por este tipo de proteína procesada. Este trabajo tiene como objetivo evaluar la calidad microbiológica de la carne de pollo condimentada comercializada en la ciudad de Rio Verde, Goiás, utilizando como microorganismos diana Salmonella spp. y Escherichia coli, y considerando como variables el tipo de establecimiento, la fecha de vencimiento y la presencia de sello de inspección. municipal. De las 80 muestras analizadas, se observó que el 30% (24/80) de las muestras fueron positivas para Salmonella spp. y el 55% (44/80) para E. coli. En cuanto al tipo de establecimiento, se observó que el 27,45% (14/51) de las muestras de supermercados y el 34,48% (10/29) de las muestras de carnicería estaban contaminadas por Salmonella spp., y para el agente E. coli, 49,01% (25/51) de las muestras de supermercados y 65,55% (19/29) de las muestras de carne fueron positivas. El 80% (64/80) de las muestras tenían el sello de inspección municipal (SIM) y el 83,75% (67/80) contenía la fecha de vencimiento en la etiqueta. La legislación en su narrativa garantiza protección al consumidor frente a la presencia de serotipos patógenos de estos agentes, sin embargo, cabe mencionar que es necesaria la acción efectiva de vigilancia sanitaria, y la investigación constante de laboratorio de los productos.

Palabras clave: Salud pública; Seguridad alimentaria; Vigilancia de la salud.

1. Introduction

The production of high-quality food that is safe for consumers represents the greatest challenge for food sector. Facing this challenge requires a high degree of commitment of all

those involved in food production and supply processes (Gonçalves, 2014). Diseases of food transmission have been the cause of investigations and researches for etiological agents and related factors to be identified and analyzed (Medeiros et al. 2017). Foodborne diseases have triggered investigations and researches to identify and analyze the etiologic agents and related factors (Medeiros et al. 2017).

With large-scale production, food contamination can begin in the production of the raw material itself. During the manipulation a number of factors related to the lack of hygiene of the equipment and even of those who manipulate them, can occur. Contamination can extend to storage, packaging and distribution stages, as these allow direct exposure to the environment (Pereira et al. 2016).

Chicken meat is a key product in the diet of practically all countries and for this reason there is a constant growth in world production of chicken. Brazil has become the second largest chicken producer in the world, however this meat can become a vehicle for the transmission of numerous microorganisms, if the sanitary procedures are neglected during the housing of poultry on farms, the pre-slaughter management phase and slaughtering operations (Brazil, 2018).

Sanitary hygienic conditions in which food is produced, manipulated and marketed, directly interfere with the microbiological quality of these foods, and consequently, the lack of care in these aspects can bring risks to consumer health (Zuldt, 2014). The biological agents most commonly cited in cases of Foodborne Diseases are bacteria, viruses, toxins, parasites and toxic substances. Bacteria are the agents most related to the cases and among them the most common are *Salmonella* spp., *Escherichia* coli, *Staphylococcus aureus*, *Shigella* spp., *Bacillus cereus* and *Clostridium perfringens*. Therefore, *Salmonella* spp. and *Escherichia coli* are relevant pathogens in public and animal health worldwide (Brazil, 2014; Lammie & Hughes, 2016).

Salmonella is one of the most prominent genera in the family Enterobacteriaceae, present in the intestinal microbiota of animals and humans. It is a pathogen of worldwide significance, widely distributed in the environment, whose main transmission vehicles are food. There are more than 2,600 identified *Salmonella* serovars, all of which are considered as potentially pathogenic (Brazil, 2018). *E. coli* are Gram negative bacteria that normally inhabit the intestinal microbiota of man and animals, and are typically non-pathogenic. However, subgroups of *E. coli* have virulence factors that make them capable of causing disease (Caldorin et al., 2013).

The sanitary hygienic control of food producing establishments is supervised and inspected by inspection services. The sanitary and industrial inspection service of products of animal origin is structured according to the area covered by the commercialization of production. The municipal inspection service (MIS) must control the quality of products of animal origin marketed in the municipality, in addition to encouraging small companies and entrepreneurs to leave clandestinity (Brazil, 1989).

The requirement that municipalities implement this service is old, but the reality is different from the legislation. Adherence to MIS is extremely important for the agribusiness, as it is a guarantee of product quality. In addition to the hygienic-sanitary issue, there is a need to enable producers to participate in federal programs such as the National School Feeding Program (NSFP) and the Food Acquisition Program (FAP) (Prezotto & Nascimento, 2013).

Rio Verde/Goiás, for more than 20 years, has stood out nationally in agribusiness activity, growing disorderly from a population and structural point of view. Thus, changes based on operational control and minimization of possible risks and health problems to the population were necessary. In 2006, the municipality implemented the municipal inspection service, being the first in the state, but the service was only regulated in 2009, and the first establishment with a MIS certificate was registered only in 2011 (Portilho, 2016).

The determining factors for the emergence of foodborne diseases are numerous, as well as the understanding of the real dangers during the ingestion of foods of animal origin. Therefore, the present study is quali-quantitative to evaluate the microbiological quality of seasoned chicken meat marketed in the city of Rio Verde, Goiás, using *Salmonella* spp. and *E. coli* as target microorganisms, considering as variables the type of establishment, the presence of a municipal inspection seal and the expiration date on the label.

2. Methods

Eighty samples of spiced chicken meat from 40 commercial establishments were randomly selected and evaluated. The sampling occurred in two stages in each establishment and the experiment was carried out at the Laboratory of Veterinary Practices of the Federal University of Jataí (UFJ).

Samples with an average of 200 g were purchased in bulk, as established by ANVISA (RDC 12). A form describing the date and time of collection, type of cut, labeling, presence or absence of the Municipal Inspection Seal, expiration date and type of establishment was filled

out. No questionnaire was applied and the identity of the establishments was kept confidential.

The samples were processed by conventional bacterial isolation according to the International Organization for Standartization (IOS) 6579: 2002, to confirm the agent *Salmonella* spp. In order to determine the presence of *E. coli*, the samples were processed according to the methodology provided by the Ministry of Agriculture, Livestock and Supply (MAPA - IN n° 62/2003).

The data were computed in the System of Statistical Analysis - SAS v. 9.3 (2010). The Mantel-Haenzsel chi-square for linear trend and "odds ratio" (OR) at 5% probability was used to assess the probable risk factors in the prevalence of *Salmonella* spp. and *E. coli*.

The evaluation criteria for samples of spiced chicken meat were classified based on the presence or absence of *Salmonella* spp. and *E. coli* (Barbosa et al. 2020; Arrais et al. 2020). Other variables such as the type of establishment (supermarkets and meat shop), presence or absence of municipal inspection seal and presence or absence of expiration date, the evaluation criteria for samples of spiced chicken meat were classified based on the presence or absence of *Salmonella* spp. and *E. coli*. Other variables such as the type of establishment (supermarkets and meat shop), presence or absence of municipal inspection seal (SIM) and presence or absence of expiration date, were assessed.

3. Results and Discussion

According to conventional bacterial isolation, it was observed that 30% (24/80) of the seasoned chicken meat samples were positive for *Salmonella* spp. and 55% (44/80) were positive for *E. coli*. These observations were similar with those recently presented by the Ministry of Health.

When listing the main causative agents of Foodborne diseases in Brazil, in the period between 2007 and 2017, it was observed that *Salmonella* spp. and *E. coli* lead the number of recorded and confirmed outbreak cases in the country, with a percentage of 7.5% for cases involving *Salmonella* spp. and 7.2% for *E. coli*, demonstrating the permanence of these agents in food as a potential risk for population that consumes this type of food (Brasil, 2018).

The prevalence of *Salmonella* spp. observed in this research is below that found in similar studies. Bau et al. (2001) analyzed 124 samples of chicken meat in a similar experiment developed in the southern region of the country, and observed a prevalence of 10.48% (13/124) of a sample of chicken meat contaminated by *Salmonella* spp. Moreira et al.

(2014), in a study conducted in the city of Goiânia, identified, through analytical tests (screening + confirmation), 21 *Salmonella* spp. isolates in 100 chilled chicken carcasses. Portilho (2016), evaluating the production of seasoned chicken cuts in establishments in Rio Verde, Goiás identified the presence of *Salmonella* spp. in 80% (24/30) of the visited establishments. The difference in the observed results can be justified by several factors inherent to the facilities found in the different visited establishments, as well as by the existence or absence of good manufacturing practices, and in particular by the scenarios and diagnostic methodologies used for detection of this microorganism.

The results for *E. coli* showed considerable differences when compared with other studies. Souza et al. (2002) determined that in 60 cuts of poultry meat purchased in the retail trade in the city of João Pessoa - PB, 95% (57/60) of the samples were positive for *E. coli*. However, lower prevalence was reported by Beraldo-Massoli et al. (2014), who observed that 18.5% of samples were contaminated by Escherichia coli, when evaluating 54 chicken samples in the city of Jabuticabal - São Paulo. Different results were described by Younis et al. (2017). When analyzing 120 samples of chicken meat in Egypt, they determined that 11.66% (14/120) of the samples were contaminated with *E. coli*. These differences between the results, emphasize that different forms of processing, storage and commercialization of the samples, are determinant for the microbiological quality of the final product.

Considering the place of samples origin, it was observed that 63.75% (51/80) were from supermarkets, while 36.25% (29/80) were from meat stores. Stratifying only positive samples for *Salmonella* spp., it was noted that 27.45% (14/51) of the supermarket samples and 34.48% (10/29) of the meat store samples were contaminated. On the other hand, from positive samples for *E. coli*, 49.01% (25/51) came from supermarkets and 65.55% (19/29) from meat stores.

In the present study, samples from meat stores had a higher prevalence of contamination but without statistically differentiating from supermarket samples (P > 0.05 - table 01). In the experiment proposed by Bau et al. (2001) 6.06% (4/66) came from supermarkets and 15.51% (9/58) came from meat shops, suggesting that the increase in positive samples for the bacteria in question is due to differences in meat handling in both types of establishments. In meat shops, inappropriate handling practices and variations in storage temperatures are common, which favors the multiplication of microorganisms. While in supermarkets, chicken products are packaged in plastics and are well refrigerated or frozen, which reduces cross-contamination and slows down bacterial multiplication.

Regarding the presence of the municipal inspection seal and the presence of the expiration date on the label, 80% (64/80) of the samples had the seal and 83.75% (67/80) had an expiration date. Evaluating the effect of the variables type of establishment, validity and product inspection on the presence of *Salmonella* spp. and *E*. coli, using the complete logistic regression model, it was found that there was no significant difference for any factor analyzed (Table 1).

Table 1.	Presence	of each	bacterium	according to	establishment,	validity a	nd inspection	(P <
0.05).								

Bacteria	***	Establishment	Validity	Inspection
Salmonella spp.	P-value	0.6431	0.5458	0.3083
Escherichia coli	P-value	0.2126	0.6791	0.2718

Source: Authors.

There was no correlation between food contamination and the different types of establishment, expiration date and inspection (P > 0.05). The samples in question were found in bulk, characterizing the reprocessing of the product when arriving at the establishments, thus losing the guarantee of the inspection seal of its place of origin. Although 80% (64/80) of the establishments visited presented municipal inspection seal, none of the establishments had labelling indicating the conditions of preparation, storage and possible risk of raw food consumption. This mandatory prerogative is provided for by DRC 13.

The presence of these microorganisms in the final product raises concerns about possible failures in the control of these bacteria, which begins in the rearing of animals, where the permanence of infected animals may trigger contamination throughout the production chain, as well as bringing these bacteria to refrigeration plants and therefore to the final consumer. Lázaro et al. (2008) consider that the route of transmission of these microorganisms to humans may be associated with the food chain, and noted that the most prevalent factor is infected animals without clinical signs, which makes detection difficult before or during slaughtering, and turn these animals in constant sources of contamination of the environment and consequently of food.

Moraes et al. (2014) evaluated the distribution of enteropathogens such as *Salmonella* spp. throughout the poultry production process, considering meconium and broiler chicks as a sampling unit; as well as ingluvies and caecum obtained from slaughterhouses and trawl swabs from breeding and slaughterhouses in the state of Goiás. All studied samples were

contaminated with *Salmonella* spp. The presence of *Salmonella* spp. in one-day-old chicks, before housing, compromises the entire health process of the flock, as they spread the bacteria throughout the entire production chain, compromising the health aspect of the aviaries, as well as the industry and its final product.

The problem of contamination of refrigeration units is an important point to be noted once, since the place where these animals are processed must ensure microbiological safety, due to the intense handling of the carcasses at this stage of processing, which can be condemned even in the meat processing plant causing significant financial losses for animal protein processing companies (Moraes et al. 2014). Souza et al. (2014) assessing the origin of the microbial load of chicken carcasses and their derivatives reported that the origin of the microbiota present in poultry carcasses, came mainly from live birds or incorporated in any of the slaughter stages, the most critical being scalding, plucking and evisceration.

Oliveira et al. (2016) when conducting an epidemiological survey to characterize the main causes of condemnation of poultry slaughter in slaughterhouses registered with the Brazilian federal inspection service in the period between 2006 and 2011, noted that among the main causes, contamination stands out (1.80%), followed by contusion / traumatic injury (1.57%), dermatosis (0.74%) and cellulite (0.50%). Zweifel et al. (2015); Belluco et al. (2016), after applying related studies, on the effects of slaughter operations on the microbiological contamination of chicken carcasses in three slaughterhouses over a period of four months, pointed out that the highest counts of microorganisms in the poultry slaughter are evidenced between the scalding and evisceration stages.

Pacholewicz et al. (2016) stressed that contamination of carcasses during slaughtering is undesirable, however it is inevitable that it will occur, so it is necessary to apply strategies to prevent and reduce such contamination, such as the appropriate regulation of evisceration machines and trained labour in the operation of these machines.

Although the focus of this research is the final product, such information reinforces that the care with food safety must start even in the initial stages of production, thus ensuring the safety of the final product. Pereira et al. (2016) pointed out that in large-scale production the contamination of food starts in the production of the raw material itself, because during the manipulation can occur a series of factors related to the lack of hygiene of the equipment and even of those who manipulate them, extending to the stages of storage, conditioning and distribution, as they allow direct exposure to the environment.

The prerogative of cross-contamination of food is reported annually by the Ministry of Health. The outbreak notification data for Foodborne diseases by *Salmonella* spp. and

Escherichia coli demonstrate that the main site of contamination in food outbreaks are households, highlighting the importance of the care that people should take when preparing food at home, especially when handling and preparing raw and cooked food (Brazil, 2018).

4. Final Considerations

The presence of *Salmonella* spp. and *Escherichia coli* in products destined for final trade is a concern when we evaluate the potential risks of cross-contamination that these foods can generate in home environments, schools, hospitals and or nursing homes. This impact is real and has been treated as a crucial point for reducing cases of Foodborne diseases outbreaks caused by this type of food. There are several factors that may interfere in the microbiological quality of food and this study addressed some factors related to the product disposed in the final consumption establishments. However based on the literature used for this discussion, it is understood that contamination of food can still occur in the primary food production stations.

The legislation is a protection for the consumer, but an even more effective supervisory action is necessary. The monitoring should be applied in the primary industries of chicken processing and/or in the commercialization in retail establishments, since the numerous health factors apply to all phases of processing, storage and commercialization process.

Further studies will be carried out in order to assess the presence of virulence factors in *E. coli* and Salmonella spp. isolates.

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Percentage of contribution of each author in the manuscript

Flávio Barbosa da Silva – 50% Bruna Ribeiro Arrais – 10% Marcos Roberto Alves Ferreira – 10% Iderval da Silva Júnior Sobrinho – 10% Marcia Dias – 10% Cecília Nunes Moreira – 10%