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ORIGINAL ARTICLE

# OCCURRENCE OF *SALMONELLA* SPP. IN BROILER CHICKEN FECES IN THE CENTRAL REGION OF THE STATE OF MINAS GERAIS, BRAZIL

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## ABSTRACT

Bacteria of the genus *Salmonella* are gram-negative rods, facultative anaerobic non-spore formers, which may or may not be flagellated. These bacteria are frequently detected in poultry products and are important in animal and public health. The purpose of this study was to investigate the occurrence of *Salmonella* spp. in feces from broiler chicken litters located in municipalities of the central region of the state of Minas Gerais, Brazil. The study analyzed 845 feces samples from different poultry farms collected between September 2016 and March 2017. Pre-enrichment broths, specific selective broths and selective indicator agar were used for the analyses. The suspected isolates were submitted to biochemical testing and serotyping with "O" and "H" antisera to identify the *Salmonella* serotypes. The results showed that 213 feces samples were contaminated with *Salmonella*, and the serotypes found were: *S.* Minnesota, *S.* Sandiego, *S.* Schwarzengrund, *S.* Infantis, *S.* Hadar, *S. enterica* subsp. *enterica* (O: 4.5), *S.* Montevideo, *S.* Miami, *S.* Heidelberg, *S.* Cerro, *S.* Ndolo, *S.* Panama, *S.* Anatum, *S.* Tennessee, *S.* Agona, *S.* Newport *and S.* Muenster. The Minnesota serotype was predominant among the isolates investigated. Actions to improve the biosafety of commercial poultry farms are necessary to prevent possible contamination of poultry products that endanger human health.

KEY WORDS: broiler chicken; antisera; serotyping; *Salmonella* serotypes.

## INTRODUCTION

The genus *Salmonella* are Gram-negative rods, facultative anaerobic non-spore forming bacteria which can be motile or nonmotile (Le Minor, 1988; Grimont & Weill, 2007). They are classified in two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the most common species, subdivided into six subspecies: *enterica, salamae, arizonae, diarizonae,*

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*houtenae* and *indica.* In each subspecies, different types are recognized, totaling about 2,500 serotypes (Grimont & Weill, 2007). The identification of the serotypes is done by means of the Kaufmann & White scheme, based on the antigenic composition of the *Salmonella* in relation to its somatic (O), flagellar (H) and capsular (Vi) antigens (Le Minor, 1988).

Their habitat is the intestinal tract of humans and many domestic and wild animals (Wan Norhana et al., 2010). Broiler chickens can be infected with *Salmonella* spp. through vertical transmission or in the breeding environment through contaminated water, feed meal, litters, and excrements of other animals. Many serotypes may survive for weeks in the bedding of commercial flocks of broiler chickens, increasing the rate of infection in poultry breeding stock (Dai Pra et al., 2009).

In order to keep up the high productivity of Brazilian broiler chickens, measures are required to control and prevent infectious agents in commercial poultry, with *Salmonella* as one of the target agents (Sousa et al., 2013). According to the Ministry of Agriculture Livestock and Food Supply Normative Instruction 20, (MAPA) of October 21, 2016, broiler chicken farms must be monitored to detect *S.* Pullorum*, S.* Gallinarum*, S.* Enteritidis *and S.* Typhimurium*,* with the purpose of enabling industrial plant health certification and ensuring the production of healthy meat for both internal and external markets (Brasil, 2016; Santos et al., 2013). These serotypes are responsible for salmonellosis, and are considered harmful to the health of birds and humans. Birds can be affected by three types of diseases: pullorum disease, caused by *S.* Pullorum; avian typhus, by *S.* Gallinarum; and paratyphoid, which is caused by the other serotypes of *Salmonella* (Berchieri, 2000).

In public health, salmonelloses are among the major zoonoses that affect world population due to their endemicity, high morbidity and difficulties in establishing control measures (Guerin et al., 2005). Strains of paratyphoid *Salmonella* are of great interest in animal health and public health and are frequently detected in poultry products. Most serovars can colonize the abdomen without causing disease, but in humans *S. typhimurium* and *S. enteritidis* may cause severe food poisoning (Cardoso & Tessari, 2008).

Therefore, due to the great importance of some *Salmonella* serotypes in public health and the economic losses generated by the infections they cause, this study aimed to investigate the occurrence of different serotypes of *Salmonella* in broiler chickens in the central region of Minas Gerais State. This Brazilian State ranked as the fifth largest producer of broiler chicken meat exported in 2016, according to the Brazilian Animal Protein Association (ABPA, 2017).

## MATERIAL AND METHODS

The samples were collected from September 2016 to March 2017 from commercial flocks of broiler chickens, located in cities in the central region of the State of Minas Gerais, totaling 845 samples. The analyses were performed at the Microbiology and Animal Pathology Laboratory of a poultry company, located in the city of Montenegro, Rio Grande do Sul, Brazil.

Samples from the broiler manure were placed in wire closed 300mL sterile plastic packages. The collection was performed by the technician responsible for each commercial flock of broiler chickens. The feces samples were placed in thermal boxes, with recyclable ice, and sent to the laboratory. The analyses of the samples followed the recommendations described in Ordinance nº 126 of November 3rd, 1995, with modifications in the use of some culture media (Brasil, 1995).

As soon as the feces samples arrived in the laboratory, they were weighed in covered sterile bowls, hydrated with 1% peptone water (pre-enrichment broth) at 1:10 (25g of sample and 250mL of peptone water) and incubated at 36ºC for 18 to 24 hours. Subsequently 1mL from each bowl was pipetted into a tube containing 10mL of Rappaport Vassiliadis Broth and incubated at 41ºC for 18 to 24 hours. Then, an aliquot of each broth culture was streaked onto XLD Agar (Xylose-Lysine-Desoxycholate Agar) and onto Hektoen Enteric Agar. The plates were incubated at 36ºC for 48 hours. Next, samples with non-characteristic colonies were considered negative for *Salmonella* spp.

The suspicious colonies grown on the XLD or Hektoen agar media were inoculated in a preliminary biochemical testing media, composed of TSI (Triple Sugar Iron), LIA (Lysine Iron Agar), SIM (Sulfide Indole Motility), and Urea Broth and incubated at 36ºC for 18 to 24 hours. Isolates growing in TSI and LIA with characteristic base and apex for *Salmonella*; indole negative and motility in SIM; and negative urease were submitted to complementary biochemical testing for identification.

The complementary series of biochemical tests evaluated the metabolic reactions of suspected isolates, such as fermentation of carbohydrates (dulcitol, glucose, lactose maltose, mannitol, and sucrose); decarboxylation of lysine and ornithine; phenylalanine deamination; Methyl Red and Voges Proskauer tests.

After incubation at 36ºC for 18 to 24 hours, isolates showing biochemical profiles compatible with *Salmonella* spp. were submitted through the process of serotyping with "O" and "H" antisera obtained from the States Serum Institute (SSI®). According to the method recommended by SSI® (2016), 10µL of "O" antiserum were homogenized with an aliquot of a bacterial isolate grown on nutrient agar, and the formation, or not, of clumps, was expected to occur within 10 seconds of the reaction. When a positive result was obtained with the "O" antiserum, the isolate was subjected to serotyping with "H" antisera phases 1 and 2, in order to identify the specific flagellar antigens and reveal the *Salmonella* serotype.

## RESULTS AND DISCUSSION

The results, shown in Table, indicate the occurrence of *Salmonella* spp. in the region investigated in Minas Gerais. Of the 845 feces samples analyzed, 213 presented positive results for *Salmonella*.

*Table.* Isolation of *Salmonella* from feces samples collected from bedding of commercial flocks of broiler chickens located in cities in the central region of Minas Gerais State

| Serotypes | n (%) of the positive samples |
|---|---|
| *S.* Minnesota | 171 (20.2) |
| *S.* Sandiego | 9 (1.1) |
| *S.* Schwarzengrund | 7 (0.8) |
| *S.* Infantis | 6 (0.7) |
| *S.* Hadar | 4 (0.5) |
| *S. enterica* subsp. *enterica* (O:4,5) | 2 (0.2) |
| *S.* Montevideo | 2 (0.2) |
| *S.* Miami | 2 (0.2) |
| *S.* Heidelberg | 2 (0.2) |
| *S.* Cerro | 1 (0.1) |
| *S.* Ndolo | 1 (0.1) |
| *S.* Panama | 1 (0.1) |
| *S.* Anatum | 1 (0.1) |
| *S.* Tennessee | 1 (0.1) |
| *S.* Agona | 1 (0.1) |
| *S.* Newport | 1 (0.1) |
| *S.* Muenster | 1 (0.1) |
| Total | 213 (25.2) |

Brazil has presented an exponential development in poultry production, and is among the countries with the highest production and export capacity regarding poultry products in the world (Cardoso & Tessari, 2015). An increase in consumption of poultry meat has led to the expansion of commercial broiler chicken raising to supply the market. This means the poultry farms are housing a greater number of birds per square meter, possibly causing sanitation hazards in the facilities, leading to the contamination and proliferation of pathogens, such as *Salmonella* spp. (Berchieri, 2000).

The present study showed the occurrence of *Salmonella* spp. in 213 of 845 feces samples obtained in poultry farms located in the investigated region. The isolates were distributed in 17 different serotypes and *S.* Minnesota was distinctly predominant. The increasing detection of *S.* Minnesota in slaughterhouses was observed in 2009 and 2010, when 3.4% of positive samples proved to belong to this serotype (Muniz et al., 2015). Machado et al. (2017) evaluated the presence of *Salmonella* in broiler chicken cutting facilities in an industry located in the State of Mato Grosso do Sul, Brazil, and noted that 90% of the positive samples were identified as *S.* Minnesota. In an investigation of 1,543 drag swab samples from broiler chicken farms in the States of Santa Catarina, Paraná and Mato Grosso, Brazil, 82 samples were found to be positive for *Salmonella*, with the most common serotypes being *S.* Minnesota and *S.* Infantis (Voss-Rech et al., 2015).

An investigation performed by Boni et al. (2011), using drag swab samples from poultry, carcasses, viscera and water from slaughterhouses in Mato Grosso do Sul, Brazil, indicated the occurrence of seven serotypes of *Salmonella* in 11.3% of the 257 samples analyzed. In another study, from August 2004 to January 2006, feces were collected from eight groups of laying hens located in Fortaleza, Brazil, totaling 32 pooled samples from one hundred fresh feces samples, showing positive results in 6.3% of the samples analyzed (Salles et al., 2008). In a study by Borsoi et al. (2010), 180 carcasses of cooled broiler chickens from retailers in the northeastern region of Rio Grande do Sul, Brazil, were analyzed, and the presence of these bacteria was observed in 12.2% of the samples.

The dissemination of *Salmonella* spp. can also occur through feed intended for commercial flocks of broiler chickens, produced with contaminated raw material (Berchieri, 2000). This was shown in the study by Hofer et al. (1997) with positive samples detected during 29 years of investigation in Brazil (1962 to 1991). Serotyping of 2,123 *Salmonella* spp. cultures recovered from raw material and feed for various bird species, identified 151 different serotypes. *S.* Infantis ranked as the sixth most frequent serotype in the study by Hofer et al (1997), while it was the fourth most common in the present study.

Negative results for *Salmonella* spp. are also documented, as showed by an investigation done in Western Parana, Brazil, performed with drag and cloacal swab samples collected in broiler chicken farms, where *Salmonella* was not detected in any of the samples, probably due to the reduced number of confined birds, which facilitates site maintenance (Teixeira & Lima, 2008; Ravagnani et al., 2012). The dissemination of this bacterium, both in the farms and in the industry, may be due to the poor sanitation of the facilities, inadequate bird transportation and deficiencies in slaughter operations (Tirolli & Costa, 2006).

*Salmonella* from sorotypes Heidelberg, Senftenberg, Infantis and Minnesota also gained importance in human and animal health joining the *typhimurium* and *enteritidis* serotypes (Lourenço et al, 2013). According to Cardoso and Tessari (2015), among the most prevalent serotypes in Brazil in recent years, Typhimurium, Minnesota, Mbandaka, Senftenberg, Agona, Schwarzengrund, Infantis and Panama are responsible for outbreaks that increase bacterial dissemination, resulting in losses to the poultry industry.

Alternative methods for detecting *Salmonella* are also frequently applied. Tests using conventional methodology together with PCR (Polymerase Chain Reaction) and ELISA were carried out in a study performed with slaughterhouse samples artificially contaminated with *S.* Enteritidis*, S.* Typhimurium*, S.* Gallinarum and *S.* Pullorum (Dickel et al., 2005). The analyses showed a higher index of positive results for the conventional technique, proving superior efficiency in relation to the other techniques evaluated in the study. An analysis conducted by Coelho (2012) using samples of feces and boot swabs from poultry environments contaminated with *S.* Typhimurium and *S.* Enteritidis compared the efficiency of the conventional Ordinance nº126 method and the molecular method used in the BAX® System. The results were satisfactory for both techniques presenting equivalent performances. These studies show that the conventional methodology is as efficient as modern ones using molecular tests.

More efficient biosafety of commercial poultry farms is necessary to avoid potential contamination of poultry products that endanger human health. Normative Instruction Nº 20, from 2016, lays down laboratory test rules to be used in broiler chicken breeding grounds, in order to control Salmonella, and especially the most relevant serotypes in regard to public health (*S.* Typhimurium*, S.* Enteritidis*, S.* Pullorum and *S.* Gallinarum) which were not detected in the present study, evidencing efficient sanitary control in these combinations of cut broiler chicken.

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