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Salmonella Enteritidis and Typhimurium in informally sold broilers

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In order to evaluate the sanitary conditions of fresh chicken carcasses sold in public markets in the city of Feira de Santana, Bahia, a study was conducted in carcasses collected from six different establishments, during ten weeks, totaling up to 60 samples, to evaluate the occurrence of Salmonella in broilers. Results show a 28.3% occurrence of the pathogen and current investigation underscored the diagnosis of Salmonella Enteritidis and Salmonella Typhimurium in the samples. The serotype Enteritidis was isolated in establishments A, B and C and the serotype Typhimurium in establishments E and F. The results of this study indicate that many chicken products sold in Feira de Santana, Bahia, Brazil, are contaminated with different serotypes of Salmonella, especially Salmonella Typhimurium and Salmonella Enteritidis and should, therefore, be suitable for cooking and handling prevention of salmonellosis outbreaks.

Key words: food, microbiology, veterinary public health.

INTRODUCTION

Salmonellae are widely distributed in nature and are capable of infecting both humans and animals. Poultry infected by paratyphus salmonellae may develop the disease either clinically or asymptomatically by harboring the agents and becoming potential sources of salmonellosis for humans (Nagaraja et al., 1991; Barrow, 1993; Santos et al., 2000).

From the 1980s henceforth, food toxic-infections caused by *Salmonella* increased and Rodrigue et al. (1990) and Santos et al. (2000) attribute this fact to intake of eggs and other sub-products contaminated by *Salmonella enterica*. However, *Salmonella* in broilers´ carcasses should never be discarded (Rampling et al., 1989; Boer and Zee, 1992; Giessen et al., 1992; Poppe, 1994; Scuderi et al., 1996; Costa, 1996; Sakai and Chalermchaikit, 1996; Ward and Threlfall, 1997; Santos et al., 2000).

Chicken meat in England and Wales caused outbreaks

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and sporadic cases of the disease (Rampling et al., 1989; Santos et al., 2000), with approximately 30,000 cases of food toxic-infections in humans (Ward and Threlfall, 1997; Santos et al., 2000). Tavechio et al. (1996) reported an increase in the isolation of *S. enterica* in Brazil as from 1993. Between 1991 and 1994, Italy experienced 1699 food-caused outbreaks, with *Salmonella* causing 81% of which 34% were of *S. enterica* (Scuderi et al., 1996; Santos et al., 2000).

Animal-originating products, especially from poultry, are an important source of human protein. In the Brazilian retail market, cooled or frozen poultry carcasses are available, even though cooling fails to eliminate bacteria as those of the genus *Salmonella* (Santos et al., 2000).

Scarcity of analysis on this subject is a highly notorious fact in Northeastern Brazil where a fast development in poultry industries is currently occurring. New researches on the occurrence of *Salmonella* spp. in the poultry chain of this region are urgently required. Further, regional differences that affect the development of these micro-organisms should also be taken into account.

Current assay investigates the occurrence of *Salmonella* spp. in several food establishments in Feira de Santana BA Brazil, which exhibits the 'hot chicken' commercialization. Results in current analysis may contribute towards the identification and introduction of more specific and effective control measures directed towards local specificities. Greater visibility will also be established with regard to the risks that the population and consumers undergo by providing the controlling authorities with highly useful microbiological data.

MATERIALS AND METHODS

Current assay was performed in Feira de Santana BA Brazil, a city in the central-north meso-region of the State of Bahia, Brazil. The method comprised the collection of samples of poultry carcasses from illegal abattoirs. The carcasses were bought from several commercial firms in one of the city districts. The methodology included certain advantages such as low cost, easiness in collecting the material and microbiological analyses developed without any further costs.

Six firms, called A, B, C, D, E and F, which sell broiler carcasses directly from the illegal abattoir, were chosen at random. A sampling plan was established by which sixty samples were analyzed. Carcasses from each firm were collected weekly, in the morning, during 10 weeks, between September and October 2013.

Internal temperature was measured at the carcass´s thermal center, or rather, at the supracoracoid region and pectoral muscles. Data were collected by a portable thermometer immediately after the collection of the carcasses and on the same premises. Samples were then conditioned in an isothermal container and sent to the Laboratory of Food and Water Microbiology of a food-processing industry in the city.

The samples were collected in the morning and the material was sent to the microbiology laboratory for the analysis of *Salmonella* spp immediately after collection. Analysis was based on the methodology following Norm 62 by MAPA (Brasil, 2003). Samples had the same visual standard, characterized by a yellowish color, with wrapped offal placed within the celomatic cavity of the carcass.

Results were given following proposal in the two-class sampling plan, or rather, when the sample could be acceptable or not (Brasil, 2001). The plan was applied in the *Salmonella* research due to the bacterium´s epidemiological characteristics.

A long thermometer (stainless steel rod, 120 mm long and scale ranging between -50 and 200°C) verified the temperature of each carcass on the collection site. Temperature was taken in the pectoralis muscle with the thermometer rod penetrating some 4 cm in the region. Room temperature of the premises where collection was performed was also measured.

RESULTS AND DISCUSSION

Ten samples from each firm were evaluated during 10 weeks (a sample collected per week) in August, September and October 2013. Results for research on *Salmonella* for each firm are given, coupled to the temperature at collection.

Tables 1, 2 ,3, 4, 5 and 6 show frequencies for each firm, with firms A, B and C demonstrating critical results due to their high positive frequency. Firms E and F showed similar frequencies, with lower rates than the others. On the other hand, firm D failed to show any positive frequency for the pathogen under analysis, between the start and end of the investigation. Table 6 provides results for positive samples of *Salmonella* during the whole assay.

Current investigation underscored the diagnosis of *Salmonella* Enteritidis and *Salmonella* Typhimurium in the samples. The serotype Enteritidis was isolated in establishments A, B and C and the serotype Typhimurium in establishments E and F.

The above fact probably suggests other species of *Salmonella*, similar to those reported by Hofer et al. (1997), who, in their studies on serovars of *Salmonella* isolated from poultry in Brazil, registered *Salmonella* Gallinarum*, Salmonella* Pullorum*, Salmonella* Heidelberg and *Salmonella* Infantis*,* besides *Salmonella* Enteritidis and *Salmonella* Typhimurium*.*

Increase of sporadic cases and outbreaks of human salmonellosis in several countries is related to the increase of infection by *Salmonella* due to the intake of poultry meat, eggs and other contaminated derived products. Several studies show that the occurrence of *Salmonella* spp. in broiler carcasses may vary between 0.024 and 85.0%. It is actually an important transmission vehicle of the bacterium (Aloccer et al., 2006).

Studies by Sakugawa et al. (2008) revealed that *Salmonella* spp. (71.7%), *Escherichia coli* (95%) and *Staphyloccocus aureus* (43.35%) were extant in samples on the retail market of João Pessoa PB Brazil. Although quality is underscored, several species of *Salmonella a*re present in broilers' meat sold in the market.

In Brazil, some more commonly found in birds are serovars *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Derby, *Salmonella* Heidelberg, *Salmonella* Senftenberg, *Salmonella* Agona and *Salmonella* Mbandaka. *Salmonella* Enteritidis and *Salmonella* Typhimurium are among the most prevalent in chickens

Table 1. Results of microbiological analyses and temperature, per week, at firm A. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (°C)
1	0	0	35.9
2	1	10	37.7
3	0	0	34.7
4	0	0	37.8
5	1	10	32.7
6	1	10	33.4
7	1	10	32.6
8	0	0	36
9	1	10	34.4
10	1	10	31.5
Total	**6**	**60**	**36 (Mean)**

Table 2. Results of microbiological analyses and temperature, per week, at firm B. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (ºC)
1	1	10	36.6
2	0	0	34.8
3	0	0	36
4	0	0	35
5	0	0	30
6	0	0	32.2
7	1	10	33.4
8	1	10	35.1
9	1	10	32.3
10	0	0	31.5
Total	**4**	**40**	**33.7 (Mean)**

Table 3. Results of microbiological analyses and temperature, per week, at firm C. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (°C)
1	1	10	33.8
2	0	0	35
3	1	10	35.8
4	1	10	34.1
5	0	0	31.3
6	0	0	32.2
7	1	10	35.6
8	1	10	36
9	0	0	34.1
10	0	0	31.1
Total	**5**	**50**	**35.5 (Mean)**

and have great importâmcia to public health (Aloccer et al., 2006; BACK, 2010).

From the mid-80's in Europe and after the beginning of the 90s in Brazil, *Salmonella* Enteritidis has become one of the most prevalent serotypes in cutting and laying birds. At the beginning of this new century, the *Salmonella* Enteritidis in Brazil has shown significant reduction. It is possible that the widespread use of killed

Table 4. Results of microbiological analyses and temperature, per week, at firm D. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (ºC)
1	0	0	33.3
2	0	0	30
3	0	0	36
4	0	0	32
5	0	0	32
6	0	0	36
7	0	0	32.2
8	0	0	33.7
9	0	0	34.9
10	0	0	35.3
Total	**0**	**0**	**33.2 (Mean)**

Table 5. Results of microbiological analyses and temperature, per week, at firm E. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (°C)
1	0	0	36.5
2	0	0	31.4
3	0	0	34.3
4	0	0	32
5	0	0	34
6	0	0	31.1
7	0	0	35
8	0	10	32.2
9	0	0	34.5
10	0	0	32.2
Total	**1**	**10**	**33.6 (Mean)**

Table 6. Results of microbiological analyses and temperature, per week, at firm F. Feira de Santana, BA, Brazil, 2014.

Week	Number of positive samples	Frequency (%)	Temperature of carcass (°C)
1	0	0	31
2	0	0	30.5
3	1	10	34
4	0	0	34
5	0	0	35
6	0	0	30.1
7	0	0	38.4
8	0	0	33.8
9	0	0	35.7
10	0	0	32
Total	**1**	**10**	**33.2 (Mean)**

vaccine in broiler breeders contributed to this fact. The predominant phage type 4 in *Salmonella* Enteritidis birds is considered one of the most pathogenic to humans, although there is variation in pathogenicity between different samples of this phage type. Although the transmission of *Salmonella* Enteritides has not been demonstrated in a classical way, there are investigations showing that of 10,000 eggs positive plot, one can isolate *Salmonella* Enteritidis internal contents of 2 to 3 eggs, which indicates that some chicks may be born infected. It

is very difficult to eradicate the *Salmonella* Enteritidis from an infected batch and the bird stands for eliminating the bacterial life. The biosecurity system and monitoring should be strict to prevent the infection establishment in the lot and to identify lots that eventually become positive, respectively (BACK, 2010).

Current analysis provides visibility on the occurrence of *Salmonella* sp*.* in poultry products (carcass of whole broilers) sold in Feira de Santana BA Brazil, or rather, precisely the products derived from illegal abattoirs with no official sanitary certificate on commercialization. This boils down to the fact that the origin, production methods and hygiene processes are unclear.

Tables 1, 2, 3, 4, 5, and 6 clearly demonstrate that temperatures of all the broilers' carcasses from all the firms did not meet the standards demanded by current legislation during the ten weeks in which these conditions were investigated. The above is of great concern since the carcasses with such temperature are prone to the growth of mesophyll micro-organisms (high rates of deteriorating and pathogenic micro-organisms) at a best growth temperature between 25 and 40°C. It should be emphasized that the best temperature for the growth of most pathogenic bacteria, among which may be mentioned *Salmonella* sp*.*, is approximately 37°C (Jay, 2005). Therefore, the consumer of such poultry meat is exposed to high biological risk.

Results of the microbiological analysis of the commercial firms A, B, C, E and F, respectively provided in Tables 1, 2, 3, 5 and 6, definitely co-relate them to the origin of the slaughtered animals, the possibility of cross-contamination during slaughter and meat processing and exposure to poor storage and conservation conditions.

Similar storage conditions were evidenced in all the commercial firms under analysis. All the meat products were exposed on tables without any temperature control and, therefore, subjected to oscillations in room temperature. No specific refrigeration or otherwise was detected in any commercial firm that would maintain the temperature of the meat products under control. Commercial firms must comply with Law 304 published in 1996 based on beef and pork, or rather, in the case of cattle and swine abattoirs they must deliver only cooled meat and offal for retail at a temperature up to 7°C; also, commercial firms must maintain such temperature (Brasil, 1996).

It is actually a highly relevant factor since meat products should not be left to deteriorate due to inadequate handling within the distribution chain, which may in fact, be evidenced during transport and unloading at the selling depot (Brasil, 1996). In the case of poultry meat, the technical regulation for technological inspection is provided by Law 210 (Brasil, 1996) which underscores two conservation methods, namely, the temperatures and the procedures that abattoirs and commercial firms should comply with.

Law 210 deals with the conservation methods by cooling, or rather, the process of refrigeration and maintenance of temperature between 0 and 4°C in the case of poultry meat (carcasses, cuts, offal and other derived products), with a tolerance of 1°C, measured within the interior of the products. It also deals with the freezing process and the maintenance of a temperature not higher than -12°C for poultry products (carcasses, cuts, offal and other derived products), with a tolerance of up to 2°C, measured within the interior of the products (Brasil, 1996).

The results from Firm D, reported in Table 4, reinforce a monitoring process. In spite of the adverse conditions mentioned above, there was a 0% frequency for *Salmonella* sp., although this fact may be co-related to the occurrence of the pathogen at the poultry origin and not to storage conditions.

When all the samples in all the commercial firms have been analyzed, it may be evidenced that 17 samples were positive for *Salmonella* sp. The contaminated samples amounted to 28% frequency for all the samples evaluated. Microbiological analyses revealed a higher number of positive samples in commercial firm A, with 35%, followed by C with 29%, B with 24%, E and F with 6% each and D without any contamination. Figure 1 shows the results.

Maldonado (2008) identified the percentage of bacteria in carcasses and offal of cooled broilers sold in fairs and in a municipal market in the western region of São Paulo SP Brazil, in May 2007, featuring a high index of *Salmonella.* During the period mentioned above, 75 samples were analyzed, of which 65% were contaminated with some species of bacteria and 35% by *Salmonella*.

Another investigation by Silva et al. (2006) showed that in a sample of 60 birds sold in commercial firms and in open fairs in the western region of São Paulo SP Brazil, approximately 40% of the poultry were positive to *Salmonella* spp.

Figure 2 demonstrates the percentages of positive samples detected during the 10 weeks, taking into account the ten weeks of repetition. Highest frequency occurred on weeks 1, 3, 7 and 9 within the interval proposed.

According to Silva et al. (2006), *Salmonella* spp. is redundant in wild poultry breeding when compared to poultry in breeding houses. This fact may be corroborated by research in granges in the northern region of the State of Paraná, Brazil and in a poultry abattoir in Jacarezinho, PR Brazil.

According to Silva (2014), Silva and Duarte (2006) and Silva et al. (2006), illegal poultry slaughter is a high concern for public health due to exposure to infectious and parasite agents transmitted to humans by animals in the intake of quality-lacking food and by environmental contamination. The unknown origin of animals and breeding methods place impediments and challenges on the monitoring process and exposes workers to zoonosis.

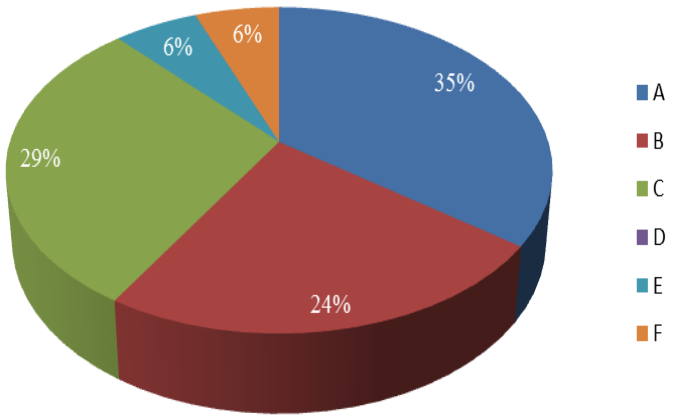

Figure 1. Frequency of total positive samples per commercial firm. Feira de Santana, BA, Brazil, 2014.

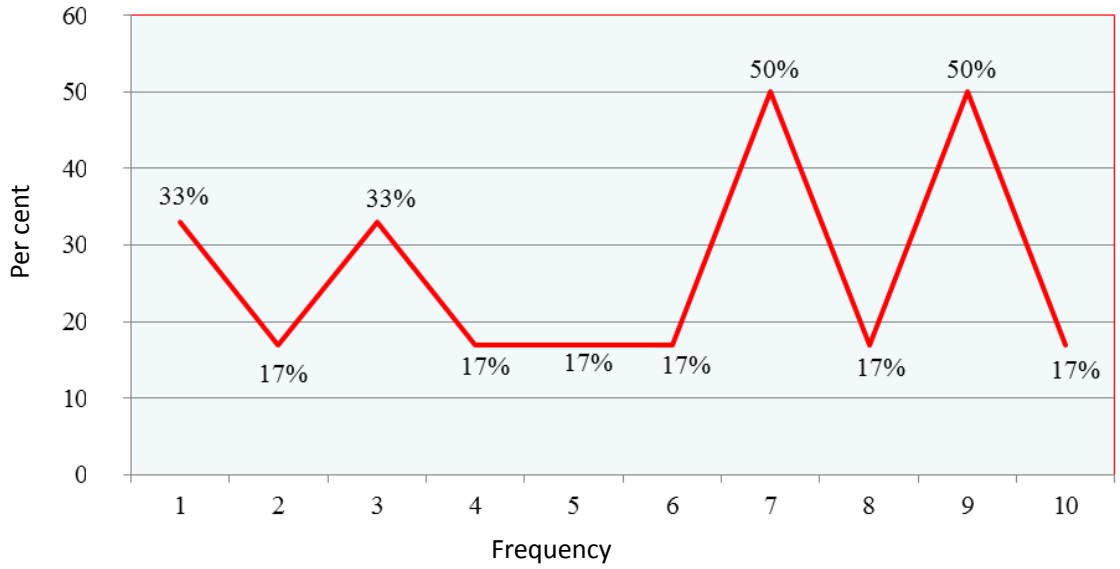

Figure 2. Frequency of positive samples throughout the period (weeks). Feira de Santana, BA, Brazil, 2014.

It should be underscored that illegal poultry slaughter is a crime against consumers, which must be hindered by all means by the health authorities. The dissemination of activities based on sanitary education by the conscience-awareness of consumers in all the social classes should be underscored.

Food in general, especially from animal origin, such as meat, eggs and milk, is the receiving end of many bacteria, resulting in Food-Borne Diseases (FBD). Rampling et al. (1989), Boer and Zee (1992), Giessen et al. (1992) and Maldonado (2008) reports that the inherent characteristics favor the presence and multiplication of bacteria, collaborated by the occurrence of FBDs with the intake of such food.

Salmonella is one of these microorganisms in products of animal origin, such as cattle, swine and poultry, which contribute heavily in the contamination of humans, with a subsequent issue for public health, even in developed countries which are also responsible for FBDs. According to Poppe (1994), Scuderi et al. (1996), Costa (1996) and Maldonado (2008), FBDs, especially those caused by *Salmonella* spp., currently represent one of the main concerns of health authorities due to the difficulty in controlling the production chain and in accessing the number of people affected by the pathogen.

In the case of meat control, the biosafety process should start during breeding where control should be drastically undertaken. According to Sakai and Chalermchaikit (1996), Ward and Threlfall (1997), Santos et al. (2000) and Maldonado (2008), *Salmonella* in poultry

breeding without aiming any type of industrialization is rife due to the absolute lack of control and quality. As has been remarked by Tiroly and Costa (2006), “in Brazil, the occurrence of *Salmonella* is relevant due to deficiencies in basic health conditions and to bad hygiene and sanitary conditions in most of the population, coupled to poor quality control of certain food industries and of small poultry abattoirs”.

To have better control of pathogens detected in samples from the commercial firms mentioned above, a monitoring process in the production chain should be established by research on the site of slaughtering process up to poultry breeding houses.

Conclusion

The results of this study indicate that many chicken products sold in Feira de Santana, Bahia , Brazil , are contaminated with different serotypes of *Salmonella*, especially *Salmonella* Typhimurium and *Salmonella* Enteritidis and should , therefore, be suitable for cooking and handling prevention of salmonellosis outbreaks.

Conflict of Interest

The authors have not declared any conflict of interests.

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