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Salmonella Serovars in Laying Hen Flocks and Commercial Table Eggs from a Region of São Paulo State, Brazil
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ABSTRACT

Salmonella spp. is the main originator of human foodborne diseases worldwide and is mainly transmitted by food containing eggs. In Brazil, as a result of the lack of studies and data collection very little is known about the prevalence of Salmonella spp. in laying hen flocks and commercial table eggs. Consequently the present study was elaborated and aimed at generating data about Salmonella spp. in part of the Brazilian egg production chain. Eight flocks of day-old chicks, eight flocks of adult laying hens (four vaccinated with bacterin against Salmonella Enteritidis and four unvaccinated) and commercial table eggs from four supermarkets were examined. Salmonella spp. was isolated in 50 % of the newly hatched chicks, 25 % of the adult flocks and 1.5 % of egg samples examined. S. enterica subsp. enterica 4,12:r:-, S. Mbandaka, S. enterica subsp. enterica 6,7:z10:-, S. Enteritidis and S. Havana were the serovars isolated in birds. In commercial table-eggs S. Mbandaka, S. enterica subsp. enterica 6,7:z10:- and S. Braenderup were isolated. These results show that Salmonella spp. is present in laying hen flocks and consequently in eggs destined for human consumption. Probably, some of the Salmonella serovars are being introduced in egg farms by vertical via.

INTRODUCTION

Salmonella spp. is a major zoonotic pathogen being the originator of several foodborne diseases outbreaks worldwide each year. These microorganisms may cause human suffering as well as economic losses to food production and to the food industry (Foley et al., 2008; IFAH, 2012). Poultry and eggs remain the major sources of Salmonella spp. in developed countries. Eggs and dairy products accounted for 42 % of the outbreaks caused by Salmonella spp. in Europe (EFSA, 2009). According to the Brazilian health surveillance service, from 1999 to 2009, 6,349 foodborne diseases outbreaks were notified, and eggs and foods containing egg were responsible for 15 % of them (COVEH, 2009).

There are over 2,600 Salmonella serovars identified but about 90 are responsible for human and animal salmonellosis (EFSA, 2009, CDC, 2011). In 2009, Enteritidis, Typhimurium, Newport, Javiana and Heidelberg were the five most frequently reported Salmonella serovars from human sources in the United States (CDC, 2011). Food of animal origin is the main source of Salmonella spp. for human beings (Pires et al., 2010). Over the last decades, there has been an increase in reported cases of human salmonellosis, making the public health authorities focus attention on controlling Salmonella spp. in livestock, poultry, and their products (Newell et al., 2010).

To take measures able to reduce human foodborne salmonellosis is essential. It is also important to quantify the burden of human illness and
also to identify the sources, route of infection and the main serovars involved (Pires et al., 2010). Considering
eggs and food containing eggs as the major sources of human foodborne salmonellosis, measures and control
programs to reduce Salmonella spp. and minimize the human exposure must be addressed to chicken farms
(Barrow, 2007; Gast, 2007). However, to elaborate a control program it is necessary to know the prevalence
and epidemiology of the main Salmonella serovars in chicken flocks.

Little is known about prevalence of Salmonella serovars in Brazil. A piece of work was done by Zancan
et al. (2000) who analysed meconium samples from transport cardboard boxes of chicks. According to
this study, Salmonella Heidelberg and Mbendaka were detected in broiler breeder chicks. Meanwhile,
Enteritidis, Mbendaka and Cerro were isolated in laying hen chicks. Moreover, Kanashiro et al. (2005)
isolated S. Enteritidis, Heidelberg, Kentucky, Infantis, Mbendaka, Typhimurium, Senftenberg and other
serovars in samples of faeces, meconium and eggs of breeders and broilers of several regions of Brazil from 1997
to 2004. S. Enteritidis was the most recovered serovar in the mentioned study.

Although few studies on the prevalence of Salmonella serovars have been carried out in Brazil
(Zancan et al., 2000; Kanashiro et al., 2005; Fernandes et al., 2006; Kottwitz et al., 2008), the information
about these microorganisms in humans, animals and food of animal origin are not updated and remain
underestimated. The present study was carried out in order to generate data which could support the
elaboration of new Salmonella spp. control programs for the poultry industry or to help in evaluating the
existing ones. This study aimed at surveying for Salmonella serovars in flocks of newly hatched chicks,
adult commercial laying hens and a representative amount of commercial table-eggs from supermarkets
of a region of São Paulo state, Brazil.

**MATERIAL AND METHODS**

**Samples and sampling**

The presence of Salmonella spp. was assessed in flocks of laying hens from eight farms (A, B, C, D, E, F,
G and H) and in commercial table-eggs commercialized by four supermarkets (I, J, K and L); all establishments
from the same region of São Paulo State, Brazil.

To investigate whether the chicks were getting farm
infected with Salmonella spp., samples of meconium from eight newly hatched chick flocks (one flock
from each farm) were collected from transport boxes following the methodology described by Zancan et al. (2000). Each flock had about 3,000 birds and were transported in cardboard boxes with 100 birds each.
In total, 240 boxes were examined using a large gauze
swab moistened in 1 % buffered peptone water (BPW) (Oxoid® CM0509). Swabs from five boxes were placed
into sterile glass with 225 mL of BPW and corresponded to one sample. Samples were taken to the laboratory
under refrigeration.

Salmonella spp. was also searched in faecal samples
of other eight flocks (four vaccinated with oil-emulsion bacterin against S. Enteritidis and four unvaccinated)
of adult laying hens (aged twenty-three to thirty-eight weeks). Each chicken house was divided into four
parts. Fresh samples of cecal faeces from each part were collected under the cages using sterile swabs
moistened in BPW, which were placed into sterile glass
and corresponded to one “pool” of samples. In total,
sixty-four pools of samples, taken at different times
from the eight flocks, were stored in sterile glasses with 225 mL of BPW and transported to the laboratory
under refrigeration.

A total of 1,700 eggs from four supermarkets (I,
J, K and L) of the same region were examined. Each egg sample consisted of five eggs (shell and contents);
a total of 340 samples were examined. Eggs were bought in packages of a dozen at different moments
(1, 2, 3 and 4).

**Sample processing**

**Bacteriological exams**

Glasses containing the samples in BPW were left at
room temperature for one hour, followed by overnight incubation at 37 °C. Then, 2.0 ml of the BPW was
transferred to tube containing 20 ml of selenite (Oxoid, CM 395) plus Novobiocin at concentration
of 40 mg/L (Merial, 8041706) (SN) and Tetrationate (Biolife, 402125) plus Novobiocin (40 mg/L) broths and
0.2 ml to 20 mL of Rappaport – Vassiliadis (RV) (Oxoid,
CM 669) broth. Broths were incubated overnight at 37 °C (Davies & Wray, 1994). After removed from the
package, “pools” of 5 eggs were placed into sterile
glass jars, broken and homogenised with a sterile
wooden stick and then incubated overnight at 37 °C.
After that, amounts of 2.0, 2.0 and 0.2 mL of this
content were inoculated into tubes containing 20 mL
of SN, TN and RV broths, respectively, and were then incubated overnight at 37 °C. Subsequently, broths
from all samples were plated onto the following
media: Brilliant Green agar (Oxoid, CM 0263), Mac
RESULTS AND DISCUSSION

Salmonella spp. was isolated from transport boxes used to deliver newly hatched chick flocks in four different farms. S. Mbandaka was isolated in flocks of farms B, C, F and G; Salmonella enterica subspecies. enterica 4,12: r: - was recovered in flock from farm C and Salmonella enterica subsp. 6,7 enterica: Z10: - on farm G (Table 1).

Table 1 – Salmonella serovars isolated in meconium samples from transport boxes of newly hatched chicks.

<table>
<thead>
<tr>
<th>Flocks of newly hatched chicks from FARMS</th>
<th>serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Salmonella Mbandaka</td>
</tr>
<tr>
<td>C</td>
<td>Salmonella Mbandaka</td>
</tr>
<tr>
<td></td>
<td>S. enterica subsp. enterica 4, 12: r: -</td>
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<tr>
<td>D</td>
<td>-</td>
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<td>E</td>
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<tr>
<td>F</td>
<td>Salmonella Mbandaka</td>
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<tr>
<td>G</td>
<td>Salmonella Mbandaka</td>
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<tr>
<td></td>
<td>S. enterica subsp. enterica 6, 7: z10: -</td>
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<td>H</td>
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</tbody>
</table>

* = Absence of Salmonella spp.

It is known that when poultry become infected with Salmonella spp. at the beginning of life is more difficult to control because newly hatched chicks are very susceptible, and they may shed this bacterium in high amount and for long periods (Barrow et al., 1988). Although many efforts have been done in breeder farms and hatcheries, Salmonella spp. still can be found in newly hatched chicks inside the hatchery or at the moment of arrival on the farm (Cox et al., 1991; Cox et al., 2000; Snow et al., 2008). Reports have shown that the detection of Salmonella spp. may vary from 11% to 77%, being S. Enteritidis the most common one (Cox et al., 1990; Zancan et al., 2000; Cox et al., 1991; Gama et al., 2003; Rocha et al., 2003). The work done by Zancan et al. (2000) that reported positive results in day-old bread birds, suggesting that also grand-parent flocks would be infected by Salmonella serovars calls our attention. In the present study, four of the eight farms received chicks infected with four Salmonella spp. showing that the control program in breeder farms and hatcheries should be improved.

S. Havana was isolated in cecal faeces from flocks of farms C and G, meanwhile S. Enteritidis was detected only in flock from farm G (Table 2). Faeces are the potential vehicle in transmitting Salmonella spp. to birds and are also an important source of contamination for eggs (Gast & Beard, 1992, Gast, 2003). Infected birds can shed intermittently about 10⁸ cells of Salmonella spp. per gram of feces (Bryan & Doyle, 1995). In this work, 25 % of the adult flocks were shedding Salmonella spp. Similar results were reported by Salles et al. (2008), who detected Salmonella spp. in 25% of the inspected flocks and by Kottwitz et al. (2008) that detected these microorganisms in 23% of examined flocks. These findings are higher than those reported by Castellan et al. (2004) who found Salmonella spp. in 10.5% of laying hen flocks in The United States and also higher than those presented by Snow et al. (2007), who isolated S. Enteritidis, Typhimurium, Mbandaka, Havana and other serovars in 11.7% of commercial flocks in the United Kingdom. It is known that factors such as weather, geographic location of the farm, farming conditions and management of the flock can influence the prevalence of Salmonella spp. in poultry (Khakharia et al., 1997; Angulo & Swerdlow, 1999).

Table 2 – Salmonella serovars isolated in cecal faeces of layers in flocks vaccinated and unvaccinated against Salmonella Enteritidis.

<table>
<thead>
<tr>
<th>Flocks of adult laying hens from FARMS</th>
<th>Serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Salmonella Havana</td>
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<tr>
<td>D</td>
<td>-</td>
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<td>E</td>
<td>-</td>
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<td>F</td>
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<td>G</td>
<td>Salmonella Enteritidis</td>
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<tr>
<td></td>
<td>Salmonella Havana</td>
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</table>

* = Absence of Salmonella spp.

There are few reports mentioning the isolation of S. Havana in faeces of commercial layers (Hussein et al., 2010), even though it was isolated from faeces of two flocks examined in the present study. S. Havana was isolated from poultry food and supplements (Berchieri Junior et a., 1989; Okamura et al., 2001, Davies & Breslin, 2004), and this could be the source of contamination for the chickens in the present...
study. Hygiene and biosecurity in addition to the administration of an appropriate form of oil emulsion bacterin against S. Enteritidis in flocks of farms A, B, C and D could be some of the factors that contributed to the absence of this serovar. In a study conducted in Japan, S. Enteritidis was isolated in several samples of faeces, eggs and the environment from farms containing vaccinated and unvaccinated flocks and the percentage of isolation was lower in flocks where the oil-emulsion bacterin against S. Enteritidis was administered (Toyota-Hanatani et al., 2009). Freitas Neto et al. (2008) also reported reductions in shedding of S. Enteritidis and egg contamination in laying hens experimentally vaccinated with bacterin. In the present study, the isolation of S. Havana in faeces of birds from farm C, vaccinated against S. Enteritidis, demonstrates a lack of cross protection between these serovars.

In this study, three of the four supermarkets had at least one egg sample positive for Salmonella spp. Among 340 egg samples examined, five (1.47%) were contaminated. It was isolated S. Mbandaka and S. enterica subspecies enterica 6,7: z10:- in the supermarkets I and J, and S. Braenderup in the supermarket L. No Salmonella spp. was recovered in egg samples from supermarket K (Table 3).

<table>
<thead>
<tr>
<th>Sample collection</th>
<th>SUPERMARKETS</th>
<th>Serovar</th>
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<td>I</td>
<td>J</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>+</td>
<td>+</td>
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<tr>
<td>4</td>
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</tbody>
</table>

- = Absence of Salmonella spp.

Lower percentages of Salmonella spp. in eggs have been described. For instance, Ebel & Schosser (2000) reported 0.005% of contamination in eggs examined in The United States. In European countries, the percentage of Salmonella spp. in eggs was about 0.8% (EFSA, 2010). On the other hand, percentages of Salmonella spp. similar to what was found in the present study have also been reported by Humphrey (1994) and Okamura et al. (2001). According to Humphrey (1994) and Gast (2003) there are many factors that can contribute to variations in the percentages of Salmonella spp. in eggs. Factors such as the sample size, season of sampling, bacteriologic method adopted for examination and the bacterial load present in eggs would be some of them.

During the 2000s, S. Enteritidis was one of the most frequent serovars associated with worldwide outbreaks of diseases transmitted by food containing poultry or eggs (EFSA, 2007; CDC, 2011; Fernandes et al., 2006; O’Brien, 2012). Although, in lower numbers, reports of human food-borne salmonellosis caused by S. Havana, S. Mbandaka, S. Braenderup (other serovars isolated in this study) have been described (Taunay et al. 1996; Tavechio et al. 1996; Fernandes et al., 2006; CDC, 2011). Therefore, their presence in table eggs and laying hen flocks represent risk for public health.

The results of this study demonstrate that, in addition to S. Enteritidis, other serovars are present in commercial laying hens and table eggs. As could be noticed in our results, some of these serovars are possibly being introduced in the egg production chain by infected newly hatched chicks when they arrive at the farm. In order reduce the prevalence of Salmonella serovars in the laying hen flocks and eggs, the control programs should be improved not only in egg farms but also in hatcheries and breeder farms.

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