

Research Paper

Commercially laid eggs vs. discarded hatching eggs: contamination by *Salmonella* spp

Luciana B.M. Kottwitz¹, Joice Aparecida Leão², Alberto Back², Dalia dos P. Rodrigues³, Marciane Magnani¹, Tereza C.R.M. de Oliveira¹

¹Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Agrárias, Universidade Estadual de Londrina, PR, Brazil.

²Centro de Diagnóstico Veterinário Brasil Sul Ltda, Cascavel, PR, Brazil.

³Laboratório de Enterobactérias, Departamento de Bacteriologia da Fundação Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil.

Submitted: February 11, 2011; Approved: September 10, 2012.

Abstract

Salmonella enterica is frequently associated with outbreaks of human salmonellosis, and products of avian origin, such as eggs and chicken meat, are the main vehicles of its transmission. The present study describes the occurrence of different serovars of *Salmonella enterica* and phagotypes of *S. enterica* serovar Enteritidis in eggs destined for human consumption. Four thousand eggs obtained from commercial egg laying farms and one thousand discarded hatching eggs from broiler farms, which were acquired at farmers' markets and informal shops, were analyzed. *Salmonella* spp. was isolated from 52.0% of the discarded hatching eggs, in which the predominant serovar was Enteritidis (84.6%), and the predominant *Salmonella* Enteritidis phagotype (PT) was PT7 (26.9%). *Salmonella* spp. was not isolated from eggs obtained from commercial egg laying farms. The antimicrobial resistance profile showed that 23.1% (n = 6) of the SE strains were resistant to nalidixic acid. The results suggest that the consumption of discarded hatching eggs represents an important source of *Salmonella* transmission to humans.

Key words: discarded eggs, *Salmonella* Enteritidis, antimicrobial resistance, salmonellosis.

Introduction

Salmonellosis is considered an important zoonosis and one of the most prevalent food-borne diseases. In Brazil, *Salmonella enterica* serovar Enteritidis (SE) is frequently associated with food-borne infections that generally involve the consumption of poultry meat and eggs as well as their derivatives (Kottwitz *et al.*, 2010). According to the Central Laboratory of Parana State (LACEN), egg-based foods were involved in 45.0% of the salmonellosis outbreaks that occurred in the state in the period from 1999 to 2008. *S. enteritidis* predominated in samples isolated from patients (87.8%) and foods (80.6%).

S. enteritidis has adapted to the breeding of birds in commercial poultry farming; this pathogen probably occupies the niche formerly occupied by the serovars *S.*

gallinarum and *S. pullorum*, which were the most important sanitary problems before the adoption of control and prevention measures. SE can infect birds without resulting in clinical manifestations, which makes its detection difficult and facilitates its spread (Vaz *et al.*, 2010). Moreover, SE persists in the poultry industry due to its capacity for vertical and horizontal transmission (Cardoso and Tessari, 2008).

Commercial eggs are from small laying hens reared in cages. Hatching eggs are destined for the production of 1-day-old chicks and are produced by broiler breeders in nests with wood shavings. In Brazil, discarded hatching eggs are donated or sold to food-producing establishments; they are even sold at farmers' markets for human consumption. Although studies have found contamination by SE in less than 1% of commercial eggs (Gast and Holt, 2000), this

serovar can multiply in the yolk if eggs are exposed to temperatures close to 25 °C (Grijpspeerdts and Herman, 2003).

Brazil is the seventh leading world producer of commercial eggs, and production in 2009 was approximately 580 million dozen eggs, of which 224 million were produced in the state of Parana (IBGE, 2010). The objective of the present study was to determine the occurrence of *Salmonella* spp. in commercial eggs and discarded hatching eggs destined for human consumption, to identify the most common serovars and phagotypes, and to evaluate the antimicrobial resistance profile of the strains isolated.

Materials and Methods

Eggs

A total of 5000 eggs collected in northwest region of Paraná State, Brazil were analyzed, between August 2003 and December 2006. Three thousand eggs were obtained from the commercial laying hen houses of poultry farms, registered with the Federal Inspection Service of the Ministry of Agriculture, Ranching and Supply. One thousand commercial laying hen eggs were acquired from small rural producers, and 1000 discarded hatching eggs from broiler hens were obtained in farmers' markets and informal shops.

Isolation of *Salmonella* spp.

The isolation of *Salmonella* spp. from the eggs was performed as described by the AOAC (1995). The eggs samples were divided into 250 samples comprising 20 eggs each. Each sample was placed in sterile plastic bags and added 200 mL of 1% buffered peptone water (Merk, Germany). After washing by hand the surface of the eggs, the rinse was placed in sterile plastic bag and incubated for 20 h at 36 °C. For selective enrichment, 1 mL of pre-enrichment was added to 10 mL of tetrathionate broth (Merk, Germany) and 0.1 mL to Rappaport-Vassiliadis broth (Merk, Germany). After incubation for 24 h at 41 °C, the enrichments were plated onto xylose-lysine deoxycholate agar (XLD) (Merk, Alemanha) and brilliant green agar with 4% novobiocin (VB) (Merk, Germany), followed by incubation for 18 h at 36 °C. After the washing of surface, the eggs of each sample were broken in a sterilized container for the aseptic separating of the yolks. The yolks were pooled and an aliquot of 25 g of the mixture was added in 225 mL of 1% buffered peptone water (Merck, Germany) and incubated at 36 °C for 16-20 hours. Selective enrichment and differential plating were performed using the same procedures described for the eggshell rinses.

The colonies that were lactose negative and H₂S producers were submitted to biochemical screening for the following biochemical tests: growth in Simmons citrate (Biobras, Brazil), motility and production of indole in sulfur indole motility medium (Biobras, Brazil), production of H₂S and gas in triple sugar iron agar (Biobras, Brazil),

decarboxylation of lysine (Difco, USA) and hydrolysis of urea (Difco, USA).

Serotyping and phagotyping were performed by the Laboratory of Enterobacteria, Department of Bacteriology of the Foundation Instituto Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil.

Evaluation of the antimicrobial resistance profile

Susceptibility to antimicrobials was evaluated by the disk-diffusion technique of Bauer *et al.* (1966) based on the recommendations of the Clinical and Laboratory Standards Institute (2006). The following antimicrobial agents were tested (OXOID[®]): ampicillin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg) and trimethoprim-sulfamethoxazole (25 µg).

Statistical analysis

Analysis of variance (ANOVA) of the results was carried out with Statistica 5.0.

Results and Discussion

In the 200 egg samples from commercial laying hen houses, *Salmonella* spp. was isolated neither from the culture of the pooled yolks nor from the eggshells rinses. In contrast, in the 50 samples of discarded hatching eggs, *Salmonella* spp. was detected in the pooled yolks (n = 3) and in the rinses of eggshells (n = 23).

Contamination can occur from infection of the ovary and oviduct of the hens (Thiagarajan *et al.*, 1994, Myamoto *et al.*, 1997), but it usually occurs through the fecal elimination of bacteria, which later come into contact with the egg (Gast and Beard, 1993). The risk of *Salmonella* transmission is greater when the contamination occurs by the deposition of the bacteria on the eggshell with later penetration into the egg, or even when the bacteria remain on the shell providing cross-contamination (Téo and Oliveira, 2005). The results presented here suggest that commercially laid eggs that had been inspected and previously cleaned present a lower risk to public health (Jones *et al.*, 1995).

Discarded hatching eggs are produced in nests with a bed of wood shavings and are not incubated when they have shell defects, cracks, dirt or thin shells. The condition of these eggs facilitates the passage of *Salmonella* on the surface to internal egg structures, increasing the risk of contamination (Oliveira and Silva, 2000).

According to the Instrução Normativa No. 78/2003 (Brasil, 2003), at poultry farms where breeder hens are monitored and at which positivity for *S. enteritidis* and *S. typhimurium* has been confirmed, a certificate of "controlled establishment" is only given after treatment of the flock and subsequent negative laboratory results. In addition, during the treatment of the birds, the incubation of their eggs is suspended. Therefore, it is essential that dis-

carded eggs from poultry farms positive for SE be identified and correctly handled because such eggs could be inadvertently destined for human consumption, thus constituting an important source of salmonellosis.

Another aspect that should be considered in comparing commercial eggs and discarded hatching eggs is the selection of specific lines, based on zootechnical characteristics of interest. Kingsley and Baëumler (2000) reported that the ability of *Salmonella* serovars to cause infections, or even to determine a carrier state, was correlated with the genetic resistance of the poultry line.

Studies have been conducted to evaluate the capacity of invasion, colonization and persistence of SE in different lines of birds. Some researchers have noted that rapid-growth birds, such as ROSS, which is the predominant line among the broilers used in the poultry industry in Brazil, are more susceptible of SE, resulting in a greater possibility of spreading this pathogen (Kingsley and Baëumler, 2000, Kramer *et al.*, 2001, Berthelot-Hérault *et al.*, 2003, Wigley, 2004, Calenge *et al.*, 2010).

In the present study, the results of the isolation of *Salmonella* from discarded hatching eggs showed a significant difference ($p \leq 0.05$) between the isolation of *Salmonella* spp. from the shell rinses ($n = 23$; 46.0%) and from the pool of yolks ($n = 3$; 6.0%). Similar findings have been reported in studies involving the isolation of *Salmonella* from the eggs of commercial laying hens. Fehlhaber and Janetschke (1995) analyzed 5.339 eggs from birds infected with *Salmonella* spp. and were able to isolate the microorganism in 4.19% of the samples from the shells, while the pathogen was detected in only 0.20% of samples taken from the inside the eggs. Similarly, Oliveira and Silva (2000) examined 124 egg samples and isolated *Salmonella* in 9.6% of the eggshells samples but only in 3.20% of the yolk samples.

The serotyping and phagotyping data from the isolates of *Salmonella* spp. found in the discarded hatching eggs are presented in Table 1. The predominant serovar was

Enteritidis, which was present in 84.60% ($n = 22$) of the samples. SE emerged as a critical poultry problem and public health concern in Brazil beginning in 1993. Epidemiological studies suggest its entrance in Brazil most likely occurred at the end of the 1980s via the importation of contaminated genetic material from a broiler poultry farm. As a result, the growth of Brazilian poultry farming in the 1990s created conditions that were favorable for the maintenance and proliferation of SE in poultry flocks (Silva and Duarte, 2002).

The phagotyping of the SE strains detected three phagotypes (PT), namely PT1, PT7 and PT9. Among the 14 typeable strains, 7 (50.0%) were PT7 (Table 1). Although, in various countries, infection by SE is related to the consumption of contaminated products of avian origin, the prevalent PTs are not the same (Cox, 1995). According to Laconcha *et al.* (1998), PT1 and PT7 can be derived from PT4 by conversion. In the state of Parana, in the period from 1999 to 2003, PT4 predominated as the strain responsible for outbreaks of salmonellosis. However, in the period from 2003 to 2006, there was a change in prevalence; PT9, for which data are still scarce (Kottwitz *et al.*, 2010), is currently the predominant strain.

Analysis of the antimicrobial resistance profile of the samples revealed that of the 26 strains of SE isolated, 6 (23.10%) showed resistance to nalidixic acid. The others, which included strains of other serovars, were found to be susceptible to all the antimicrobials tested (Table 1). Unlike the other serovars of *S. enterica*, SE was still sensitive to the majority of the antimicrobials until recently (Molbak *et al.*, 2002). However, with the spread of SE in poultry farming, antimicrobials have been utilized more intensively to mask the infections by this pathogen (Silva and Duarte, 2002). As a consequence, an increase in SE strains resistant to antimicrobials, especially quinolones, has been observed (Vaz *et al.*, 2010).

Since 2009, according to data from the São Camilo Laboratory, Maringá, Paraná State, Brazil, in the state of

Table 1 - *Salmonella* serovars and SE phagotypes according to the method of isolation and antimicrobial resistance profile.

Serovar / phagotype (PT)	Isolation method		Antimicrobial resistance profile	
	Shell rinse N° (%)	“Pool” yolk N° (%)	Resistant to nalidixic acid N° (%)	Susceptible** N° (%)
SE UT*	7 (26.9)	1 (3.8)	2 (7.8)	6 (23.2)
SE PT7	7 (26.9)	-	1 (3.8)	6 (23.2)
SE PT1	4 (15.4)	-	1 (3.8)	3 (11.2)
SE PT9	1 (3.8)	2 (7.8)	2 (7.8)	1 (3.8)
<i>S. Heidelberg</i>	2 (7.8)	-	-	2 (7.8)
<i>S. Mbandaka</i>	1 (3.8)	-	-	1 (3.8)
<i>S. Anatum</i>	1 (3.8)	-	-	1 (3.8)
Total	23 (88.4)	3 (11.6)	6 (23.2)	20 (76.8)

*UT: Not typeable; ** Susceptible to all antimicrobial agents tested: ampicillin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg) and trimethoprim-sulfamethoxazole (25 µg).

Parana, there has been a significant reduction in the isolation of Enteritidis from samples of avian origin, while serovars such as *S. Minnesota* and *S. Mbandaka* have been frequently detected. Nonetheless, the destination of discarded hatching eggs needs to be discussed.

Just as SE emerged in poultry farming when strategies were adopted to eradicate other serovars, it is possible that current measures aimed at its elimination from broiler hen farms will favor its substitution. Therefore, the presence of SE in discarded eggs observed in this study suggests that this foodstuff poses a significant public health concern, regardless of the serovar that predominates in poultry flocks.

The spread of *Salmonella* may be controlled by appropriate breeding conditions and decontamination of eggshells. The absence of *Salmonella* spp. in the commercial laying hen eggs analyzed in the present study, alert to the type of the eggs that really represents risk to the public health. The isolation of *Salmonella* in 52% of the discarded hatching eggs analyzed, the prevalence of the serovar Enteritidis and the identification of SE PT9, which has been implicated in human outbreaks of salmonellosis, suggest that discarded hatching eggs are an important vehicle of *Salmonella* spp. transmission when destined for human consumption.

Acknowledgments

The authors thank FIOCRUZ for the *Salmonella* serotyping and phagotyping and the National Council for Scientific and Technological Development (CNPq) for the doctorate scholarships awarded to M.M.

References

- AOAC. Association of Official Analytical Chemists (1995) Official Methods of Analysis, 6.ed., Washington, DC.
- Bauer AW, Kirby MMW, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45:493-496.
- Berthelot-Hérault F, Mompert F, Zygmunt MS, Dubray G, Duchet-Suchaux M (2003) Antibody responses in the serum and gut of chickens lines differing in cecal carriage of *Salmonella enteritidis*. *Vet Immunol Immunopathol* 96:43-52.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento - MAPA. Secretaria de Defesa Agropecuária. Instrução Normativa nº 78, de 03 de Novembro de 2003. Diário Oficial da União, Brasília, seção I, p. 3, 05 nov. 2003.
- Calenge F, Kaiser P, Vignal A, Beaumont C (2010) Genetic control of resistance to salmonellosis and to *Salmonella* carrier-state in fowl: a review. *Genetics Selec Evol* 42:11.
- Cardoso ALSP, Tessari ENC (2008) *Salmonella* na segurança dos alimentos. *Biológico* 1:11-13.
- Clinical and Laboratory Standards Institute (2006). Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S16. CLSI, Wayne.
- Cox JM (1995) *Salmonella enteritidis*: virulence factors and invasive infection in poultry. *Trends Food Sci Technol* 6:407-410.
- Fehlhaber K, Janetschke P (1995) Higiene Veterinária de los Alimentos. Acribia, Zaragoza.
- Gast RK, Beard CW (1993) Research to understand and control *Salmonella enteritidis* in chickens and eggs. *Poultry Sci* 72:1157-1163.
- Gast RK, Holt PS (2000) Influence of the level and location of contamination on the multiplication of *Salmonella enteritidis* at different storage temperatures in experimentally inoculated eggs. *Poultry Sci* 79:559-563.
- Grijnspeerdt K, Herman L (2003) Inactivation of *Salmonella enteritidis* during boiling of eggs. *Int J Food Microbiol* 82:13-24.
- IBGE. Instituto Brasileiro de Geografia e Estatística. Estatística da Produção Pecuária. Indicadores IBGE. Available at www.ibge.gov.br Accessed November 22, 2010.
- Jones FT, Rives DV, Carey JB (1995) *Salmonella* contamination in commercial eggs and eggs production facility. *Poultry Sci* 74:753-757.
- Kingsley RA, Baëumler AJ (2000) Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. *Mol Microbiol* 36:1006-1014.
- Kottwitz LBM, Oliveira TCRM, Alcocer I, FARAH SMSS, Abrahão WSM, Rodrigues DP (2010) Avaliação epidemiológica de surtos de salmonelose ocorridos no período de 1999 a 2008 no Estado do Paraná, Brasil. *Acta Sci Health Sci* 32:9-15.
- Kramer J, Visscher AH, Wagenaar JA, Boonstra-Blom AG, Jeurissen SHM (2001) Characterization of the innate and adaptive to *Salmonella enteritidis* PT1 infection in four broiler lines. *Vet Immunol Immunopathol* 79:219-233.
- Laconcha I, López-Molina N, Rementeria A, Audicana A, Perales I, Garaizar J (1998) Phage typing combined with pulsed-field gel electrophoresis and random amplified polymorphic DNA increases discrimination in the epidemiological analysis of *Salmonella enteritidis* strains. *Int J Food Microbiol* 40:27-34.
- Miyamoto T, Baba E, Tanaka T, Sasai K, Fukata T, Arakawa A (1997) *Salmonella enteritidis* contamination of eggs from hens inoculated by vaginal, cloacal, and intravenous routes. *Avian Dis* 41:296-303.
- Molbak K, Gerner-Smidt P, Wegener HC (2002) Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. *Emerg Infect Dis* 8:5.
- Oliveira DD, Silva EN (2000) *Salmonella* em ovos comerciais: ocorrência, condições de armazenamento e desinfecção da casca. *Arq Bras Med Vet Zootec* 52:655-661.
- Silva EM, Duarte A (2002) *Salmonella* Enteritidis em aves: retrospectiva no Brasil. *Ver Bras Ciên Avicol* 4:85-100.
- Téo CRPA, Oliveira TCRM (2005) *Salmonella* spp.: The eggs as vehicle of transmission and the implications of resistance for public health. *Semina: Ciências Agrárias* 26:195-210.
- Thiagarajan D, Saeed AM, Asem EK (1994) Mechanism of transovarian transmission of *Salmonella enteritidis* in laying hens. *Poultry Sci* 73:119-127.
- Vaz CSL, Streck AF, Michael GB, Marks FS, Rodrigues DP, Dos Reis EMF, Cardoso MRI, Canal CW (2010) Antimicrobial resistance and subtyping of *Salmonella enterica* subspecies *enterica* serovar Enteritidis isolated from human outbreaks and poultry in southern Brazil *Poultry Sci* 89:1530-1536.
- Wigley P (2004) Genetic resistance to *Salmonella* infection in domestic animal. *Res Vet Sci* 76:165-169.