Comp Clin Pathol
DOI 10.1007/s00580-011-1238-z

ORIGINAL ARTICLE

Study on *Salmonella* contamination of traditionally produced edible poultry eggs

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Received: 18 November 2010 / Accepted: 5 April 2011

Abstract One of the important issues of food hygiene is *Salmonella* contamination of eggs that may cause food-borne infection and disease in humans. The aim of this study was to investigate *Salmonella* contamination of traditionally produced poultry eggs in Tehran. Contamination of eggs to other *Enterobacteriaceae* was also investigated. For this purpose, 200 eggs including 131 egg samples from chickens and 69 from quails, geese, and ducks (23 samples from each species) were investigated. After conventional isolation procedures, *Salmonella* contamination was detected in five chicken eggs. Serological tests revealed that all of the isolated genera belonged to D serogroup and multiplex PCR showed that all strains carried *spv* and *sefA* genes and also random sequence (specific for the genus *Salmonella*); therefore, all strains confirmed as *Salmonella* Enteritidis. Antimicrobial susceptibility test revealed sensitivity of all *Salmonella* isolates to florfenicol, ceftriaxone, trimethoprim–sulfamethoxazole, chloramfenicol, and oxytetracycline. In addition, except the quail eggs, 29 enteric bacteria were isolated from eggs including 21 *Enterobacter* spp., 4 *Klebsiella* spp., 3 *Escherichia coli*, and 1 *Proteus* spp. This study indicated that traditionally produced poultry eggs were highly contaminated by *Enterobacteriaceae*. Moreover, the chicken eggs were contaminated by *Salmonella* Enteritidis; therefore, non-commercial chicken eggs can be considered as an important threat for public health.

Keywords Poultry eggs · *Salmonella* · Multiplex PCR · Antimicrobial susceptibility · *Enterobacteriaceae*

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Introduction

The numerous motile serovars of *Salmonella* are often referred to as paratyphoid (PT) *salmonella*. This organism can infect a wide variety of hosts including invertebrate and vertebrate wildlife, domestic animals, and humans to yield either asymptomatic intestinal carriage or clinical disease. Since the first report of avian salmonellosis in 1895 due to outbreak of infectious enteritis in pigeons, PT infections have long been known to cause significant disease losses in young poultry. More recently, PT *salmonella* have been additionally identified as important agent of food-borne disease in humans. Although *Salmonella* spp. are generally inactivated during standard cooking practices, improper handling and preparation of contaminated poultry eggs and meat continue to contribute to human salmonellosis. Raw meat and poultry products are recognized as the primary source for transmitting *Salmonella* species to humans, and 40% of the clinical cases attributed to the consumption of egg and poultry products (Li et al. 2007). Advances in poultry production practices, changes in consumer lifestyles and preferences, and increase in nutritional awareness have all elevated poultry products importance as the leading source of animal proteins worldwide. The tendency of societies to consume natural and organic food products has been resulted in selling of traditionally produced food stuffs

Published online: 20 April 2011

in the cities. Non-commercial poultry eggs are produced in rural areas and are also available in the cities for human consumption. Controlling PT infections in poultry flocks has thus become an important objective from both the economic and public health perspectives (Dyda et al. 2009; Madadgar et al. 2009; Gast 2008).

The aim of this study was to estimate the prevalence of *Salmonella* contamination in traditionally produced poultry eggs (chickens, quails, ducks, and goose) in Tehran. The serovars and antibiotic susceptibility of the isolated strains were determined and contamination by other enteric bacteria was also assessed.

Materials and methods

Poultry eggs

In this study, 131 samples were taken from chicken eggs, and 69 from duck, goose, and quail eggs (23 from each species) which were collected from different parts of Tehran.

Sample preparation and bacterial isolation

Samples were taken from the surface of the egg shells and its contents separately. First, samples were collected from the surface of the shells using wet sterilized swabs and then cultured into two separate primary enrichment media including lactose broth and tripticasein soy broth. Then, the eggs were brushed, soaked in 70% ethanol solution for 10 min, and cleansed. The contents were taken by breaking the shells and mixed thoroughly. In the next stage, approximately 25 g of each sample was weighed, added to 225 ml of lactose broth, and was shaken for 25–30 times. If the sample was less than 25 g, the whole egg contents were used for enrichment. One gram of each sample was also cultured into tripticasein soy broth. After overnight incubation at 37°C, 1 ml of the previously inoculated lactose broth medium was transferred into selenite cystine as an enrichment media.

After 12–18 h of incubation at 37°C, a loopful was taken from tripticasein soy broth and selenite cystine and streaked on xylose lysine deoxycholate (XLD) and MacConkey agar and incubated overnight at 37°C. Finally, the suspected colonies were checked using differential media including TSI, urea, SIM, citrate, MR-VP, and LIA (lysine iron agar). Any types of bacterial colonies which were grown on MacConkey or XLD agar were also identified by means of conventional biochemical tests (Downes and Ito 2004; Holt 1994; Gast 2008).

Serogrouping of *Salmonella* isolates

Serogrouping was performed by conventional serotyping method using commercial antisera (Difco, BD, Detroit, MI, USA) and comparison with Kauffmann-White schema.

Multiplex PCR test for *Salmonella* Enteritidis

PCR test were carried out according to Pan and Liu (2002) method. This test was performed in 25 μl reaction: PCR buffer included 50 mM KCL and 10 mM Tris–HCl (pH=8). Amounts of each primer, *Taq* DNA polymerase, DNA template, dNTPs, and $MgCl_2$ were 0.5 μM, 1 unit, 5 μl, 250 μM, and 1.5 mM, respectively. PCR products were electrophoresed on 1.2% agarose gels at 110 V for 60 min and stained with ethidium bromide (0.5 μg/ml). Primer sequences and target genes of multiplex PCR are shown in Table 1.

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed based on standard disk diffusion method (Kerby-Bauer) and analyzed according to CLSI recommendations (2005). Antibiotics used for this process were nalidixic acid, tiamulin, erythromycin, colistin, penicillin, streptomycin, neomycin, lincomycin, kanamycin, cefalotin, flumequine, gentamicin, ciprofloxacin, ampicillin, tetracycline, furazolidone, oxytetracycline, chloramfenicol, trimethoprim–sulfamethoxazole, ceftriaxone, and florfenicol.

Results

Salmonella contamination of eggs

Salmonella strains were isolated from five chicken eggs of which four strains were isolated from the shells and one from the egg contents. Therefore, *Salmonella* contamination

Table 1 Primers sequences used for determination of *Salmonella* Enteritidis isolates by multiplex PCR (Pan and Liu 2002)

Primer	Target gene	Primer Sequences(5′-3′)	Product (bp)
ST11	Random	GCCAACCATTGCTAAATTGGCGCA	429
ST14	Sequence	GGTAGAAATTCCCAGCGGGTACTGG	
S1	*spv*	GCCGTACACGAGCTTATAGA	250
S4		ACCTACAGGGGCACAATAAC	
SEFA 2	*sef A*	GCAGCGGTTACTATTGCAGC	310
SEFA 4		TGTGACAGGGACATTTAGCG	

Table 2 *Enterobacteriaceae* contamination of poultry eggs

Genus	Chicken		Goose		Duck	
	Shell	Content	Shell	Content	Shell	Content
Enterobacter spp.	4	2	0	4	10	1
Klebsiella spp.	0	1	0	0	3	0
Escherichia coli	2	0	1	0	0	0
Proteus spp.	1	0	0	0	0	0

in chicken eggs was 3.8% (5/131), while no *Salmonella* contamination was found in other tested poultry eggs.

Other *Enterobacteriaceae* bacterial contamination

Bacteriological study showed that the most contaminated poultry eggs were the duck eggs (60.8%) and the most frequent isolated bacteria belonged to genus *Eneterobacter*. No germ was isolated from the quail eggs. *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *E. coli* were generally isolated from the eggs (Table 2).

Serogrouping and multiplex PCR

Based on the serogrouping test results, all of the five isolated *Salmonella* belonged to D serogroup. Multiplex PCR revealed the presence of genes including *spv*, *sefA*, and random sequence (specific for the genus *Salmonella*) in all of the isolates; therefore, all strains confirmed to be *Salmonella* Enteritidis genotypically (Fig. 1).

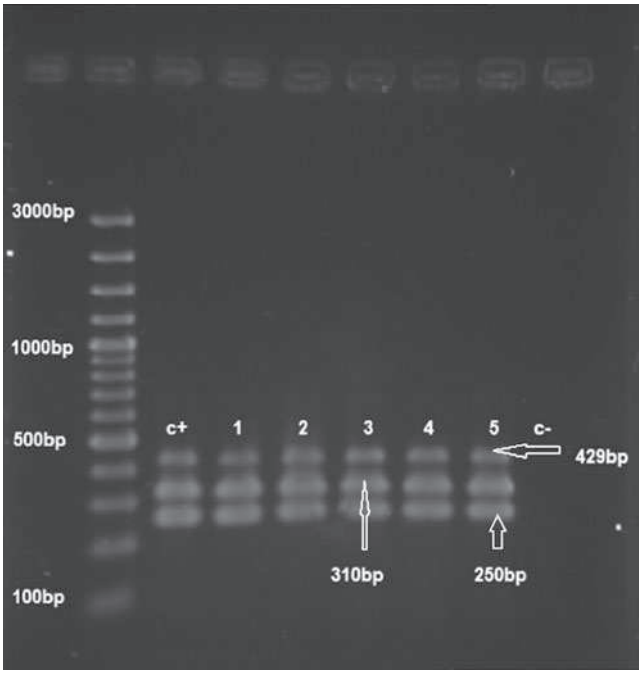

Fig. 1 Results of multiplex PCR for detection of *Salmonella* Enteritidis on five isolates of this study. *1–5* isolates of this study, *C+* positive control which was a poultry clinical isolate of *Salmonella* Enteritidis confirmed by reliable antisera (Difco, BD, Detroit, MI, USA), and *C−* negative control. The 250, 310, and 429 bp band sizes indicate *spv* (*Salmonella* plasmid virulence), *sefA* (*Salmonella* Enteritidis fimbria), and random sequence (for detection of *Salmonella* genus) genes, respectively

Antimicrobial susceptibility test

Antibiotic susceptibility of five *Salmonella* isolates were evaluated using 21 various antibiotic disks, and the results showed 100% resistance to nalidixic acid, tiamulin, erythromycin, colistin, penicillin, streptomycin, neomycin, lincomycin, kanamycin, cefalotin, flumequine, and gentamicin; in contrast, all of the isolates were sensitive to oxytetracycline, chloramfenicol, trimethoprim–sulfamethoxazole, ceftriaxone, and florfenicol. According to the antibiogram results, four distinct resistance patterns were observed in five *Salmonella* Enteritidis isolates (Table 3).

Discussion

From 1985 to 1996, 79% of *Salmonella* Enteritidis infections were caused by consumption of contaminated eggs (Gast 2008). The results of the present study revealed that *Salmonella* Enteritidis was isolated from the chicken eggs at the rate of 3.8%. According to the Center for Disease Control and Prevention (2004), *Salmonella* Enteritidis and Typhimurium are the two most common serovars associated with human disease and therefore are of high importance to public health.

Table 3 Resistance patterns of five *Salmonella* Enteritidis strains isolated from poultry eggs

Number (%)	Resistance patterns
1 (20)	AM, CF, CP, NA, S, P, FM, TM, TE, E, GM, N, CL, LM, K, FR
1 (20)	AM, CF, CP, NA, S, P, FM, TM, E, GM, N, CL, LM, K
1 (20)	AM, CF, NA, S, P, FM, TM, E, GM, N, CL, LM, K
2 (40)	CF, CP, NA, S, P, FM, TM, E, GM, N, CL, LM, K

AM Ampicillin, CF Cefalotin, *CP* Ciprofloxacin, *NA* Nalidixic acid, *S* Streptomycin, *P* Penicillin, *FM* Flumequine, *TM* Tiamulin, *TE* Tetracycline, *E* Erythromycin, *GM* Gentamicin, *N* Neomycin, *CL* Colistin, *LM* Lincomycin, *K* Kanamycin, *FR* Furazolidone

Some other works which were conducted in other countries indicated the existence of *Salmonella* in eggs. Arnold et al. (2010) estimated the prevalence rate of *Salmonella* Enteritidis and Typhimurium to be 14% in egg-laying holdings of UK. In a survey by Hadian (1998), 1,200 egg yolk samples in Tehran were checked and 8% *Salmonella* contamination was reported and the most contaminating serotype was *Salmonella* Enteritidis. However, in a report presented by Bozorgnia et al. (2008) in the 6th Convention of Iranian Veterinary Clinicians, no contamination was detected in a total of 120 commercial eggs in Tabriz. In the present study, five *Salmonella* strains were obtained from chicken eggs of which four strains were isolated from the shells and one from the contents. Prevalence rate of *Salmonella* Enteritidis infection in this study was 3.8% in traditionally produced chicken eggs. These findings indicated noticeable contamination of non-commercial eggs with *Salmonella* Enteritidis in Iran. According to the results of these studies, rate of *Salmonella* contamination is variable in different studies, but contamination of eggs still occurs, and considering the importance of *Salmonella* contamination of eggs, the problem should not be unnoticed. This may be resulted from the lack of hygiene management implemented by farmers. *Salmonella* was not isolated from goose, duck, and quail eggs in the present study, but it may be either resulted from the limited number of samples or lower sensitivity of these species to *Salmonella* infection. In this research, the number of chicken eggs was twice than other birds because availability and consuming traditional chicken eggs are much more common than other kinds of poultry eggs. The most frequent serovars detected among avian and poultry birds belonged to B, C, and D serogroups in different studies (Gast 2008; Mirzaie et al. 2010), and five *Salmonella* isolates in this study belonged to D serogroup.

In the present work, two different isolation protocols were carried out to isolate *Salmonella* from poultry eggs at the same time, and most of the isolates were recovered in the method that tripticasein soy broth (TSB) used as an enrichment and XLD as selective medium. This may be due to the presence of more enriching nutrient materials in TSB and more sensitivity of XLD agar for isolation of *Salmonella*.

Nowadays, the enteric bacteria like *Enterobacter*, *Serratia*, *Klebsiella*, and *Escherichia* are considered as an important cause of nosocomial and opportunistic infections in humans. Immunocompromised patients like HIV-infected individuals, the patients receiving medication for cancers, and graft recipients who receive immunosuppressive agents are particularly susceptible to opportunistic enterobacterial infections. Urinary tract infections and infections of the oral cavity have been reported in immunocompromised patients (Diz Dios et al. 1993; Manfredi et al. 2001). Twenty-nine bacteria that belonged to the family *Enterobacteriaceae* in this study were isolated from the eggs surfaces and also from the eggs contents. Surprisingly, no bacterium was isolated from the quail eggs. This finding is in contrast to Erdugrul et al. (2002) which reported the isolation of *Salmonella* Enteritidis from quail eggs. However, other poultry eggs were highly contaminated by *Enterobacteriaceae*, and due to their pathogenic capabilities, they may infect the patients if they overcome the immune system. Besides, as there are many stresses and immunosuppressive diseases and conditions like AIDS, cancers, hepatitis, etc., it may be necessary to control these food-borne pathogens by public health surveillance authority systems.

Bajaj et al. (2003) studied antimicrobial susceptibility of 66 *Salmonella* isolates and indicated that the most resistance was observed against penicillin and then to vancomycin, erythromycin, trimethoprim, streptomycin, tetracycline, and gentamicin with 96.9%, 83.3%, 81.8%, 42.4%, 24.2%, 9%, and 3% of frequencies, respectively. In addition, in 2005 report presented by National Antimicrobial Resistance Monitoring System 27.1%, 19.5% and 0.4% of *salmonella* isolates from chickens in year 2003 were resistant to tetracycline, streptomycin and nalidixic acid respectively. In another study, the most contaminant *Salmonella* was *Salmonella* Typhimurium that was resistant to several antibacterials and the least frequent serovar was *Salmonella* Kentucky which was sensitive to most antibiotics. Additionally, 63.4% of isolated *Salmonella* were resistant to tetracycline, 63.4% to nalidixic acid and 61% to streptomycin (Musgrove et al. 2006). In a study performed by Sepehri et al. (2007) on broiler chickens, 2.5% of the isolated bacteria belonged to genus *Salmonella* and all isolates were sensitive to florfenicol, lincospectin, and enrofloxacin but were resistant to tylosin. Antimicrobial resistance analysis of five *Salmonella* isolates in the present study against 21 antimicrobials indicated 100% resistance to nalidixic acid, tiamulin, erythromycin, colistin, penicillin, streptomycin, neomycin, lincomycin, kanamycin, cefalotin, flumequine, and gentamicin, but 100% sensitivity to oxytetracycline, chloramfenicol, trimethoprim–sulfamethoxazole, ceftriaxone, and florfenicol.

In conclusion, the results of the current study indicated that non-commercial poultry eggs are noticeably contaminated by *Salmonella* and other *Enterobacteriaceae*; therefore, monitoring by the related authorities may be a practical and beneficial approach to prevent possible food-borne and opportunistic infections in societies.

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