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ARTICLE

First Report on Isolation of *Salmonella* Enteritidis from Eggs at Grocery Stores in Korea

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Abstract

Salmonella Enteritidis is responsible for causing foodborne diseases upon consumption of egg products. While cases of *S.* Enteritidis isolation from eggs have been reported in other countries, no such cases have previously been reported in Korea. In this study, we report the first isolation and identification of *S.* Enteritidis from domestically distributed eggs in Korea. Eggs were collected from eight countrywide grocery stores during different seasons between 2011 and 2012. Egg contents and washing solution of egg shells were incubated in buffered peptone water, and the enriched broth was further enriched in tetrathionate broth and Rappaport-Vassiliadis. The secondary enriched broth was streaked on xylose lysine desoxycholate agar. The suspected colonies were confirmed to *S.* Enteritidis by a biochemical test, serotyping, and PCR test. Genetic relatedness among the isolates was analyzed using Diversilab *Salmonella* kit. Three strains of *S.* Enteritidis were isolated from egg contents and egg shells collected from grocery stores of the Eumseong-city in the fall of 2011. All three stains showed resistance to chloramphenicol, streptomycin, nalidixic acid, and ampicillin by the disk diffusion method. In addition, the isolates showed more than 99% DNA homology, indicating that they were presumably identical strains. Therefore, there is a requirement to monitor and control against *S.* Enteritidis from eggs in Korea.

Key words: egg, *Salmonella* Enteritidis, isolation, antibiotic resistance

Introduction

Food poisoning caused by *Salmonella* is an important source of foodborne disease worldwide. In particular, food poisoning by *Salmonella* upon consumption of eggs and egg products occurs annually in the United States, the Netherland, Canada, Germany, and other countries, thereby posing a risk to public health (Amedo *et al.,* 1998; Henzler *et al.,* 1998; Humphrey *et al.,* 1989; Lee *et al.*, 2002; Rowe, 1989; Wilson *et al.,* 1998). In Korea, eggs are considered as an ideal food product and they contain various nutrients, including protein; thus, they are an integral part of a healthy and nutritious diet. Because of a greater availability of less expensive and functional eggs, more eggs are being consumed and there is an increased concern regarding food poisoning by *Salmonella* (Chun, 2009; Jones *et al.,* 1995; Lee *et al.,* 2002; Stadelman, 1995).

In the United States, *Salmonella* spp. infection was reported in one of 10,000 eggs, and *Salmonella* spp. infection was identified in one of 15,000 eggs in the United Kingdom (Duguid and North, 1991). In the United Kingdom, where an average person eats three raw eggs per week, 1 in 100 people was found to be infected with *Salmonella* and suffer from food poisoning every year (Duguid and North, 1991). Because *S.* Enteritidis is specifically responsible among all other *Salmonella* species for food poisoning by consumption of eggs (Cowden *et al.,* 1989; Coyle *et al.,* 1988; Lin *et al.,* 1988), standards have been established internationally to control the spread of *S.* Enteritidis by eggs (Codex, 1976, FDA, 2000).

In Korea, regular tests are annually conducted at eggs from farms to detect *S.* Enteritidis (MIFAFF Notice 2009-169). In countries other than Korea, including the United States, cases of *S.* Enteritidis isolation have been reported (Baker, 1980; Humphrey *et al.,* 1989); however, between

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2000 and 2011, no such cases have been reported either eggs from farms or distributed stage in Korea (Chun, 2009; Lee *et al.,* 2002; Woo, 2005). In contrast, *S.* Enteritidis has been recently found more frequently from domestically slaughtered chickens (Lee *et al.,* 2007), In addition the demands for eggs that are safe to consume are increasing with an increase in the annual production of eggs. In this study, therefore, eggs were collected during each season from eight countrywide grocery stores and subjected to regular testing to determine the *S.* Enteritidis infection ratio in domestically distributed eggs.

Materials and Methods

For isolating *Salmonella* from eggs, 20 eggs were pooled according to the FDA Bacteriological Analytical Manual (BAM) (BAM, 1998), and the *Salmonella* test was conducted according to the processing standards and ingredient specifications for livestock products (QIA Notice 2012-162).

From fall, winter in 2011 to spring, summer in 2012, 100 eggs each were collected from four grocery stores in Gyeonggi Province (Hwasung-city, Icheon-city, Anseong-city, and Pocheon-city) and four stores in Eumseong-city in Chungbuk Province, Gunsan-city in Jeonbuk Province, Gumi-city in Gyeongbuk Provice, and Yangsan-city in Gyeongnam Provice, respectively.

Salmonella isolation tests were conducted five times with 20 eggs each time, corresponding to a total of 100 eggs. Sterilized saline solution was used to wash the egg shells of all 20 eggs, and the contents of all disinfected eggs were placed in a sterilized pack and agitated, before being enriched in buffered peptone water (BD, USA). The enriched broth was further enriched in tetrathionate (TT) broth (BD) and Rappaport-Vassiliadis (RV) broth (Merk, Germany). The secondary enriched broth was streaked on xylose lysine desoxycholate (XLD) agar (BD), and suspected colonies were transferred to triple sugar iron (TSI) agar (BD). Biochemical testing was conducted using an automated microbiological identification system (Vitek II®, Biomeriux, France) to identify *Salmonella* spp.

For serotyping of the isolation strain, Edwards and Ewing's identification of Enterobacteriaceae procedure (Ewing, 1986) was used. Somatic (O) antigen was confirmed by slide agglutination test, which was performed using commercial antiserum (BD). Flagella antigen was inoculated into GI motility agar (BD) to activate the flagella, after which, it was inoculated into veal infusion broth (BD), cultured overnight, fixed with 0.6% formalin, and checked using tube agglutination test. The serotyped strain was checked again for *S.* Enteritidis by PCR (Soumet *et al*., 1999). *Salmonella* spp. was checked using ST11(5'-GCCAACCA TT GCTAAATTGGCGCA-3') a ST15(5'-GGTAGAAATTCCCAGCGGGTACTGG-3') primers (429 bp), and *S.* Enteritidis was checked using S1(5'-GCCGTACACGAGCTTATAGA-3') & S4(5'-ACCTACA GGGGCACAATAAC-3') primers (250 bp), respectively.

Rep-PCR typing was conducted to verify the genetic relationship between the isolates. DNA was extracted using an Ultra-Clean Microbial DNA Isolation kit (Mo-Bio Laboratories, Canada) and amplified using a Diversilab kit (bioMerieux) along with positive and negative control DNA. Genetic homology was analyzed for each sample by locating and analyzing the intensity of DNA bands by Rep-software (bioMerieux) and Pearson's correlation method.

Antibiotic resistance testing was conducted using the disk diffusion method. Bacterial concentration was adjusted to McFarland No. 0.5 and applied to Muller-Hinton agar (BD) using a sterilized swab; antibiotic discs were subsequently inoculated using a dispenser (BD). Fifteen antibiotics were tested and resistance was judged as per the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012).

Results and Discussion

Of the eggs collected from the eight countrywide regions in spring, summer, fall, and winter, two strains of *Salmonella* from the egg shells and one strain from egg contents collected from the Eumseong-city region in the fall in 2011 were identified to *S.* Enteritidis by serological test (Table 1). In addition, we confirmed by PCR that all isolates were *S.* Enteritidis (Fig. 1). Based on the antibiotic resistance test, all three stains showed resistance to chloramphenicol, streptomycin, nalidixic acid, and ampicillin (Table 2). Upon analyzing the genetic relationship, three stains showed more than 99% homology, indicating that they were identical strains (Fig. 2).

S. gallinarum (Woo, 2005) has been previously found from domestically distributed eggs, and *S.* Enteritidis has been isolated from chicken, pork, and duck (Cho *et al.,* 2011; Choi *et al.,* 2008; Lee *et al.,* 2007). However, this is the first study to report the isolation of *S.* Enteritidis from egg shells and egg contents of eggs distributed at grocery stores in Korea. *S.* Typhimurium, *S.* Agona, and *S.* Enteritidis have already been isolated from egg shells

Table 1. Isolation of *S.* Enteritidis from eggs collected from grocery stores between 2011 and 2012 in Korea

Grocery stores of sample collection	Isolation of *S.* Enteritidis							
	Spring		Summer		Fall		Winter	
	Egg shell	Content	Egg shell	Content	Egg shell	Content	Egg shell	Content
Pocheon-city	-	-	-	-	-	-	-	-
Hwasung-city	-	-	-	-	-	-	-	-
Anseong-city	-	-	-	-	-	-	-	-
Icheon-city	-	-	-	-	-	-	-	-
Eumseong-city	-		-	-	+	+	-	-
Gunsan-city	-	-	-	-	-	-	-	-
Gumi-city	-	-	-	-	-	-	-	-
Yangsan-city	-	-	-	-	-	-	-	-

*Tests for *S.* Enteritidis were conducted five times with pooled egg contents and washing solution of egg shells of 20 eggs per grocery store.

Table 2. Antibiotic resistance of *S.* Enteritidis isolated from eggs in Korea

Antibiotics		*S.* Enteritidis isolates			Reference
Classification	Names	Egg 1[1)]	Egg 2[2)]	Egg 3[1)]	ATCC25922
Aminoglycosides	Gentamicin	S[3)]	S	S	S
	Streptomycin	R[4)]	R	R	S
	Neomycin	S	S	S	S
Aminopenicillin	Ampicillin	R	R	R	S
β-lactam / β-lactamase inhibitor combinations	Amoxicillin / clavulanic acid	S	S	S	S
Cephalosporin I	Cephalothin	S	S	S	S
Cephamycin	Cefoxitin	S	S	S	S
Cephalosporin III	Ceftiofur	S	S	S	S
Fluoroquinolone	Ciprofloxacin	S	S	S	S
Folate pathway inhibitors	Trimethoprim / Sulfamethoxazole	S	S	S	S
Penicols	Chloramphenicol	R	R	R	S
	Florfenicol	S	S	S	S
Polymyxins	Colistin	S	S	S	S
Quinolone	Nalidixic acid	R	R	R	S
Tetracyclines	Tetracycline	S	S	S	S

[1)]Egg 1, 3: *S.* Enteritidis isolated from egg shell
[2)]Egg 2: *S.* Enteritidis isolated from egg content
[3)]S: Sensitivity
[4)]R: Resistance

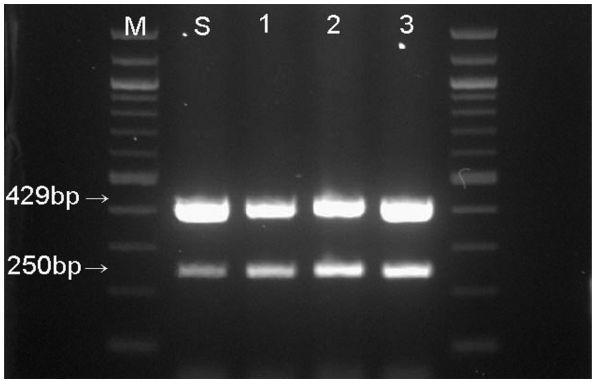

Fig. 1. Detection of *Salmonella* isolates from eggs by PCR with two primer sets: ST11-ST15 (429 bp) and S1-S4 (250 bp). Lane 1: *S.* Enteritidis ATCC13076; Lanes 2-4: *S.* Enteritidis (1, 3: *S.* Enteritidis isolated from egg shell; 2: *S.* Enteritidis isolated from egg content), respectively.

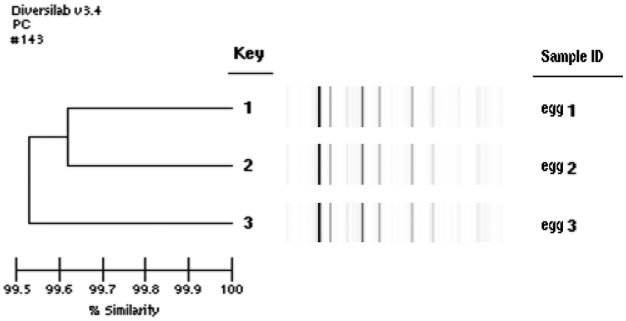

Fig. 2. Rep-PCR generated dendrogram for three *S.* Enteritidis isolates (Egg 1, 3: *S.* Enteritidis isolated from egg shells, Egg 2: *S.* Enteritidis isolated from egg contents)

and egg contents in the United States, United Kingdom, and other countries (Baker *et al.,* 1980; Cowden *et al.,*

1989; Coyle *et al.,* 1988; Henzler *et al.,* 1998; St. Louis *et al.,* 1988). The 0.66% of eggs imported into Albania from European countries such as Bulgaria, Italia, Greece, and Turkey were reported to be infected with *S.* Enteritidis (Altin *et al.,* 1999).

In addition, it has been reported that *Salmonella* present on the egg shell surface enters the egg through stomas or cracks (Cho, 2011; Henzler *et al.,* 1994; Jang *et al.,* 1999). As evident from the test results conducted in the present study, bacteria were isolated from both egg contents and egg shells, identical results were obtained from antibiotic resistance tests, and the isolates showed more than 99% homology. Therefore, it is possible that bacteria present on the egg shell surface may have entered the egg through stomas and contaminated the egg contents. Hence, it is very important to wash and disinfect eggs before distribution to ensure the safety of consumers from eggs.

S. Enteritidis is responsible for serious food poisoning through consumption of eggs. Our study reports the first known isolation of this bacterium from domestically distributed eggs at grocery stores in Korea and it is important in the context of public health and also demonstrates the necessity for preventing and controlling the spread of pathogens in future.

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