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Prevalence of *Salmonella* spp. in Poultry Eggs from Different Retail Markets at Savar Area, Bangladesh

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Abstract

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. The aim of this study is to determine the prevalence *Salmonella* spp. in poultry eggs from different retail markets of Savar area, Bangladesh. A total of 103 poultry eggs were examined to investigate the prevalence of *Salmonella* spp. The isolated samples were presumptively identified by established conventional cultural method and biochemical tests for *Salmonella* spp. detection. The average prevalence of *Salmonella* was found to be 86% in poultry eggs. Among these, 83% from outer shell of eggs and 3% (3 isolates) from egg contents were identified. In this study, we also observed that the prevalence of *Salmonella* spp. that was higher in spring (91%) season than in winter (82%) season. Prevalence of *Salmonella* spp. was significantly higher in egg shell compared to egg contents ($p<0.01$). The present study represents that poultry eggs are potential reservoir of *Salmonella* spp. Human illnesses may be associated with pathogenic *Salmonella* spp. during consumption of contaminated poultry eggs. Intervention strategies are hence important to control *Salmonella* infection from farm to fork.

Keywords

Prevalence, *Salmonella* spp., Poultry Egg, Poultry Retailers

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1. Introduction

Egg industry is primarily based on poultry eggs that are valuable foodstuffs for humans. Consuming contaminated eggs has been associated with negative health impacts. Poultry are considered an important source of foodborne disease and the illnesses were associated with the consumption of contaminated eggs. Eggs and egg products when improperly handled can be a source of food borne diseases, such as Salmonellosis. *Salmonella* is one of the major bacterial agent that cause food borne infection in humans leading food borne disease worldwide (Herikstad et al. 2002). Raw egg or lightly cooked eggs implicated in 78% of outbreaks (Control & Prevention, 2000). *Salmonella* alone affects about 1.4 million people each year in the United States with about 16,000 hospitalizations and more than 500 deaths annually (Mead et al. 1999). There are 16 million annual cases of typhoid fever, 1.3 billion cases of

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gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia, 2007).

Salmonella infection is the most frequent food-borne gastrointestinal disease transmitted from animals to humans, mainly through water, meat, eggs and poultry (Riyaz-Ul-Hassan et al. 2004). There are a large number of *Salmonella* serotypes that can cause a variety of diseases in different hosts. Some serotypes (*S.* Typhi , *S.* Gallinarum) are host adapted, while others (*S.* Typhimurium, *S.* Enteritidis) may cause disease in a large variety of hosts. This bacterium is able to invade cells, including cells from gut epithelium (Galan et al. 1992).

Salmonella serotype associated with poultry reproductive tissues that are of public health concern include *Salmonella* Enteritidis*, Salmonella* Typhimurium. Among the different serotypes, *Salmonella* Enteritidis may be better able to achieve invasion and as a consequence may be found more frequently in reproductive tissues.

There are two possible routes of bacterial contamination of egg shells: either vertical or horizontal (De Reu et al. 2006). Vertical transmission takes place in the transovarian route where the yolk, albumen and membranes are directly contaminated as a result of bacterial shedding from the infection of hen's reproductive organs, which takes place before the shell covers the eggs (Messens et al. 2005). Horizontal contamination begins with the passage of the eggs through the highly contaminated cloaca area at the moment of lay and leads to the shells be penetrated by microorganisms (De Reu et al. 2006). Egg shells may additionally become contaminated from any surface with which it comes into contact. When laid, the egg shells are wet and at the same temperature as the hen's body temperature (Patterson et al. 2008). They are then released in an environment with a temperature of approximately 20°C, where they cool immediately and their content contracts creating a negative pressure inside which can allow for the contaminant to move through the shell (De Reu et al. 2006).

Salmonella infection is one of the major constraints of poultry farming that hindered its development in Bangladesh (Das, 2005; Kamaruddin, 2003a, 2003b). In recent years, the prevalence of Salmonellosis in breeder flock, commercial broiler, and layer flocks is increasing day by day. However, very limited research works had been carried out in Bangladesh concerning Salmonellosis in poultry so far. Therefore, Salmonellosis status of a farm needs to be determined for its proper control and management (Ahmed et al. 2008). The human health concern is the risk of direct transfer of resistant human pathogens via the food chain. In view of this circumstances, the aim of this study was to investigate the prevalence of *Salmonella* spp. in eggs shell and egg content of poultry at Savar area of Bangladesh.

2. Materials and Methods

2.1. Sample Collection Area

The study was accomplished during the period from November 2011 to March 2012. The egg samples were collected from different egg retailers and farm house of Savar area, Bangladesh.

2.2. Collection of Samples

A total 103 egg samples were collected from different egg retailers and farm laying house of Savar region. Aseptic techniques were strictly maintained during sample collection. Unwashed eggs were collected in sterile bags within the ice box and immediately transported to the microbiology laboratory.

2.3. Sample Processing and Isolation of *Salmonella*

Collected samples are carried into laminar air flow bench. Sterile cotton swabs dipped in sterile buffered peptone broth were used to swab the entire surface area of the egg shell then added to the 9 ml pre-enrichment broth (buffered peptone broth) incubate in at 37ºC for 24 hours. Outer shell surface washed with 70% alcohol and air dried in a sterile chamber for 10 minutes then crack with a sterile knife. Each egg's content was mix thoroughly and homogenized. 1 ml of egg content was inoculated into 9 ml pre-enrichment broth (buffered peptone broth) and incubate at 37ºC for 24 hours. After pre-enrichment, 1ml and 0.1 ml of egg shell and egg content for each sample separately inoculate into 9 ml selective enrichment broth (Tryptic soy broth and buffered peptone water) and incubate at 37º C for 24 hours. After pre-enrichment, 1 ml and 0.1 ml of the pre-enrichment cultures (Tryptic soy broth and buffered peptone water) of all sample types were transferred into 9 ml of selective enrichment broth such as Tetrathionate (TT) broth and Rappaport-Vassiliadis (RV) broth, respectively, and incubate at 37°C for overnight (18-24 hours). At the end of selective enrichment, a loop full of broth culture (from TT and RV broth) were spread onto selective media such as Xylose lysine deoxycholate (XLD) agar, Salmonella-Shigella (S-S) agar and Bismuth Sulfite Agar (BSA) plates and incubate at 37° C for overnight (18-24 hours). Plates were examined for the presence of colonies that may resemble Salmonella spp.

2.4. Identification of *Salmonella*

The typical *Salmonella* colonies on XLD, S-S agar, BSA agar plate were identified by morphological characteristics. To obtain pure culture of *Salmonella* spp., each selective media

cultures were subcultured into nutrient agar. Presumptive *Salmonella* isolates were subjected to biochemical tests such as Gram's staining, carbohydrate fermentation test, motility, triple sugar iron test, citrate utilization, simmons citrate test, nitrate Reduction test, methyl red test, voges proskauer test, urease Test.

2.5. Statistical Analysis

Data were subjected to chi-square statistics using Microsoft Excel 2007 program. Significant differences of the data were established by least significant difference at the 5% level of significance.

3. Results

A total 103 poultry egg samples were examined, among them 89 (86.4%) isolates were considered positive as *Salmonella* spp. Among these 89 eggs 86 eggs (97%) were positive for *Salmonella* spp. from outer shell surface and 3 eggs (3%) were found positive from egg content (Table 1). In Gram's staining under microscope, the organism's revealed gram-negative, pink color, small rod shaped appearance. Pure *Salmonella* colonies are identified by morphological characteristics and biochemical tests.

Prevalence of *Salmonella* spp. was significantly higher in egg shell compared to egg contents (p<0.01).

Table 1. Prevalence of *Salmonella* spp. in egg shell and contents of Poultry at Savar area of Bangladesh

Sample collection site	Number of sample	Number of *Salmonella* positive	Percentage (%)
Outer shell	103	86	83**
Egg content	103	03	3
Total Isolation	103	89	86
Chi-square Test (P-value)	0.000**		

**Significant at (p<0.01)

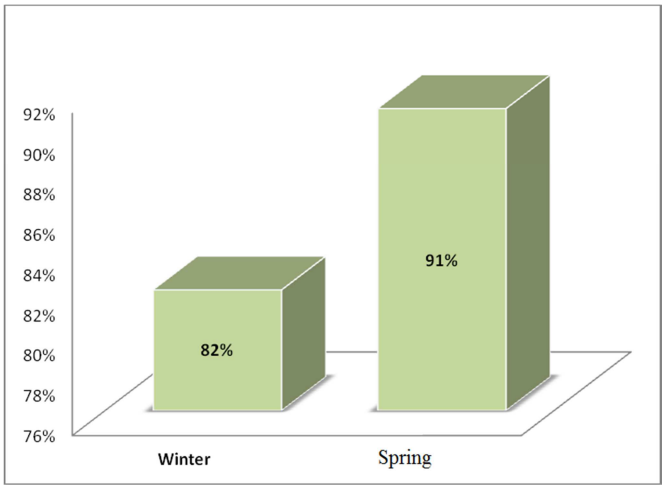

Figure 1. Seasonal variation of isolated *Salmonella* spp. at Savar area

The study was also concerned with investigation of *Salmonella* infection prevalence according to seasonal influence. *Salmonella* infection was higher (91%) in spring than winter (82%) season (Figure 1).

4. Discussion

Salmonella is a leading cause of food-borne illness in many countries with eggs and poultry being important vehicles of transmission. During the past two decades *S.* Enteritidis has became a leading serotype causing human infections, with hen eggs being a principal source of the pathogen. Egg and egg products have been associated with the occurrence of different *Salmonella enteritidis* and non-enteriditis mediated food borne outbreaks. The emergence of *S. enteritidis* as the leading cause of human Salmonellosis in many countries was attributed to this serotypes unusual ability to colonize the ovarian tissue of hens and be present within the contents of intact shell eggs (Threlfall et al., 2014; Himel et. al., 2014).

The present study was conducted to determine the prevalence of *Salmonella* spp. in the poultry eggs. In this present study, 103 poultry eggs were examined for the isolation and identification of *Salmonella*. Among them 86 (97%) samples were found positive for *Salmonella* spp. isolation from outer shell of the eggs. Only 3 (3%) sample were found positive for *Salmonella* isolation from 103 egg content. Total 89 (86.4 %) samples were positive from 103 egg shell and content. Eggs are considered to be the major sources of confirmed salmonellosis (Lepoutre, 1994). Infected ovaries and oviducts of the hen are the major sources of contamination (Abdel Karem and Mattar, 2001). Eggs can become contaminated also on the surface, either from faeces or the environment. Cracked eggs are those that could not be sold at the market because of being damaged by the end of laying or during transportation.

In egg contents, the low and sporadic incidence of egg contamination was probably because of the protective effect of the egg's complex system of membrane barriers and the antibacterial components of the albumen. Penetration of the shell by *Salmonella* Enteritidis has been suggested (Nascimento et al. 1992; Schoeni, Glass et al. 1995) and the *Salmonella* Enteritidis, *Salmonella* Typhimurium, or *Salmonella* Heidelberg present in feces could penetrate to the interior of eggs and grow during storage.

It was reported the shells and contents of 2,090 packs of six raw eggs from shops in Northern Ireland were examined and nine isolates of *Salmonella* were detected from separate packs of eggs (0.43 %) (Wilson et al. 1998). One of the isolates was from egg contents (0.05 %) and eight of the isolates were detected on the shell of eggs. In 2002, from five countries of European Union (Denmark, Finland, Ireland, Sweden and

Norway), the prevalence of *Salmonella* spp. in laying hens producing table eggs was from 0- 25% and S. Enteritidis from 0-1.9%, have been reported (EC, 2004). The findings of the present work are to some degree consistent with the results obtained by others. It is assumed that in the USA one in 10,000 eggs is infected with *Salmonella* spp., in Great Britain one in 15,000 eggs (Radkowski, 2001). In a survey done in New Zealand by Environmental Science and Research Limited (Swallow et al. 2004) in 1994 found that no *Salmonella* were detected on the shells of 341 samples of 6 eggs (2,046 eggs in total) or in the contents of 339 samples of 6 eggs (2,037 eggs in total). It was reported *Salmonella* spp. was detected from 13.3% and 0.6% of eggs samples that were produced in Spain and France, respectively (Little et al. 2007).

Animal based foods, especially chicken, have been associated with the occurrence of *Salmonella* in humans for the consumption of egg and egg product. The aim of this study was to determine the occurrence of *Salmonella* spp. in eggs. This study also indicates the prevalence of *Salmonella* increase during the spring season (91%) than winter (82%). Hossain et al. (2010) reported that the prevalence of *Salmonella* infection was the highest (18.5% ± 11.9% = 30.4%) in summer, followed by winter (11.6% ± 12.1% = 23.7%), rainy season (14.2% ± 10.8% = 25.0%), and autumn (13.3% ± 10.0% = 23.3%). Similar findings also reported by Mahmud et al. (2011).

5. Conclusion

Eggs associated Salmonellosis is an important public health problem in the world. We have to consider some of the point that eggs offered for sale must be free of faeces, dirt and stains. Premises and equipment for handling and storage of eggs must be maintained in a sanitized state fit for the production of food for human consumption. Egg farms must be regularly visited by field inspectors to monitor bird health by recording feed and water intake, rate of lay, egg quality, bird behavior and appearance. Continuous monitoring and control methodologies, which should be applied in poultry farms for the control of spread and eradication of this pathogen, where possible, are strongly recommended. Efforts including critical control point programs in food production are needed to reduce the incidence of *Salmonella* in food. Consumers-awareness efforts would protect public health from food borne and Salmonellosis.

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