

## PREVALENCE OF *Salmonella* spp. IN CHICKEN EGG FROM KHULNA CITY

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### ABSTRACT

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Chicken eggs from wholesale market in Khulna City were examined for the presence of *Salmonella Typhimurium* in the year of 2005 during summer season. Out of eighty (80) eggs (40 fresh & 40 cracked) tested, four (4) were found to be contaminated with *Salmonella Typhimurium*. Among four, three were isolated from cracked eggs sample while the other one was from fresh eggs. The ratio of *Salmonella Typhimurium* contaminated and contamination free egg is 1:40 in fresh egg, while 1:13.33 for cracked egg. However, this ratio is remarkably high in comparison to the normal ratio 1:20,000. Most of the poor peoples consume cracked egg and become sick by various types of enteric diseases associated with *Salmonella Typhimurium*. So, consuming cracked egg should be prevented for the welfare of public health.

**Keywords:** *Salmonella* spp., Salmonellosis, Enterobacteriaceae, Chicken egg

### INTRODUCTION

*Salmonella* spp is a group of organisms that are closely related to one another and probable should be considered as a single species. *Salmonella* spp infections appear to be one of the most typical examples of an enteric disease (Satorres *et al.*, 1998) that is transmitted from animals to humans. The transmission may occur through food, such as meat, dairy products, and egg by-products (Holmberg *et al.*, 1984; Gast and Beard, 1993; Suzuki *et al.*, 1981; Koidis and Bori, 1999). More than 2,000 serovars of *Salmonella* spp have been described, and all are considered to be potentially pathogenic for animals, including humans (Borrego *et al.*, 1992). Salmonellosis in humans can produce symptoms ranging in severity from intestinal disturbances (Hogue *et al.*, 1997; Mason, 1994; Anonymous 1990, Anonymous 1998) to death (Taylor *et al.*, 1984). Infections caused by *Salmonella* strains, mainly *S. enteritidis*, have increased over the last few years (Rodrigue *et al.*, 1990).

Salmonellosis is characterized by diarrhoea, fever, abdominal pain or cramps, vomiting, headache and nausea. The incubation period ranges from 8 to 72 hours. Symptoms can last up to a week. *Salmonella* spp infections vary from mild to severe, and are occasionally fatal. Fatalities are more often seen in susceptible populations, which include infants, the elderly and the immuno-compromised (Anonymous, 2002).

Even though the original population of *S. enteritidis* in liquid egg seems to be low, there is potential for these populations to increase to levels capable of causing disease. Temperature abuse of the egg product can lead to higher numbers of organisms that may not be completely eliminated by current pasteurization protocols (Erdgrul, 2004).

A bacterium, *Salmonella Typhim* can be inside perfectly normal-appearing eggs, and if the eggs are eaten raw or undercooked, the bacterium can cause illness. However, the nutrients that make eggs high-quality foods for humans are also a good growth medium for bacteria. For this Vulnerable groups such as pregnant women, the elderly and babies are additionally advised to consume only eggs where the yolk and white have been cooked until they are solid (Anon, 1988, 1998).

The majorities of reported Salmonellosis outbreaks involving eggs or egg-containing foods have occurred in food service kitchens and were the result of inadequate refrigeration, handling and insufficient cooking. Egg trays have also been shown to be contaminated in other studies (Viora *et al.*, 1993) and may be the element of cross contamination. If not properly handled, *Salmonella* Spp bacteria can double every 20 minutes and a single bacterium can multiply into more than a million in 6 hours. But, properly prepared egg recipes served in individual portions and promptly eaten are rarely a problem. The egg industry, the public health community and government agencies have been working diligently to deal with *Salmonella* spp.

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So far our knowledge goes no work has been carried out in Khulna to check the prevalence of *Salmonella* spp in commercial supplies of chicken eggs. So our present work was aimed at to:

- i) Isolation of *Salmonella Typhimurium* from fresh and cracked chicken egg.
- ii) Characterization and identification of isolated organism.

## MATERIALS AND METHODS

### ***Collection of Eggs***

The study was carried out from different place of Khulna city. The study area included Boro-Bazer, Nirala & Gallamary Bazer. Both fresh & crack eggs are collected from the market that supply to various fast food center & other type of food stoles.

The study was conducted to determine the presence of *Salmonella Typhimurium* bacteria in eggs collected from different market.

### ***Sampling Procedure***

Special care was taken of the eggs to be examined, so that no contamination would occur during sampling. Collected egg samples were put into an autoclaved flask containing glass beads then mixed well to prepare egg solution.

### ***Isolation of Salmonella spp***

#### **1. Pre-enrichment**

After making the egg solution by mixing well into a sterile flask, it was inoculated into the buffered peptone water (BPW), enrichment medium. Incubated overnight at 37 °c.

#### **2. Selective Enrichment**

Selenite F broth enhances the isolation of *Salmonella* spp. Tetra-thionate broth can also be used as an alternative. The selenite F broth was inoculated with 10ml of egg solution. The broth was incubated with egg solution for 24 hours. An excellent alternative-plating medium of high selectivity for *Salmonella* spp is brilliant green; the combination of this medium with tetra-thionate brilliant green-bile salt broth for enrichment gave excellent results for non-typhoid *Salmonella* spp.

Then plating medium of low selectivity and one of moderate or high selectivity were inoculated. Macconkey Agar was used as mediums of low selectivity incubated at 35-37°C for 18-24 hours for *Salmonella* spp. As a medium of moderate or high selectivity for the isolation of *Salmonella* spp, xylose-lysine-desoxycholate (XLD) agar was used. Media of high selectivity was inoculated with heavy inoculums of egg suspension and media of low selectivity with light inoculums (1 loopful). After inoculation, incubation was done at 35-37°C. Bismuth sulfite agar was also used for isolating *Salmonella typhi* (also useful for other *Salmonella* spp). For this large inoculums was inoculated and incubate for 48 hours.

### ***Biochemical test for Salmonella spp***

Various procedures are available to demonstrate the essential characteristics for identification of these bacteria. Two differential media are recommended for screening the isolates:

Urease test broth (UTB)

Kligler's iron agar slant (KIA).

Identification of Colonies on Plating Media

- Identification of different species by colony appearance was referred.
- Well-separated colonies of typical appearance were transferred to identify.

Each of the plating media for further test was marked on the bottom of the Petri-plate. Plate contained more than one colony-type were processed to get at least one colony of each type. Suspected colonies on plating media were screened on UTB and KIA as described below, and illustrated in the flow chart in figure 1.

Performance of Urease Test and KIA

Bacteria from each colony were inoculated into separate tubes of UTB. Observed after inoculation for 24 hours at 35°C. Urease positive tubes were discarded and urease negative tubes were subcultured to KIA. Incubated overnight at 35°C. All members of the family enterobacteriaceae ferment glucose and produce acid (yellow butt) or acid and gas on KIA. For this when lactose was simultaneously fermented, both the butt and the slant become yellow (acid), described as A/A. Blackening along the stab line or throughout the medium indicates hydrogen sulfite production. The results were recorded

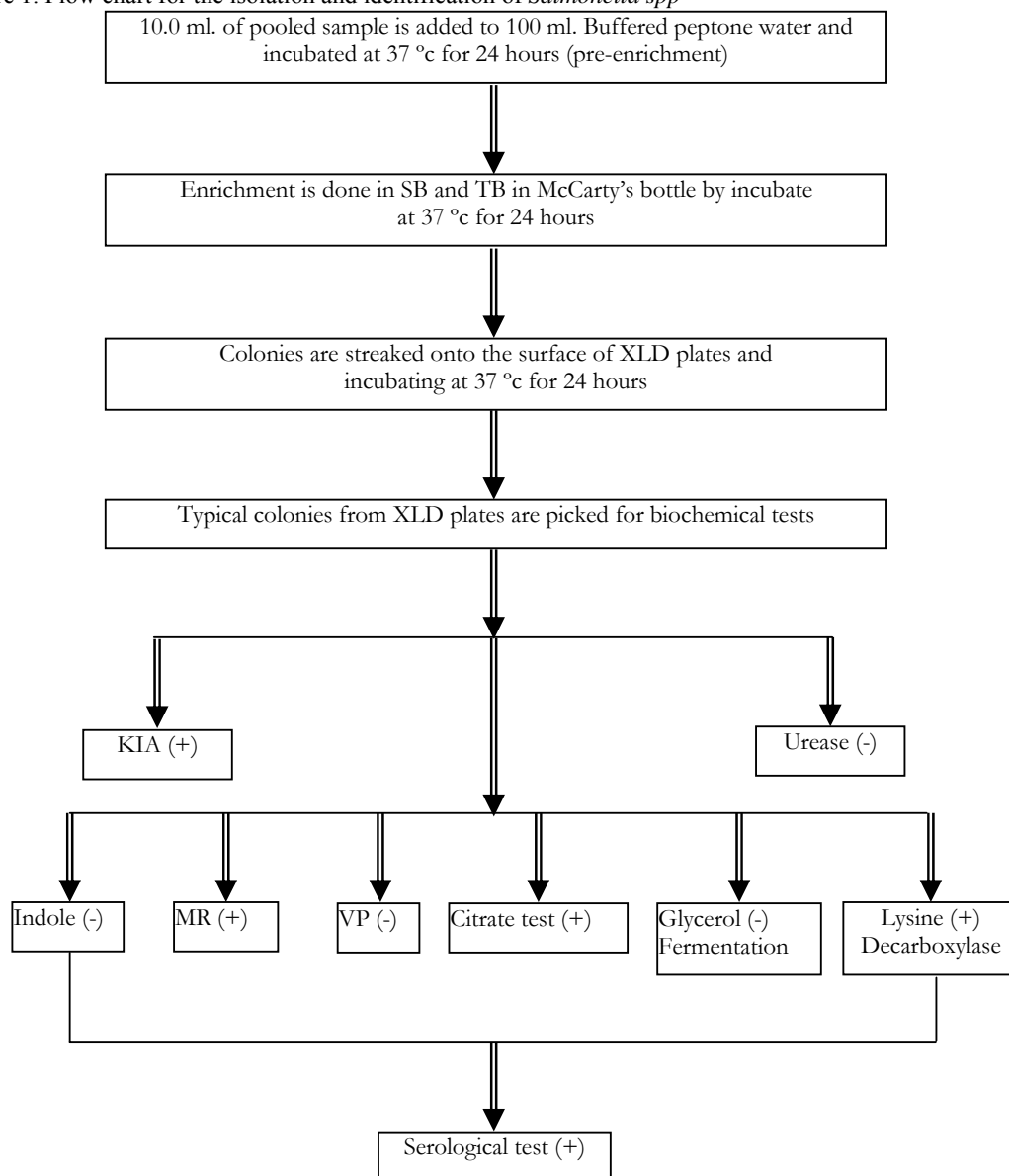
For presumptive identification of isolates from the above urease and KIA reactions motility agar, peptone water for Indole test, glycerol peptone water, and perform oxydase test were done.

**Serological Test for *Salmonella* spp**

The preserved *Salmonella* spp. that was isolated from the egg samples placed in slide containing normal saline with known serum of mice blood. Blood was from the mice that were injected with known *Salmonella typhi*. The serum coagulates with the bacteria indicating the presence of *Salmonella typhi*.

Table 1. Biochemical properties of *Salmonella* spp. isolates

<b>Tests</b>	<b>Results</b>
KIA	Alkaline slant, acid butt; with or without H <sub>2</sub> S
Urease	-
Indole	-
MR	+
VP	-
Citrate	+
Glycerol fermentation	-
Lysine decarboxylase	+

Figure 1. Flow chart for the isolation and identification of *Salmonella* spp

## RESULTS

The study was under taken to study the prevalence of *Salmonella typhi* from chicken eggs in wholesale market of Khulna City Corporation. Eighty (80) egg samples were collected from the wholesale market. Among these samples half was fresh and another half was cracked chicken egg.

Out of 80 eggs samples tested 38 (17 cracked & 21 fresh) were screened out for the absence of typical characteristics on the XLD plate. The rest 42 eggs sample produce characteristics on XLD plate. The colonies on XLD plate were selected for its flat shape, large size and black center. But in some samples two (2) types of colonies are selected: type (a) Flat or rough shape, large size and black center; type (b) Smooth shape, small size and red in color. Out of 42 isolates 38 was urease negative and followed up for biochemical characterization. Among them 25 samples were showing the KIA positive reaction i.e. slant red (alkaline) and butt yellow (acidic) reaction and the rest were discarded. Then 25 isolates were followed up for indole test, citrate utilizing test, methyl red test and voges-proskaur test.

Out of 25 isolates 19 isolates produced typical biochemical reaction like *Salmonella* spp (negative indole test, positive or negative citrate test and positive MR test & negative VP test). Out of these 19 isolates 15 were from cracked egg sample and 4 were fresh egg sample. These 19 isolates were preserved for serological test.

Out of 19 samples, finally 4 were reported as *Salmonella typhi* after serological test. Between these 4 samples, three (3) were reported from the cracked egg samples while the rest one was from the fresh egg samples. The ratio of *Salmonella* spp contaminated and contamination free egg is 1:40 in fresh egg, while 1:13.33 for cracked egg. From the above result, it can be concluded that prevalence of *Salmonella typhimurium* in chicken eggs both fresh and cracked are abnormally high in comparison to the normal ratio 1:20,000.

## DISCUSSION

Egg is one of the most nutritious foods with high food value. However most of the people consume eggs due to their nutritious value, cheap and availability. The results of our research showed that prevalence of *Salmonella* spp in chicken egg from the wholesale market in Khulna city is higher than the normal ratio. More over prevalence of *Salmonella* spp in cracked egg is comparatively very high than the fresh eggs. As, the poor people are consuming these cracked eggs because of their poverty; they are continuously suffered from various type of enteric disease. Not only poor people but also the rich who are consuming different items of fast food made by cracked or improperly handled fresh eggs are also being infected. On the other hand pasteurization process cannot eliminate *Salmonella* spp completely (Erdğrul, 2004). So the Vulnerable groups such as pregnant women, the elderly and babies are additionally advised to consume only eggs where the yolk and white have been cooked until they are solid (Anon, 1988 , 1998). Moreover, at the time of food processing formation of bacterial biofilms on equipment, particularly plastic but also stainless steel equipment, is a problem (Carpentier and Cerf, 1993; Joseph, *et al.* 2001). Organisms incorporated in these biofilms are partially protected from disinfectants and other noxious agents (Hood and Zottola, 1995). It is desirable to reduce the build-up of these biofilms by regular cleaning, but it is difficult to do this in a consistently effective way and very careful pressure washing or scrubbing is required to avoid leaving residual foci of contamination (Gibson *et al.*, 1999). There may, however, be negative aspects involved in wet cleaning of egg-handling equipment unless it can be thoroughly dried before re-use (Davies and Breslin, 2003). So, I think City Corporation and law enforcing agency, BSTI go for legal steps to avert this situation through banning on selling and buying cracked egg, making public awareness about health hazards of cracked egg, advising people not to take raw or undercooked eggs or products containing raw or uncooked eggs, maintaining cool chain through transportation and preservation.

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