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**Author**

**January, 2015**

**ABSTRACT**

The present study was conducted to know the prevalence of bacteria under the genus of Salmonella and Esherichia in the shell surface of local chicken egg in different market in Chittagong metropolitan during the period of August, 2014 to 31 August, 2014. This study presents the degree of contamination of local chicken eggs with bacteria of the genus *Escherichia*, *Salmonella* taking into account the source of the eggs. The samples were collected randomly. Samples were collected by swabbing to buffer peptone water and then cultured in MacConkey agar and Eosin Methyline Blue agar.The results of the study indicate a relatively high degree of contamination of local chicken eggs with Salmonella bacteria and Esherichia coli on egg shell surface. Results revealed that 52% of egg shell were contaminated with E. coli and 42% were contaminated with Salmonella. Only 6% eggs were found without any Salmonella or E. coli on their shell surface. All of five markets have Salmonella and E. coli in egg shell. Riaz Uddin Bazar has the highest 70% E. coli on egg shell and Karnafuli market has highest 80% Salmonella on egg shell of local chicken egg. So, egg shell of local chicken eggs are highly contaminated with Salmonella and E. coli.

**Key word**: Salmonella, E. coli, local chicken egg, isolation and identification.

 **Chapter I**

**INTRODUCTION**

Egg is one of the main sources of protein in all over the world. From the nutrition point of view, eggs were always one of the most complete foods available for man(Tunon *et al*, 1984). Besides vitamins and mineral elements eggs can provide three essential elements for a good diet: proteins, lipids and carbohydrates(Beig *et al*, 1986). Salmonella enteritidis is the only human pathogen that contaminates eggs routinely, even though the on-farm environment of the chicken is a rich source of a number of Salmonella serotypes (Soerjadi-Liem and Cumming, 1984; Caldwell et al., 1995; Byrd et al., 1999).

 Food born disease is a public health concern all over the world and can lead to chronic illness and death for the individual. For the community it is a cost for medical care, investigations and loss of productivity.

*Campylobacter* and most *Salmonella* serovars are adapted animal pathogens and do not cause illness in animal but to man when transferred through e.g. eggs and meat.

*Salmonella* contamination occurs from faeces and bacteria can survive in dry faecesfor long periods. Animal feed is an important source of the bacteria as well as domesticand wild animals. *Salmonella enteritidis* is a common strain which can contaminateeggs either by contact with the faeces or infect the egg as it passes downthe oviduct. In man it produces a toxin that causes illness (Garbutt, 1997). An infectionwith *Salmonella* can cause diarrhoea, blood infection and typhoid fever. Iftyphoid fever is untreated, mortality in humans can reach 15 % (Martinko andMadigan, 2006)

Another bacterium infecting food through contact with manure is *Escherichia coli*. This bacterium is found in the normal gut flora in humans andanimals and birds. However, there are some strains such as EHEC (0157:H7) which arepathogenic for humans (Garbutt, 1997).

In Bangladesh most of the laying hens are housed in deep-litter floor system equipped with nests, and remaining is housed in conventional cages.

**Objective:**

1. To find out Salmonella and E. coli in local chicken egg shell.
2. To see the percentage of these bacteria in egg shell
3. To know the rate of contamination in different market.

**Chapter II**

**Review of Literature**

Board and Tranter (1995) reported that when leaving the cloacae most eggs are sterile. The main bacterial contamination occurs in general after eggs have been laid. Contamination occurs when the egg is in contact with nest material, trays, dust, soils and manure.

Todd *et al* (1996) found that Cracked eggs increase the probability of contamination inside the

Egg

De Reu *et al*. (2005a) found a positive correlation between the concentrations of

Bacteria in the air of the poultry house and the initial egg shell contamination regarding total aerobic count. This study also showed that floor eggs have a high bacterial load compared to eggs laid in nest and that the egg conveyor belt is a key point for contamination of accumulated eggs.

De Reu *et al*.(2005b) reported that type of housing system can affect bacterial contamination. A higher bacterial contamination of the air from aviary systems than from cage systems and a higher total aerobic bacterial contamination on eggs from aviary system than from conventional cages were found. However, for gram-negative bacteria as *Salmonella, Campylobacter* and *E. coli* there were no higher contamination degree. The age of the hens did not affect the degree of contamination.

Kretzshmar-McCluskey *et al.* (2009) found that the micro flora load on the shell increased as the age of hens increased. This is probably due to a more contaminated housing area in the end of a production period than in the beginning in some farms when the hens are young.

Singh *et al.* (2009) found that eggs from nest-boxes and floor had a higher contamination of *E. coli* and *Campylobacter* than eggs from cage system. A significant difference regarding use of nest boxes for different bird genotypes was also found. The white strains had a lower percentage of eggs laid outside the nest compared to the brown strains and hence the study suggests that there are genotype environment interactions.

Scott and Silversides (2000) studied that Egg shell quality can be affected by bird strain as an effect of genetic selection. Brown laying strains are sometimes reported to have heavier eggs but a thinner egg shell than the white.

Smith *et al (*2000) reported that Increased excreta moisture, e.g. if hens are fed a too high concentration of salt, can lead to a higher egg shell contamination.

Board and Tranter (1995) reported that it is not possible to use a visual examination of the bacterial eggshell contamination because many studies have shown that there is no reliable correlation between visual shell contamination and bacterial contamination. Heavy soiled eggs are an exception.

Stokes et al. (1956) identified several factors that can affect the ability of *Salmonella* to penetrate an egg. Many of these factors such as porosity of the shell, thickness of shell membrane, and concentration of natural antimicrobials could be altered by genetic selection.

Forsythe and Hayes (2000) found that equipment that travels throughout a facility may increase the risk of cross-contamination

T.J Humphrey (2002) found that *Salmonella* enteritidis can contaminate the contents of clean, intact shell eggs as a result of infections of the reproductive tissue of laying hens. The principal site of infection would appear to be the upper oviduct. In egg contents the most important sites of contamination are either the outside of the vitelline membrane or the albumen surrounding it. In fresh eggs, only few salmonellas are present and as albumen is an iron-restricted environment, growth will only occur once storage-related changes to vitelline membrane permeability, which allow salmonellas to invade yolk contents, have taken place

Haines and Moran,1940; Gillespie et al 1950; Lorenz et al. 1952; McNally,1952; Forsythe et al. 1953 studied that spoilage bacteria and other microorganisms may gain entrance to the egg content as a result of washing.

Musgrove, Michael T, Jones, Deana R,Northcutt, Julie K. Cox, Nelson A. Harrison, Mark A (2004) demonstrated that commercial processing decreased microbial contamination of eggshells

Smeltzer *et al* (1979) reported that The probable mode of natural bacterial contamination of hatching eggs is the cooling of moist, freshly laid eggs from the body temperature of the hen to the air temperature in the presence of contamination on the shell. The hen brings soil and feces into the nest and these materials have been shown to contain microorganisms, including salmonellae. Eggs lay in wet, dirty nests or on the floor are more likely to be contaminated.

Baker *et al* (1974), The outer and inner shell membranes of an egg do offer some protection against bacterial penetration

Rizk *et al*. (1966b) demonstrated that the numbers of salmonellae that have penetrated an egg will greatly increase as the temperature of storage increases, and that the existing conditions during the incubation of a hatching egg promote proliferation of salmonellae

Marianne Chemally,Adelene Huneu –Salau N,Anne Labbie, Catherine Houdayer, Isabelle Petetine,2 And Phillippe Fraval showed that 39.3% of the positive flocks had at least one positive eggshell, with a total of 1.05% of eggshells testing positive for *Salmonella*. We detected the same serovars on samples taken from the farm and from eggshells within a given flock, with isolates sharing the same genetic pattern in 7 of 11 flocks. Eggshells tested positive for *Salmonella* in flocks (i) located where delivery trucks pass near air entrances of the poultry house, (ii) with high holding capacity (.30,000 laying hens), and (iii) with more than five positive samples coming from the farm environment, as well as in cases of flocks with a maximum egg-laying rate of .96% and in cases where farmers worked in other animal production.

J[. Bruce](http://link.springer.com/search?facet-author=%22J.+Bruce%22), [E. M. Drysdale](http://link.springer.com/search?facet-author=%22E.+M.+Drysdale%22) (1994) reported that Contamination of hatching eggs may reduce hatchability, be responsible for transmission of poultry pathogens and impair the quality of chicks produced.

**Chapter III**

**MATERIALS AND METHODS**

**Study area**

The study was conducted at five different markets Pahartali, Kazir dewri, Jhaotola, Riaz uddin bazaar, Karnafuli market under the Chittagong metropolitan area.

**Duration of the study**

The duration of the study was 06 August, 2014 to 31 August, 2014 during my laboratory rotation in Chittagong Veterinary and Animal Sciences University.

**Sampling plan**

The study was designed for isolation, purification, characterization and identification of Salmonella organism in local chicken egg in Chittagong metropolitan.

**Sample collection**

Samples were collected from 5 different markets, 2 shops from each market. Samples were collected from total 50 local chicken eggs. Five egg samples from every shop were selected randomly. Egg surface were swabbed by cotton swab wet with buffer peptone water. Each sample then collected in separate eppendorf tube and carried to the laboratory for culturing by ice box. All the sample collection was done by wearing hand gloves.

**Isolation and identification of bacteria**

Isolation and identification of bacteria from egg surface sample were done in PRTC laboratory under pathogen free condition in laminar air flow. Isolation and identification procedure was as follows:

**Agar preparation**

**Buffer Peptone water:**

**Composition:**

Peptone: 10g/l

Sodium Chloride: 5g/l

Disodium phosphate: 3.5g/l

Potassium dehydrate Phosphate: 1.5g/l

**Preparation:**

1. Suspend 20gm of powder in 1 liter of purified water
2. Autoclave in 121°C for 15 minutes.

 **MacConkey Agar:**

**Composition:**
Peptide digested animal tissue: 1.50g/l

Casein enzymatic hydrolyses: 1.50g/l

Pancreatic digest of gelatin: 17g/l

Lactose: 10g/l

Bile salt: 1.50g/l

Sodium chloride: 5g/l

Crystal inolate: 0.001g/l

Neutral red: 0.03g/l

Agar-15 g/l

**Preparation:**

1. Suspend the 51.3gm of powder in 1 L of purified water and mix thoroughly.

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes.

4. Cool it to 45-50 °C and pour into sterile Petridis.

**Eosin methyline blue agar:**

**Composition**

Enzymatic Digest of Gelatin: 10g/l

Di potassium hydrogen phosphate: 2

Lactose monohydrate: 5

Eosin Y: 0.4

Methylene Blue: 0.065

Agar: 13.5

**Preparation:**

1. Suspend the 36gm of powder in 1 L of purified water and mix thoroughly.

2. Heat with frequent agitation in water bath for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes.

4. Disperse the precipitate and pour to sterile Petridis.

**Brain Heart Infusion Broth:**

**Composition** (% w/w)

Brain infusion solids: 12.5

Beef heart infusion solid: 5

Proteose peptone: 10

Glucose: 2

Sodium chloride: 5

Di sodium phosphate: 2.5

**Preparation**

1. Disperse 37gm of powder in 1liter purified water
2. Heat gently to dissolve the medium.
3. Autoclave at 121 C for 15 minutes.

**Culturing on Agar Media**

For suspected cases inoculation from swab sample culturing were done at MacConkey agar and Eosin Methyline blue (EMB) agar. After overnight incubation the bacterial growth was observed as pink colonies at MacConkey and Metallic sheen colonies at EMB agar. Both lactose fermenting and non lactose fermenting colonies were found. *Salmonella* organisms were grown on differential plating media such as MacConkey and SS Agar. It has been shown that *Salmonella* occasionally fails to grow on certain selective media such as Briliant Green or *Salmonella*-Shigella Agar but grows satisfactorily on Bismuth Sulfite and MacConkey Agars (Carlson *et al.,* 1974)

**Chapter IV**

 **RESULTS AND DISCUSSION**

The results and discussion of this study are described under the following captions.

Results were Salmonella in 42%, E. coli in 52% and 6% found negative in overall all market. In different markets, Riaz Uddin Bazar, Kazir Deuri and karnafully market found 100% contamination where it is 80% in Pahartoli and 90% in Jhaotola. This may be attributed to the fact that poultry farmers do not practice strict medication and care( Board and Tranter , 1995)

Several factors have been implicated in egg contamination. Among these are faeces of the birds, litter material, egg crates, packing and storage. Others are cloths and hands of poultry workers, dust, the environment, weather conditions, transporting and marketing. (Smeltzer *et al*, 1979)

**Collection of data: Collected data are presented here in the following table**

**Table 1:** Percentage of Salmonella and E. coli in different market

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name of market | Total egg | Organisms positive | Salmonella (%) | E. coli (%) | Negative(%) |
| Pahartali | 10 | 8 | 2(20) | 6(60) | 20 |
| Jhaotola | 10 | 9 | 4(40) | 5(50) | 10 |
| Riaz Uddin Bazar | 10 | 10 | 3(30) | 7(70) | 0 |
| Kazir Deuri | 10 | 10 | 4(40) | 6(60) | 0 |
| Karnafully Market | 10 | 10 | 8(80) | 2(20) | 0 |

Here parenthesis indicates percentage. From this table we can say that E. coli percentage is higher in most market than Salmonella.

**Table 2:** Total percentage of Salmonella and E. coli in the study area

|  |  |  |
| --- | --- | --- |
| Criteria | Number | Percentage (%) |
| Salmonella | 21 | 42 |
| E. coli | 26 | 52 |
| Negative | 3 | 6 |

 **FIG 1:** Percentage of E. coli and Salmonella in different market

This chart indicates that closely related markets are more affected with E. coli than Salmonella. Higher percentage of E. coli present in Riaz uddin bazar and higher percentage of Salmonella present in Karnafully market.

 **FIG 2:** Rate of salmonella and E. coli in different market.

This chart indicates that Karnafuli market has higher rate of Salmonella when Riaz uddin bazaar has higher rate of E. coli in local chicken egg shell.

 **FIG 3:** Total percentage of Salmonella and E. coli in markets

So, microorganisms that were isolated and identified from the sampled eggs from the market include Salmonella and E. coli. This may be due to the fact that the eggs were improperly stored for a long time. As eggs stay longer, their resistance reduced enabling these organisms to penetrate into the egg content. (Etches, 1992) Escherichia coli is known to contaminate the surface of egg while mechanical process can spread the bacteria through eggs and meat. Contamination with the pathogen while in the field, occur through improperly decomposed manure, contaminated water and poor hygienic practices of the farm workers. (De Reu *et al*, 2005b) Here it is found that, eggs were brought to these market from very distant places. Transportation cause to increase the chance of contamination of egg as there is no proper hygienic protocol maintained during transport. (Forsythe and Hayes, 2000)

**Chapter V**

**CONCLUSION**

So,the study reveals that local chicken eggs are contaminated with Salmonella and E. coli in a high rate in all those market in Chittagong metropolitan. Eggs exposed to contamination due to bad storage conditions in storehouse, wrong show in market, dirty table, high temperature, dust, hand touching, and all other surrounding pollution state, also consumers should keep egg in refrigerator and cooked egg well to kill bacteria. But some constraints in this study were small population size, limited time, and lack of seasonal variation. And there was no proper data about the source of eggs and feeding history of birds. So it can be said that considering the real situation of the markets, there is a high load of Salmonella and E. coli in the egg shell of local chicken eggs in Chittagong metropolitan area. However, more study with larger studied population and different geographical region is recommended for making final comments.

**Chapter VI**

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