**Chapter I**

**Introduction**

Good egg shell quality is necessary for economical viability of the worldwide egg

Industry (Roberts, 2004). Bad egg shell quality can result in food poisoning. The

major bulk of food born outbreaks is caused by microorganisms that have the capacity to reproduce in food. Food borne 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.

The bedding and housing management also responsible for contamination of egg dhell.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.

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.

*Salmonella* contamination occurs from manure and bacteria can survive in dry manurefor 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 manure 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. 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 are housed in conventional cages. The cage system is most common within large farms and four hens are allowed in one cage. The size of the different floor and cage farms varies between 1000 birds up to lakh at biggest farms. The average farm has 2500 hens. Surfaces of equipment, packaging, and other objects that come into direct contact with food are potential sources of recontamination. The purpose of the present study was to investigate the bacterial micro flora of egg shells from cage systems and from floor systems and to get a general impression of the hygiene of egg production in Bangladesh and the factors affecting it.

**Objective:**

1. To find out Bacteria found in farm egg shell.
2. To see the percentage of these bacteria in egg shell
3. To know hygienic condition of the egg

**Chapter II**

**Review Of Literature**

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

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.

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.

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.

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

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Humphrey T.J (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 allows salmonellas to invade yolk contents, have taken place.

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

Wall *et al.* (2008) also found that the age of hens did not affect the total count or the presence of *Enterococcus*.

**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 district of Chittagong.

**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 appendorf 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

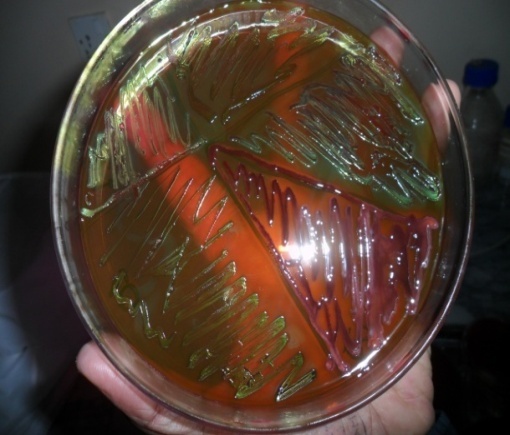
Di sodium phosphate: 2.5

**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 will grow 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)

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**Fig:Pink colony on ManConkey agar *(E. coli )*  Fig:Metallic sheen colony in EMB agar (*E. coli).***

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**Fig-Salmonella in** **ManConkey agar**

**Chapter IV**

**RESULTS AND DISCUSSION**

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

Results were Salmonella is 30 %, E. coli is 54% and 6% found negative in overall all market. In different markets, Pahartoli, Kazir dewri market found 100% contamination where it is 80% in Riaz Uddin Bazar 90% in Jhaotola and Karnafuli market. 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 :** Data of collected egg from different farms

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Total | Oraganism (Positive) | Salmonella(%) | E. coli(%) | Negative(%) |
| Pahartali | 10 | 40 | 60 | 0 |
| Jhaotola | 9 | 40 | 50 | 10 |
| Riaz Uddin Bazar | 8 | 40 | 40 | 20 |
| Kazir Deuri | 10 | 40 | 60 | 0 |
| Karnafully Market | 9 | 40 | 50 | 10 |

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

|  |  |  |
| --- | --- | --- |
| Criteria | Number | Percentage (%) |
| Salmonella | 16 | 32 |
| E. coli | 27 | 54 |
| Negative | 7 | 14 |

The table shows that the eggs more densely contaminated with *E. coli* than *Salmonella* which clearly indicate fecal contamination is very common in the farm.

The study reveals that the contamination is higher in the Kajir deuri and Pahartoli.it may caused due to long time storage in the market. As eggs stay longer, their resistance reduced enabling these organisms to penetrate into the egg content. (Etches, 1992)

Besides hygienic condition is also poor in the farm level. So contamination from farm is very common .On other hand rearing system is an important factor of bacterial contamination.

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 overall significant difference, in higher total bacterial count, *Salmonella* and coliforms/*E. Coli* for floor system compared to cage system was expected andother studies report similar results (De Reu *et al*. 2005b) (Singh *et al,* 2009).

Floor contamination also causes contamination of egg shell.

A study of McCracken *et al.* (1996) agrees with this observation. A wet excreta is a bigger problem in the floor system than in the cage system because the hens may come into contact with the manure more easily in the floor system.

**Chapter V**

**CONCLUSION**

We conclude from this study that the farm eggs are 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. Finally the trade people must be transport egg from good source and good hen farms because the type of rearing (cage or floor)greatly effect on quality of egg and also from countries empty from dangerous zoonotic diseases. This study was a short term pilot screening study designed within the frame of available time and finance resources in order to get a general picture of the hygienic conditions on some egg layer farms. In order to get more reliable results more duplicates and dilutions are needed.

**Chapter VI**

**REFERENCES**

Baker, R. C., 1974. Microbiology of eggs. J. Milk Food Technol.37:265–268.

[Bruce](http://link.springer.com/search?facet-author=%22J.+Bruce%22) J., [E. M. Drysdale](http://link.springer.com/search?facet-author=%22E.+M.+Drysdale%22) 1994 .Trans-shell transmission[Microbiology of the Avian Egg](http://link.springer.com/book/10.1007/978-1-4615-3060-2) 1994, pp 63-91

Board, R G. & Tranter, H S. (1995). *The microbiology of Eggs*. In: Haworth (Ed. 4). Egg science and technology*.* 81- 104. New York

Colins C. H., Lyne P. M. (1976).In Microbiological Methods. 4th edn. Laboratory Techniques Series. Buttenworths, London.

De Reu, K., Grijspeerdt, K., Heyndrickx, M., J., Uyttendaela, M. & Herman, L. (2005a). The use of total erobic and Gram-negative flora for quality assurance in the production chain of consumption eggs. *Food control, 16: 147-155*

De Reu, K., Grijspeerdt, K., Heyndrickx, M., Zoons, J., De Baere, K., Uyttendaele, M., Debevere, J. & Herman, L. (2005b). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. *British Poultry Science, 46:2, 149-155*

Etches, R. J. (1992). The influence of housing aspect of egg quality. In: Proceedings Of

19th Worlds Poultry Congress. Amsterdam, Netherlands. Worlds Poultry

Congress. Amsterdam, Netherlands. Worlds Poultry Science Association, 3:

164-167

Forsythe, R. H., Ayres, J. C., And Radlo, J. L. 1953. Factors affecting the microbiological populations of shell eggs. Food Technol., 7, 49-56.

Forsythe, S. J.. and P. R. Haves. 2000. Food Hygiene. Microbiology and HACCP. Aspen Publication. Gaithersburg. MD.

Garbutt, J. (1997). *Essentials of food microbiology.* Arnold a member of the Hodder Headline Group.

London

Gillespie, J. M.and scott , W. J. 1950. Studies in the preservation of shell eggs. IV. Experiments on the mode of infection by bacteria. Australian J. Appl. Sci., 1, 514-530.

Haines , R. B. and Moran, T. 1940. Porosity of, and bacterial invasion through, the shell of hen's egg. J. Hyg., 40, 453-461.

Humphrey T.J 2002 .Contamination of egg shell and contents with *Salmonella enteritidis*: a review [Volume 21, Issues 1–2](http://www.sciencedirect.com/science/journal/01681605/21/1), January 1994, Pages 31–40

Kretzshmar-McCluskey, V., Curtis, P. A., Andersson, K. E., Berry, W. D. & Kerth, L. K *.* (2009). Influence of hen age and strain on eggshell exterior, eggshell interior with membranes, and egg contents of micro flora, and on Salmonella incidence during a single production cycle. *J. Appl.* *Poult. Res. 18 :665–670*

Lorenz, F. W., Starr, P. B., Starr, M. P., And Ogasawara, F. X. 1952. The development of Pseudomonas spoilage in shell eggs. I. Penetration through the shell. Food Research, 17, 351-360.

Madigan, T M. & Martinko, M J. (2006). Brock *Biology of Microrganisms*. Pearson Education Inc. (Ed. 11). United States Of America

Marianne Chemally,Adelene Huneu –Salau N, Anne Labbie, Catherine Houdayer, Isabelle Petetine,2 And Phillippe Fravalo. Isolation of Salmonella enterica in Laying-Hen Flocks and Assessment of Eggshell Contamination in France Journal of Food Protection, Vol. 72, No. 10, 2009, Pages 2071–2077

Mcnally, E. H. 1952 .Effect of drying after washing in the incidence of eggs infected by spoilage micro-organisms. Poultry Sci., 31, 1102-1104.

McCracken, K.J., McAllister, A. & Johnaston, R. (1996). Effects of processing of layer diets on food intake, excreta production and performance. *British Poultry Science 37:61-62*

# Musgrove, Michael T, Jones, Deana R,Northcutt, Julie K. Cox, Nelson A. Harrison, Mark A. Identification of *Enterobacteriaceae* from Washed and Unwashed Commercial Shell Eggs [Journal of Food Protection®](http://www.ingentaconnect.com/content/iafp/jfp;jsessionid=pc2ynbfyab1f.alice), Number 11, November 2004, pp. 2368-2626, pp. 2613-2616(4)

Rizk, S. S., J. C. Ayres, and A. A. Kraft, 1966a. Disinfection of eggs artificially inoculated with salmonellae. Poultry Sci.45:764–769.

Roberts, R J. (2004) .Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens. J.Poultry. Science. *41: 161-177*

Scott, T A. & Silversides, F G. (2000). The effect of storage and strain of hen on egg quality. *Poultry Science, 79:1725-1729*

Singh, R., Cheng, K M. & Silversides, F G. (2009). Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poultry Science, 88: 256-264*

Smeltzer, T. I., K. Orange, B. Peel, and G. Runge, 1979. Bacterial penetration in floor and nest box eggs from meat and layer birds. Aust. Vet. J. 55:592–593.

Smeltzer, T. I., K. Orange, B. Peel, and G. Runge, 1979. Bacterial penetration in floor and nest box eggs from meat and layer birds. Aust. Vet. J. 55:592–593.

Smith, A., Rose, S P., Wells, R G. & Pirgozliev, V. (2000). The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. *British* *Poultry Science, 41: 168-173*

Stokes, J. L., W. W. Osborne, and H. G. Bayne. 1956. Penetration and growth of *Salmonella* in shell eggs. Food Res. 21:510–518

Todd, E.C.D. (1996). Risk assessment of use of cracked eggs in Canada. *International Journal of Food Microbiology, 30: 125-143*

Wall, H., Tauson, R. & Sorgjerd, S. (2008). Bacterial Contamination of Eggshells in Furnished and Convential Cages. *J. Applied Poultry Res. 17:11-16*