**Chapter -1**

**INTRODUCTION**

Ducks are considered as a second common poultry in the world. and are raised primarily for meat purpose. There are approximately 42.124 million duck populations in Bangladesh (**Bangladesh Economic Review 2012**), of which most of ducks are reared in backyard system. They are easy to raise, hardy and less susceptible to many of the common poultry diseases (**Ali and Islam 1995**). Duck raising provides subsidiary income to landless, marginal and small farmers (**M. M. Rahman *et at* 2009)**. Most of the ducks in the country are indigenous type and are reared by the rural farmers under scavenging condition with forage in water bodies like ponds, ditches, tanks etc. we know that duck rearing is superior to deshi chicken rearing to some extent because duck provides more eggs than chicken(**Isalm et al 2002).** Furthermore,the weight of ducks egg is higher (average weight 57-60g) than that of chickens egg (average weight 55g) (**Islam *et al.* (2002**), **Ravindran *et al.* (1984), Alam and Hossaion** (**1989**).). In addition, duck can be reared in flood affected area where chicken rearing is not possible. The indegenous ducks are more habituated with the ordinary feeding, management provided by the small scale as well as landless farmers.

Poultry products especially duck eggs and egg products are nutritive and vital constituent in human diet. The eggs and associated products play a crucial role in human nutrition in developing country like Bangladesh (**Vaclavic & Christian, 2014, Hasan et al., 2009**). Eggs are enriched with protein, minerals, fat and different vitamins like vitamin B12 (**Kassis et al., 2010**). Microbial contamination of egg has important outcome to the poultry industry and illness from contaminated egg is a serious public health problem around the world. The significance of these diseases in humans can vary from mild symptoms to life threatening situation [1]. The egg and its products are an important component source of necessary nutrients and a major food within the human diet. In spite of the antibacterial factors, it can be infected with different bacteria such as Salmonella spp., Listeria monocytogens, Campylobacter jejuni and Escherichia coli. Salmonella and E. coli are a major food-borne bacterial pathogen, with poultry and poultry products being a primary source of infection to humans (**Sharma and Carlson, 2000**). Poultry are considered an important source of food borne diseases and the illnesses were associated with the consumption of contaminated eggs. However, consuming inaccurately treated eggs and egg products can causes food borne diseases like Salmonellosis (**Gras et al., 2014**). It is also considered as a major public health burden in developing countries like Bangladesh, Pakistan, and India. Salmonellosis is also considered as an important food borne disease in developed world reporting million of human cases. **(Frenzen et al., 1999; Herikstad et al., 2002**). Broad range of Salmonella spp like Salmonella choleraesuis, S. enterica, S bongori, S. typhi, S paratyphi and S. typhimurium causes gastrointestinal (GIT) diseases and typhoid fever. In a rough estimation, Salmonella spp causes typhoid fever to 16 million people, gastroenteritis to 1.3 billion people causing 3 million deaths annually worldwide. Human cases of Salmonellosis caused by S. enteritidis increased recently due to ingestion of poultry products specifically eggs (**Guard, 2001**). Additionally presence of E. coli in egg shell also possess a considerable public health hazards and economic loss in poultry industry.

However, reusable egg trey is a potential source for contaminating egg shell by Salmonellosis and colibacillosis in developing country like Bangladesh (**Aoust et al., 2000**). The egg consumption is considerably increased in Bangladesh in past two decade due to promotion of egg as an ideal food by GO and NGO. So it could be act as a potential vehicle of Salmonella and E. coli transmission in human. While the EU members and other develop worlds have introduced statutory surveillance program to reduce the incidence of human Salmonellosis but monitoring of Salmonellosis in developing country like Bangladesh is still primitive type. In some cases, authority is not concern about monitoring of Salmonella and E. coli in farm level. The reasons behind the ignorance may be constrain of resource and facility.

In addition, small scale backyard duck farm is predominant in Bangladesh with minimum biosecurity practices unlike other large scale commercial production system; enhance the chance of infection to the birds. In Bangladesh, however, there are no directives to control the process of egg production (**Hope et al., 2002**) or limited study to evaluate the quality of eggs in Bangladesh. This investigative work is proposed to address this issue and was focus on the bacterial load especially Salmonella and E. coli associated with egg shell of duck.

**Objectives of current study:**

1. To estimate the prevalence of *Salmonella* from shells of duck eggs in various local markets of Chittagong.

2. To estimate the prevalence of *E. coli* present in the duck egg shell in study area.

**Chapter- 2**

**REVIEW OF LITERATURE**

Pertinent literatures on Salmonella infection in eggs and egg products in table egg of commercial layer farm and associated factors are reviewed in this chapter. The purpose of this chapter is to provide up to date information concerning the research work which is addressed here. Information related to the current study is presented below under following sub headings

**2.1 Salmonella**

**2.1.1 Taxonomy and characteristics of Salmonella:**

Salmonella have been known and responsible for causing diseases in human and animal since it was discovered by Dr Daniel Salmon .Salmonella Like other Enterobacteriaceae, are motile, non spore forming and facilitative anaerobes. Salmonella reduce nitrates to nitrites, ferment glucose and negative in oxidase .Salmonella consists of two species – Salmonella enterica and Salmonella bongori. Salmonella enterica consists of six subspecies (ssp.) under which there are 2500 serovars [11] The subspecies of S. enterica being divided as S. enterica subsp. enterica (I), S. entericasubsp. salamae (II), S. enterica subsp. arizonae (IIIa), S. enterica subsp. diarizonae (IIIb), S.enterica subsp.houtenae (IV), and S. enterica subsp. indica (VI) **(Popoff and Gheesling,**

**2003 ; Popoff et al., 2001 ; Tindall et al., 2005).** All Salmonella strains are serologically classified using Kauffmann-White scheme **(Popoff and Gheesling, 2003 ; Popoff et al., 2001 ; Tindall et al., 2005).** The majority of theSalmonella serotypes belong to S. enterica subsp. enterica (about 60%), followed by subspecies salamae (20%), diarizonae (13 %), arizonae (3.8 %), houtenae (2.8%) and indica (0.45%).Only (0.8%) belong to the second species Salmonella bongori **( Pignato .et al., 1998)** Strains that belong to S. enterica subsp. (S. enterica subsp. entericae), are frequently pathogenic to humans and mammals while those belonging to subspecies II, IIIa, IIIb, IV, VI and Salmonella bongori are usually isolated from reptiles and other cold- blooded animals**(Brenner et al., 2000).**

 **2.1.2 Salmonella in animals**

Salmonella are widely distributed in the animal kingdom, including a wide range of wild and

domestic animals and can be excreted in their feces. The degree of host adaptation varies between Salmonella serotypes and affects the pathogenicity for man and animals **(Tsolis et al., 2011).** For epidemiological reasons, it is common to place the Salmonella into three groups depending on their pathogenic reactions. The first group of serotypes is infectious and host adapted to only humans. These include serotypes such as S. typhi, S. paratyphi A and S. paratyphi C. This group includes the organisms associated with typhoid and the paratyphoid fevers, which are the most serious of all the diseases caused by Salmonella. The second group

is host adapted serotypes to animals, although some of these may also be human pathogens. Included are S. gallinarum (poultry), S. dublin (cattle), S. abortus-ovis (sheep), and S. choleraesuis (swine). The third group is unadapted serotypes with no host preference. All these serotypes are potentially pathogenic for humans and animals and they include most food borne serotypes **(Jey, 1996).** However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis, and chicken products are widely acknowledged to be a significant reservoir for Salmonella. They have frequently been incriminated as a source of Salmonella contamination and consequently thought to be major sources of the pathogen in humans Furthermore, one of the commonest causes of Salmonella infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat **(Uyttendaele and Lips, 1998 ; Panisello et al** ., **2000)** The incidence of Salmonella in poultry has been well determined in many countries such as (United States, Belgium, UK, Malaysia, Spain and Japan), and the level of contamination by Salmonella ranged from 20% to 89% from total poultry population **(Jey, 1996 ; Uyttendaele and Lips, 1998 ; Jørgensen et al., 2002 ; Rusul and Radu, 1996 ; Capita et al., 2003** ; **Limawongpranee et al., 1999)** Motile zoonotic Salmonella serovar from poultry farm was isolated in Bangladesh. But there is no specific data of outbreak related to food born Salmonellosis **(Barua et al., 2012).**

**2.1.3 Salmonellosis in human**

Salmonellosis, a food borne disease occurs through ingesting pathogenic serotypes of Salmonella. Food borne Salmonellosis is caused after penetration and passage of organism in the intestinal epithelium from gut lumen. Two toxins namely, an enterotoxin and a cytotoxin are involved in pathogenesis of Salmonellosis in animal and human **(Jay,** **Musgrove et 1996;** **al., 2006; Braden et al., 2006).**

Salmonellosis, an infectious disease in man and animal manifested in three form like gastroenteritis,( nausea-fever-vomiting and diarrhea), enteric fever (typhoid and paratyphoid) and septicemia (fever, anorexia, anemia, lesions in viscera) **(Samuel, 1996).** Human infections are usually associated with animal contact and the consumption of contaminated food products such as poultry, meat and other dairy products **(Uyttendaele et al., 1998).** Salmonellosis is often considered as asymptomatic self limiting disease which can causes fatal effect on young and immune compromised personnel **(Wilson et al., 2003).** Non-typhoidal Salmonella strains, caused by Salmonella serotypes other than S. typhi and S. paratyphi are important causes of food borne diseases in human and animal. However, the majority of cases are self-limiting gastroenteritis **(Kariuki et al., 2002).**

The clinical symptoms are usually nausea, vomiting, abdominal pain and diarrhea with or without fever which appears within 8-72 hours of pathogen contact. Few (<5%) of the patients develop invasive Salmonella infections or bacteremia and about 10% of those with invasive disease develop localized infections. During the past decade, there had been a significant world-wide increase of non-typhoidal salmonellosis especially in industrialized countries including The United kingdom (U.K), Germany, France, Austria, Denmark, and The United States of America**(Little et al.,2007; Musgrove et al., 2006; Braden et al., 2006;)**

**2.1.4 Salmonellosis outbreaks linked to eggs**

S. enteritidis is an important human pathogen though it emerged from poultry and human S.enteritidis infections showed a dramatic increase turns to become the most commonly isolated serotypes in many countries **(Rabsch et al., 2001).**S. enteritidis is prominent as a major food-borne pathogen in developed world like USA, Europe and UK **(Velge et al., 2005).** However, despite a recent reduced incidence it is considered as most common serotypes causing GIT infection in those countries. In the United States food-borne Salmonella are estimated to cause approximately 1.3 million illnesses, 15,000 hospitalizations, and 500 deaths each year. About 300,000 of these illnesses may be attributable to. S. enteritidis **(Mead et al., 1999).** Most, perhaps as many as 80%, of S. enteritidis infections are associated with eggs (Louis et al., 1998). During the 1980s and 1990s, S. enteritidis emerged as an important cause of human illness in the United States, and the rate of S. enteritidis isolates reported to CDC increased from 0.6 per 100,000 populations **(Guo et al., 2012).** Case-control studies of sporadic infections and outbreak investigations found that this increase was associated with eating raw or undercooked shell eggs **(CDC, 2000 ; Morse et al., 1994; Guo et al., 2012; Medliton** **et al., 2013).**

There were a total of 997 reported outbreaks of S. enteritidis infection in the United States from 1985–2003 which resulted in 33,687 illnesses, 3,281 hospitalizations, and 82 deaths. The number of reported outbreaks of S. enteritidis infection in the United States increased from 26 in 1985 to a high of 85 in 1990, with a gradual decrease thereafter to 34 outbreaks in 2003. In addition, the number of cases in outbreaks each year has decreased, from a high of 2,656 in 1990 to a low of 578 cases in 2003. A food vehicle was confirmed in ~ 44% of outbreaks of S. enteritidis infection in the United States. Among outbreaks of S. enteritidis infection with a confirmed food vehicle from 1985– 2003, 75% of outbreaks had vehicles that were either primarily egg-based or that contained egg ingredients **(Braden, 2006)**. There are no sufficient data in developing country like Bangladesh due to limited epidemiological study. Further, where incidence data are available these are frequently outdated. In addition, under-reporting of cases and the presence of other infectious diseases considered to be of high priority may have also overshadowed the problem of Salmonellosis.

**2.2 General characteristics of Egg shell and Microbial invasion:**

**2.2.1 Egg as a food**

Eggs are among the most nutritious foods on earth and can be part of a healthy diet. However, they are perishable just like raw meat, poultry, and fish. Unbroken, clean, fresh shell eggs may contain S. enteritidis bacteria that can cause food-borne illness. While the number of eggs affected is quite small, there have been cases of food-borne illness in the last few years .Egg proteins have a high biological value, and are often used as a standard to compare the quality of other proteins in foods. In addition, eggs contain unsaturated fatty acids, iron,

phosphorus, trace minerals, and vitamins.

**2.2.2 Egg production**

Egg formation is a process that occurs in the ovary and the oviduct of the chicken’s female reproductive system. Formation of the unfertilized egg starts with generation of the yolk (ovum) in the ovary, followed by its release to the upper part of the oviduct. Subsequently, yolk membrane, albumin, and shell are produced during the pass of the yolk through the different portions of the long tubular oviduct. Laying chickens produce a complete shell egg approximately every 24 h, which is the time required for the egg to reach its full size and shape **.**

**2.2.3 The structure of the egg**

An egg consists of a yolk at the centre, surrounded by albumin (white), both of which are enclosed within the shell. The yolk structure consists of the latebra, the germinal disc or blastoderm, and a series of layers of light and dark yolk, which are enclosed by the vitelline membrane .The albumin is made of four layers, from the inside to the outside of the egg, that includes the chalaziferous that extends as a rope-like structure and keeps the yolk in the center of the egg, the adjacent inner thin layer, the dense albuminous sac, and the surrounding outer thin layer. The outer covering consists of two keratin-like inner and outer membranes, with 0.01-1.02 mm total thickness, encircled by the shell. The egg shell is composed of 94% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate, and 4% protein. The shell is a porous structure (~10,000 pores/shell), has an average thickness of 0.31 mm, and is covered by the cuticle, which is a protein-rich coating that constitutes the most external layer of the egg **.**

**2.2.4 Contamination of eggs by Salmonella**

Bacterial infections of shell eggs and its content can occur in two different ways: either vertically or horizontally. Of these, the first is mainly associated with Salmonella spp., especially S. enteritidis.In vertical transmission, Salmonella are introduced from infected reproductive tissues to eggs prior to shell formation. Salmonella serotypes associated with poultry reproductive tissues that are of public health concern include S. enteritidis, S. typhimurium and S. heidelberg. Among the different serotypes, S. enteritidis may be better able to achieve invasion, and as a consequence, may be found more frequently in reproductive tissues **(Keller et** **al., 1995 ; Mizumoto et al., 2003 ;** **Howard et al., 2005).** Horizontal transmission is usually derived from fecal contamination on the egg shell. It also includes contamination through environmental vectors, such as farmers, pets and rodents.

Many different serotypes of the genus Salmonella can be involved. They may be able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage by Salmonella .Vertical transmission is considered to be the major route of Salmonella contamination and is more difficult to control, while horizontal transmission can be effectively reduced by cleaning and disinfection of the environment.

**2.2.5 Microbial quality of eggs**

The hen's eggs are an excellent example of a product that is normally is well protected by its intrinsic parameters. Externally, a fresh egg has three structures, each effective to some degree in retarding the entry of microorganism: the outer, waxy shell membrane, the shell, and the inner shell membrane. Internally, lysozyme is present in egg white. This enzyme has shown to be quite effective against gram-positive bacteria. Egg white also contains avidin, which forms a complex with biotin, thereby making this vitamine unavailable to microorganism. In addition, egg white has a high pH and contains conalbumin ,which forms a complex with iron, thus rendering it unavailable to microorganism. On the other hands, the nutrient content of the yolk material and its pH in fresh egg make it an excellent source of growth for most microorganism as shown in figure 2.7 **(Jey, 1996).**



**Figure (1):** The physical and antimicrobial defenses of a hen's egg (**Wilson and Powell,** **1998).**

**2.2.6 Organisms per egg at Lay**

The number of S. enteritidis in contaminated eggs varies from egg to egg. Available evidence suggests that most contaminated eggs have very few S. enteritidis bacteria within them at the time of lay. It is the initial contamination level in an egg that is influenced by subsequent distribution and storage practices. In a study of contaminated eggs produced by naturally infected hens, 32 positive eggs were detected **.** Enumeration of their contents found that 72% of these eggs contained less than 20 S. enteritidis organisms. The calculated mean number of S. enteritidis per contaminated egg was 7. However, there were a few eggs that contained many thousands of S. enteritidis bacteria following >21 days of storage at room temperature. In another study of experimentally infected hens, 31 S. enteritidis positive eggs were detected. Enumeration of their contents found that the typical contaminated egg harboured about 220 S. enteritidis organisms. Yet, there were marked differences in levels depending on storage time and temperature. Four of the contaminated eggs contained more than 400 S.enteritidis organisms per egg .

**2.3 Detection techniques for Salmonella in eggs**

Conventional culture methods used for the isolation of Salmonella from eggs include, nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspect colonies are then confirmed biochemically and serologically. These methods are time consuming and take approximately 4-7 days.Since Salmonella are closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are required**.** More recently, a number of alternative methods for the detection of Salmonella in foods have been developed including, immunoassays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques.

**2.4 Salmonella in egg production farms**

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. 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.The overall prevalence of Salmonellosis in Bangladesh is reported to 4-25% in farm level **(Barua et al., 2012).**

**2.4.1 Salmonella in eggs:**

In Great Britain a survey carried out in 2002 found 0.15% of eggs collected at retail outlets to be contaminated with Salmonella spp. and 0.12% were contaminated with S. enteritidis. .In United Kingdom, the Department of Health funded a retail survey of UK produced eggs, were detected Salmonella spp in 0.99% out of the 13,970 samples of 6 eggs (an estimated contamination rate per individual egg of 1 in every 100 boxes of 6 eggs). There was no significant change in Salmonella contamination of UK produced eggs since a previous survey in 2000.In a study done in England and Wales by Public health investigation a total of 12,615 eggs were collected from catering premises from September 2002 to November 2004. Salmonella were detected in 88 (4.2%) of 2,102 pools of eggs. Salmonella were detected from 5.5% of eggs produced in Spain, 6.3% of eggs of unknown origin and 1.1% of eggs produced in the UK but not Lion Quality (0%).Salmonella were not detected from eggs produced in other countries (0%: France, Germany,Portugal and USA) as shown in tables 2.7 and 2.8 **(Little et al., 2007; European Commission,2004**).

In another study done by Pan-London investigation from November to December 2002, 4,356 eggs (726 pooled samples of six eggs) from catering establishments and hospitals were examined within London. Salmonella were detected from seven (0.9%) of the 726 samples. Notably, no Salmonella were isolated from 341 pooled Lion Quality UK produced egg samples, nor from 45 samples produced in France. Salmonella were detected from 4.3% (6 ⁄140) eggs of unknown origin and 0.5% (1 ⁄ 200) UK eggs but not Li**(Little et al., 2007** ; **European Commission,** **200on Quality as shown intables 2.8 and 2.9 4**). In a study done in United Kingdom eggs were collected monthly from a 12 cage- layer flocks in four farms vaccinated with an S. enteritidis bacterin, where possible, hens were also taken for culture at the end of the laying period, and fecal and environmental samples were taken from the laying houses before and after cleaning and disinfection. The total level of contamination by S. enteritidis of both contents and shells found in vaccinated flocks was therefore 33 batches/13,682 eggs (0.24%) and the total of contamination for any Salmonella serotype was 92 batches/13,682 eggs (0.68%) **(Davies and Breslin, 2004).** These results contrast with the findings of testing of eggs from three unvaccinated flocks prior to this study where 21 batches of egg shells from a total of 2,101 eggs (1.0%) and six batches of contents from 2,051 eggs (0.29% ) were contaminated with S. enteritidis **(Davies and Breslin, 2004).** S. enteritidis was found in 67/699 (9.6%) of vaccinated spent hens and 64/562 (11.4%) of bulked fresh fecal samples taken from laying houses. Failure to adequately clean and disinfect laying houses and to control mice appeared to be a common feature on the farms **(Davies and Breslin, 2004).** In a survey of Salmonella contamination in eggs produced outside the UK and on retail sale in England which was carried out between March 2005 and July 2006. A total of 1,744 boxes of six eggs or more were sampled. Salmonella contamination on the egg shell was found in 157 box samples, (one box of every 30 boxes of six eggs had Salmonella contamination of 1,744 samples of six pooled eggs). Of these, 10 also contained Salmonella inside the egg. S. enteritidis was the most common type of Salmonella found. The eggs collected came from eight different countries across Europe, with two-thirds of the eggs collected (66.3%) originating in Spain, France (20.0%) or the Netherlands (7.4%). Salmonella spp. was detected from 13.3% and 0.6% of eggs samples that were produced in Spain and France. In New Zealand a survey carried out by Environmental Science and Research Limited (ESR) in 1994 examined eggs sampled from Otago, Southland and Canterbury. 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). The same survey noted that overall, 64 of 4,090 (1.5%) eggs examined were contaminated with visible fecal material. Most of these (62%)

were collected directly from the producer rather than retail sources. There was no distinction

made in this survey between free ranges, barn produced and caged bird eggs (ESR, 2004). In a study done in Poland a total of 1,200 eggs were purchased in 40 local markets in Olsztyn, Poland were examined for the presence of Salmonella between June 1997 and December 1998. Eggs were obtained from 12 commercial laying flocks laid within 2 days. Salmonella were not found on the shell or inside the eggs. From this study it appears that the incidence of Salmonella on eggs from Olsztyn shops is very low **(Radkowski, 2001)**. In Hawaii a study done on one hundred and six dozen eggs, representing 12 brands were purchased from Oahu supermarkets and cultured for Salmonella. The sampling unit was defined as a carton of 12 large grade A eggs, the eggs from each dozen were separated into two flasks, one containing the shell and other containing the magma (white and yolks). Salmonella were detected in 10 cartons (9.4 percent) of the 106 dozen eggs sample; positive samples were from shells only. In 2005 a study done in Mexico City, four hundred (400) eggs were collected from market, supermarket, and smaller grocery stores located in different zones within Mexico City. In all cases, eggs corresponding to 10 brands (40 eggs per brand). One S. enteritidis contamination egg yolk was obtained, representing 0.25% of total samples. Also, 11 additional bacterial genuses other than Salmonella spp. was found in (egg yolk, egg albumin and eggshell), including Acinetobacter spp., Alcaligenes spp., Bacillus spp., Branhamella spp., Edwardsiella spp., Hafnia spp. Klebsiella spp., Serratia spp., Shigella spp., Staphylococcus spp., and Yersinia spp**. (Martinez et al., 2005).** In Canada a study done on seven layer flocks with S. enteritidis in their environment were investigated to determine the numbers of hens infected with S. enteritidis. Environmental samples from each flock were collected and consisted of 60 randomly collected fecal droppings and 12 dust/fluff samples from egg belts (where present) or from vents, fans and walls. Salmonella spp. was isolated from all previous flocks and found that environmental isolates in each flock were recovered from fecal samples, while dust / fluff samples were culture-positive in only three flocks. Cultures of tissue of 580 hens from seven flocks detected 26 (4.5%) S. enteritidis infected hens from two flocks. In one flock 2/150 hens were infected with S. enteritidis and no Salmonella spp. were isolated from 2,520 eggs (one day old). In the second flock where 24/150 hens were infected with S. enteritidis were isolate. S. enteritidis were isolated from one sample of egg contents and from one sample of cracked shell from among 14,000 eggs (one day lay). The overall prevalence of S. enteritidis contamination of the eggs from the two flocks with infected hens was less than 0.06 % **(Poppe et al., 1992).** A study in uttrprodesh of India found 22-39% overall salmonella infection in egg from different source of backyard, poultry, duck and quail egg. But the study cited higher frequency in table egg from commercial farm up to 28%. This study is also close to our current findings of Salmonella prevalence. In an earlier study conducted in India,incidence level of 10.8% in 534 chicken eggs was observed by**( Sharma and Thakur ,2003)** with higher incidence on egg shell surface than internal contents. Begum et al., (2010) has been conducted a study on table egg in Dhaka city found overall 14-17% inner egg and 18- 31% egg shell eggs are contaminated with Salmonella Spp. Another study in Khulna city found 8% Salmonella spp contaminating the egg where 3% S. typhi and rest of Salmonella enterica.

**Chapter -3**

**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 CVASU.

**Data collection procedure**

Questionnaire had developed to know the source of egg from where it came from the retailer in the market.

**Sample collection**

Samples were collected from 5 different markets, 2 shops from each market. Samples were collected from total 50 duck eggs. Five egg samples from every shop were selected both from back yard farm and commercial duck farm.. 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 had done by wearing hand gloves.

**Isolation and identification of bacteria**

Isolation and identification of bacteria from egg surface sample were sample were done in PRTC laboratory under pathogen free condition in laminar air flow.isolaton 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).

**Chapter- 4**

**Results and discussion**

**Collection of data:**

**Tab 1:Prevalence of E.coli and Salmonella at different market in Chittagong.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Market | E.coli. | % | Salmonella | % | No results | % |
| Pahartoli Market (10)  | 7 | 70 | 3 | 30 |  |  |
| Kazir dewri Market (10) | 4 | 40 | 6 | 60 |  |  |
| Jhaotola Market(10) | 5 | 50 | 5 | 50 |  |  |
| Riaz Uddin Market(10) | 2 | 20 | 8 | 80 |  |  |
| Karnafuli Market(10) | 6 | 60 | 2 | 20 | 2 | 20 |

**Tab 2**: **Prevalence of E.coli and Salmonella at backyard and commercial duck farms in Chittagong.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type of sample | E.coli. | % | Salmonella | % |
| Backyard farm egg (35) | 19 | 39.58 | 16 | 33.33 |
| Commercial duck farm egg (13) | 7 | 14.58 | 6 | 12.5 |
| No results (2) |  |  |  |  |

Among the isolation of microorganism E.coli is higher in Pahartoli Bazar (14.58%) than other market. The presence of E.coli and Salmonella in different market has shown in diagram:

**FIG: PREVELNCE OF E.COLI AND SALMONELLA IN DIFFERENT MARKET.**

This chart indicates that closely related markets are more affected with salmonellosis than E. coli .Higher percentage of salmonella present in Riaz uddin bazaar. In backyard farming, E.coli and Salmonella present in higher than commercial duck farm. These indicate that unhygienic management lead to break out E.coli and Salmonella through the egg shell in that farm.

**Fig: PREVELNCE OF E.COLI AND SALMONELLA IN FARMING SYSTEM**

We conclude from this study that 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

**Chapter - 5**

**Conclusion**

E coli and Salmonella can transmit through the eggshell to the day old chick, which hamper the growth as well as death of embryo. In a hatchery, contaminated eggshell is a threat to produce chick. Various factors are responsible for the contamination of eggshell. Sometimes whole management system could responsible for the contamination of eggshell. Conclusion of this study is that free ranges rearing of duck are more vulnerable factor for Salmonella and E coli outbreak. Proper fumigation management system minimizes the contamination of egg and eggshell. In that case, collection of egg should be at proper time, which could minimize the contamination of egg and eggshell.

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