Determination of *Salmonella* in table eggs (shell and content) and associated factors in some selected areas of Bangladesh

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. *Salmonella* are known for its wide range host. It can cause variety of diseases in some hosts while in others, can be asymptomatic. Poultry and eggs are considered as major sources for different pathogenic *Salmonella* serotype. . Eggs produced locally under the small scale layer farm may present a hazard to consumers which may increase the spread of *Salmonella* in the environment. To investigate the occurrence of *Salmonella*, a total of 72 samples were taken from 6 poultry farm in some selected area of Bangladesh. Sampling program was executed between November and December, 2013 and samples were tested using standard laboratory methods. Data was collected through direct interview and structured questionnaire which developed and validated by academic supervisor. The study showed that the true prevalence of *Salmonella* in egg shell and egg contents were 0.093% and 0.068% respectively. The highest apparent prevalence in Udayan poultry farm (50%) and lowest in Liza poultry farm (16.67%). The average frequency of egg shell contamination is positively correlated with re-use of egg trey without disinfection. To the best of our knowledge, this is the first study in Chittagong investigating the of *Salmonella* spp. in eggs in selected local egg production farms. Further detail study is highly recommended.

Keywords: Salmonella, small scale layer farm, table egg, food borne diseases, public health

CHAPTER I
INTRODUCTION

Poultry products especially 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) However, consuming inaccurately treated eggs and egg products can causes food borne diseases like Salmonellosis (Gras *et al*., 2014)

Salmonella is a major food-borne pathogen distributed worldwide and contaminated poultry products, especially undercooked meat and raw eggs are important sources of it (Dhama *et al*., 2013).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) and typhoid fever. However, *Salmonella enterica* subspecies *enterica* is responsible for more than 99% infection in man and animal **(**Carrasco *et al*., 2012**)**.

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. Most of the infections of *Salmonella* are zoonotic in nature except *Salmonella typhi* and *S. paratyphi* . The non typhoidial salmonellosis has been increased dramatically during the past 10 years. The predominant serotypes responsible for non-typhoidal salmonellosis are *Salmonella enterica* serotype *enteritidis* and *typhimurium* (King and Strynadka*,* 2011).

Salmonella spp implicated in a wide range of foods and a food products cause's salmonellosis. Animal originated foods like poultry, poultry products and raw eggs are often contaminated by different *Salmonella* spp. However, other sources of exposure include water, vegetables, fruits, handling of farm animals and pets, and human person-to-person when hand-mouth contact occurs without proper washing of hands **(**FAO, 2002**).**

Human cases of Salmonellosis caused by *S. enteritidis* increased recently due to ingestion of poultry products specifically eggs **(**Guard, 2001**).** Additionally presences of Salmonella spp in egg shell also possess a considerable public health hazards and economic loss in poultry industry. Contamination of egg by *Salmonella* spp may causes at any stage of production like farm, collection, transportation or marketing through vertical or horizontal transmission. Vertical transmission means contamination of egg yolk, albumin, membranes or eggshells. While in horizontal transmission disease is penetrated during or after oviposition through the egg shell from the gut or contaminated feces. However, reusable egg trey is a potential source for contaminating egg shell by Salmonellosis in developing country like Bangladesh (Aoust *et al*., 2000).

Egg and egg products produced from small scale layer farm is a major protein source for people in Bangladesh. 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* 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* in farm level. The reasons behind the ignorance may be constrain of resource and facility. The actual data on prevalence of Salmonellosis in eggs and egg products is poorly documented that prone to zoonotic threat.

In addition, small scale commercial farm is predominant in Bangladesh with minimum bio-security practices unlike other large scale commercial production system; enhance the chance of infection to the birds. However, the data on prevalence and rate of infection in eggs and egg products in Bangladesh is limited. 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 table egg produced in local farms to determine the apparent and true prevalence of egg contamination by Salmonella spp. with the associated factors.

Objectives of current study:

The principal objective of this study is to investigate the presence of *Salmonella* spp. in table eggs inner content and egg shell of selected local small scale commercial farms in Bangladesh. The following specific objectives will be studied:

1. To estimate the apparent prevalence of *Salmonella* in table eggs from layer birds of small scale commercial layer farms in study areas of Bangladesh.
2. To estimate the true prevalence of *Salmonella* in table eggs from studied farms.

CHAPTER II

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 (Yan *et al*., 2003). *Salmonella* Like other Enterobacteriaceae, are motile, non spore forming and facilitative anaerobes. *Salmonella* reduce nitrates to nitrites, ferment glucose and negative in oxidase (Yan *et al*., 2003).

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. enterica* subsp. *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 the *Salmonella* 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).

2.1.5 *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).

2.1.2 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, 1996; Musgrove *et 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 (Yan *et al.*, 2003). 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;)

2.1.4.2 Types of food involved

Different types of food are involved in *Salmonella* infection depending on production chain, consumption pattern and prevail *Salmonella* serotypes (EFSA, 2004). Most food-borne *S. enteritidis* infections are associated with the consumption of raw eggs and foods containing raw eggs. In fact, 77% to 82% of *S. enteritidis* outbreaks have been associated with grade A shell eggs, or egg-containing foods undercooked eggs and products containing undercooked eggs (FAO, 2002). Salmonellosis outbreaks in the United States implicating eggs and egg products such as homemade ice cream, mayonnaise and others egg products for the transmission of *S. enteritidis* were presented in table 2.5 which contain summary of different studies (Romo, 2004).

2.1.3 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 out-dated. 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. To be safe, eggs must be properly handled, refrigerated, and cooked (USDAFS, 2005).Chicken is the most important bird used to produce eggs for human consumption around the world, and eggs are a unique well-balanced source of nutrients in the human diet. 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. Shell eggs consist of 9.5% shell, 63% albumin, and 27.5% yolk (Romo, 2004).

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 (Romo, 2004).

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 (figure 1). 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 **(**Romo, 2004**;** De Reu K, 2006; EFSA, 2005).

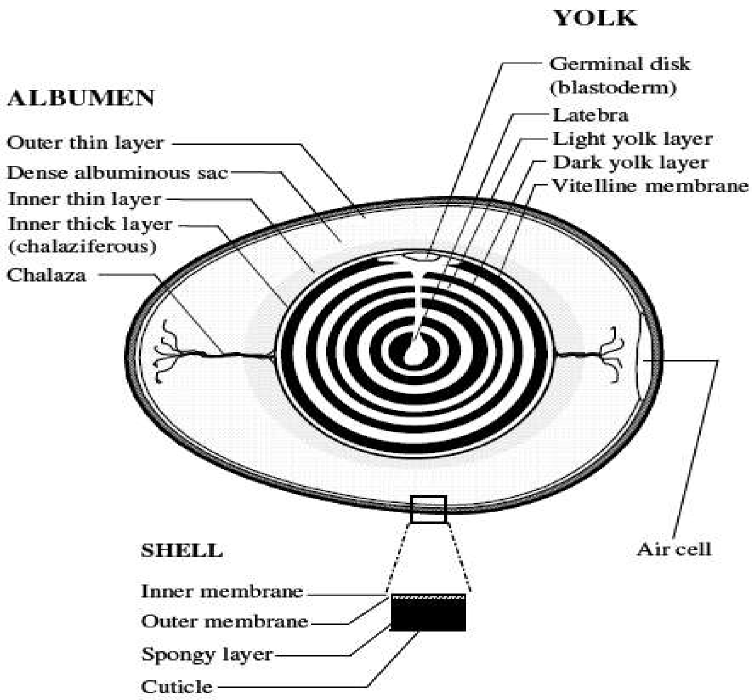

Figure (1): Schematic representation of the parts of the egg **(**Romo, 2004**).**

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* (EFSA, 2005). 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* (FAO, 2002). 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 (FAO, 2002).

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 (about 9.6) 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 (about 6.8) make it an excellent source of growth for most microorganism as shown in figure 2.7 (Jey, 1996).

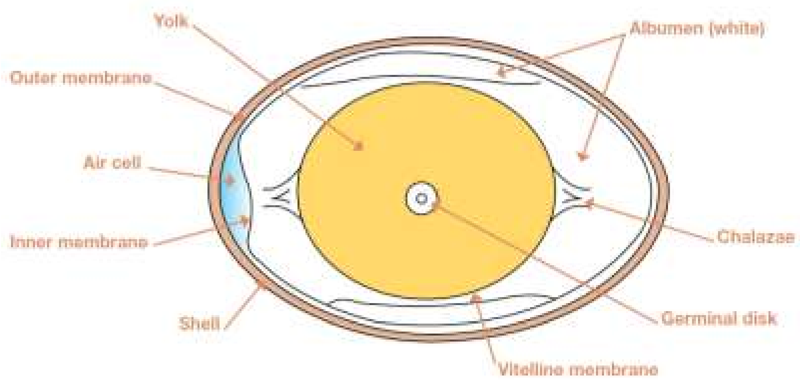

Figure (2): The physical and antimicrobial defenses of a hen's egg (Wilson and Powell, 1998).

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. If the egg is handled under conditions that allow growth of the bacteria in the egg, then the initial concentration will increase. Nevertheless, some contaminated eggs will arrive at the kitchen with the same number of bacteria within them that they contained at the time of lay.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 (FAO, 2002).

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 (FAO, 2002).

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 (Andrews *et al*.**,** 1998**;** IOS, 2002). 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 (Axelsson and Sorin, 1997)**.**

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 (FAO, 2001). 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* (ACMSF, 2004).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 (Food Standards Agency, 2004). 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 Lion Quality as shown in tables 2.8 and 2.9 (Little *et al.*, 2007 ; European Commission, 2004). 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, respectively. *Salmonella* were not recovered from eggs produced in Belgium, Germany, Portugal, Republic of Ireland or The Netherlands as shown in table 2.10 (Food Standards Agency, 2006).

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 (Ching *et al*., 1991).

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 a study done in Albania seventy-nine shell egg lots, representing a total of 22,945,520 eggs imported into Albania from many countries during the 2- years period 1996–1997 (69 lots during 1996 and 10 lots during 1997) were investigated for the presence of the *Salmonella* spp. *Salmonella* wer detected in 1 out of 79 (1.26%) analyzed pooled samples, the lot consist of 275,000 eggs, originating from Bulgaria. *Salmonella* were isolated only from the egg shell, but not from the liquid part and was belonging to *Salmonella* group c, but it was not further serotypes as shown in table 2.13 (Telo and Sulaj, 1999)**.** 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). In Brazil a study done on 614 boxes corresponding to 12 flocks (A-M) of white laying hens to investigate the presence of *Salmonella* spp. in flocks of white laying hens. Fresh samples of cecal feces were collected from different farms were inspected at arrival (one day old) and eggs were collected and placed in sterile trays. *S. enteritidis* was detected in feces from four flocks which consisted of 129 boxes (33.3%) as shown in table 2.14. *Salmonella* were studied in 500 eggs at 52 weeks from each previous four positive flocks and one negative flock. *S. enteritidis* was found in one egg from flock A (0.2 %) and from 10 eggs from flock L (2.0 %) as shown in table 2.15 (Gama and Fernandes ., 2003).

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. Another study in north India by Sing *et al.*, (2010) reported lower frequency in egg shell (1-2%) but higher frequency in egg content (8%). The collected samples were from retail and wholesale market of North India. The author reported overall prevalence of 3-5% salmonella in egg samples. 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*.

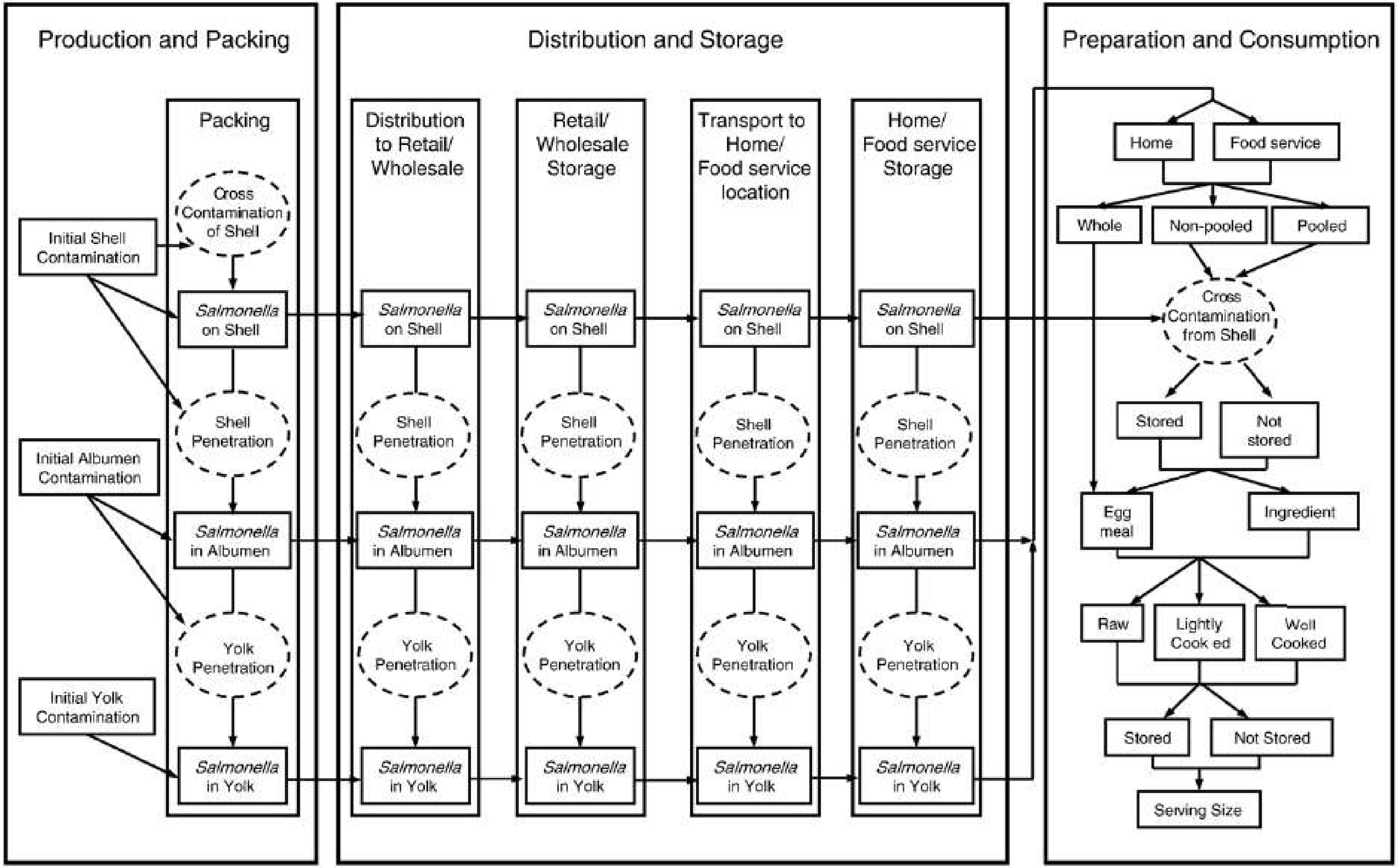

Figure: 3: Possible way of contamination in egg and egg shell from production to consuming (Martelli and Devies, 2012)

CHAPTER III

MATERIALS AND METHODS

3.1. Study area

Small scale commercial layer farm (N=6) of Chittagong (n=3) and Noakhali (n=3) were selected purposively for the study. The Jafar Paoultry farm (farm 1), Hoque Poultry farm (farm 2) and Kachwya Poultry farm (farm 3) is located in moddhom chorkakra, charparbotipur and bagtara village of Companygonj upazilla respectively. The Udayan Poultry farm(farm 4), Islam Poultry farm(farm 5) and Liza Poultry farm(farm 6) is located in Satkaniya, potiya and chandanish of Chittagong districts. The landscape characteristics of study area are consisting both high and lowland area along with coastal belt. The livelihood of the farmers depends on the poultry farming along with other income generating activity.

3.2. Study design:

A cross sectional study was conducted in four different upazilla of Noakhali and Chiitagong in order to investigate *Salmonella* spp and associated factors for prevalence of *Salmonella* spp in table eggs of small scale layer farms. The studied farm 1, 2, 3, have 4000, 2000 and 2500 birds of Noakhali of Noakhali and farm 4, 5, 6 have 1800, 7000 and 4000 birds of Chittagong. The studied farm 1, 2 and 6 having Isa brown stain while 3, 4 and 5 are rearing Hisex brown of strain of layer bird.

3.3. Study period:

The study was conducted between October to December 2013 during internship period.

3.4. Source population and sample collection:

The egg laying commercial farms were selected randomly from Chittagong and Noakhali as sourse population for the study. Only egg laying flocks of each farm were used to develop the sampling frame where smallest unit consist of 2000 birds. Initially 12 fresh eggs were collected representing the egg laying flocks from the selected farms and transported to

Microbiology Department of Chittagong Veterinary and Animal Sciences University (CVASU). Obtained samples were transferred carefully with a layer of sterile cotton for avoiding the breakage of egg. Each egg was given a unique identification number according to the farm identity and strain of the farm. The samples were preserved in 4^0C until processing.

The swabbing techniques were used to evaluate the *Salmonella* spp in egg shell. The inner masses were inoculated in media for detection of *Salmonella* in egg inner mass. The methods were followed as described by Safaei *et al.,* 2011.

Figure 1: Collection and Packaging of egg in egg trey for marketing in Liza farm

3.5. Recoding risk factor associated with egg contamination with *Salmonella* spp:

A structured record keeping sheet was developed, validated and used to collect the necessary information. The questionnaire contained closed, semi closed and open ended question. The questionnaire was grouped on: 1) basic information related to farm identity, farm composition and bird demography, 2) Farm management system related to biosecurity level of farm, 3) Egg collection, preservation and marketing procedure. Detailed questionnaire are given in appendix I. All information is collected by face to face interview to the farm owner, manager or attendance as well as physical examination by author. Though the questionnaire was developed in English it was administered in Bangla. Details questionnaire is added in apndix-1.

3.6. Laboratory evaluation

3.6.1. Preparation of inoculums:

To produce statistically reliable results, the minimum number of eggs were selected based on the number of samples from which the standard error starts converging to an asymptotic value (Safaei *et al.,* 2011). Six of 12 randomly selected egg samples from each group were subjected to laboratory evaluation,

For the preparation of inoculums of egg shell surface, a sterile cotton swab wetted in sterilized normal saline solution (NSS) was used for surface swabbing and it was re-immersed into the same tube having 10 ml normal saline solution. The surface of each of the eggs was first disinfected with 70% ethanol and the eggs were broken. Finally, the content thoroughly mixed for approximately 1min using centrifuge machine for preparation of inoculums of inner content.

3.6.2. Media used for laboratory evaluation:

Nutrient agar (Oxoid Ltd., P^H: 6.2±0.0) was used as primary enrichment media for *Salmonella* spp .Three selective media were used for the isolation of the bacteria. The XLD agar (Oxoid Ltd., P^H 7.4±0.2), SSG agar (Merck, P^H: 6.9±0.2) and TSI agar (Oxoid Ltd., P^H: 7.2±0.2) for *Salmonella.*

3.6.3. Culture protocol for isolation and identification of Salmonella spp

For the isolation of *Salmonella,* 1ml of prepared inoculums from egg shell and egg inner mass was inoculated in screw cap test tube containing nutrient agar (primary enrichment media) and incubated for 24 hours at 37^0C. After incubation a loopful of nutrient agar was streaked on to both XLD Xylose Lysine Deoxycholate (XLD) and Salmonella-Shigella(SS) agar. The agar plates then were incubated at 37^0C for 24 hours. The colonies with black center in XLD and blackish growth in SS were considered as presumptive *Salmonella* spp. and then these colonies were subjected to confirmation through biochemical test (TSI stab) for *Salmonella.*

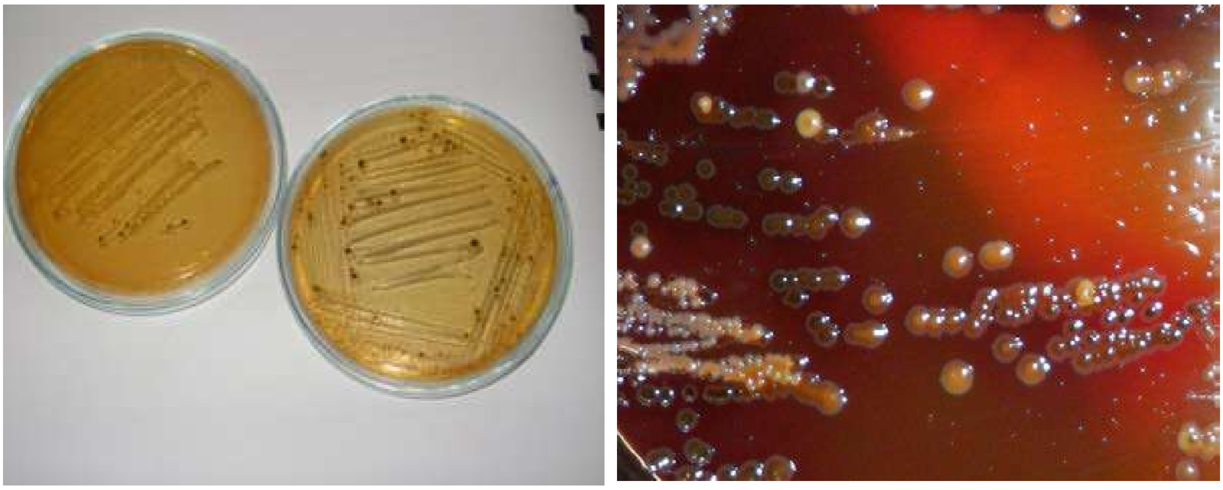

Figure: 3 Black centered colony in XLD agar suspected to *Salmonella* spp.

3.6.4. Gram's staining

Gram's staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria. Therefore**,** the suspected colonies were taken over a slide to make a thin smear that was done by sliding the edge of another glass slide across the glass slide containing the sample and then allowed it to air dry. The smear was then heat fixed by quickly passing it two to three times through a flame. After fixation the Gram's staining was done by follows: Crystal violet (primary stain) was used for two minutes, Gram's iodine (mordent) for 1 minute, Acetone (decoloriser) for 5-7 seconds and finally, Safranin (counter stain) for 1 minute. Gently rinsing was done with tape water after every step. The slide was then observed by microscope under 100X with emersion oil and characterization of bacteria was done.

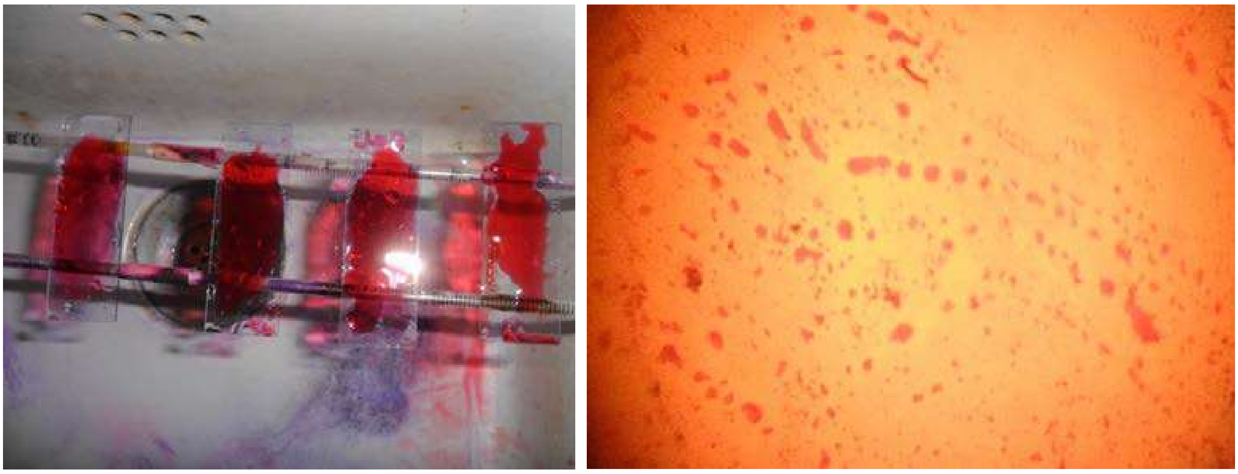

Figure: 4 Gram staining of *Salmonella spp* showing Gram-negative, pink colored, small rod shaped under microscope

3.6.5. TSI slant for Salmonella

A straight inoculating needle was used to pick up isolated colony from culture of isolates. The TSI slant was inoculated by stabbing the butt down to the bottom, and then streaked over the surface of the slant. The TSI slant was then incubated overnight at temperature of 37^{0}C. The positive result for *Salmonella* and *E. coli* were detected based on the properties.

Figure 6: TSI slant before inoculation (left) and red slant with yellow butt with blackening indicating *Salmonella spp*

3.6.6. Data analysis

Data obtained was imported to the Microsoft Office Excel-2007 and transferred to the software STATA/IC-11 for analysis. Descriptive statistics was done by using the STATA software. The associated factor was correlated with high frequency of egg contamination within farm level through descriptive statistics.

CHAPTER IV

RESULTS AND DISCUSSIONS

The total population of the studied farm, ages of the bird, egg production performance and epidemiological data of the farm during study period is added in appendix-2 as reference table. The result of egg shells and egg contents culture are furnished in the table-1. Out of 72 eggs samples, from 36 samples inoculums were made for the three different medias: XLD, SS and TSI for culture.

Table 1: Result of sample culture on XLD, SS agar and TSI for Salmonella isolation and identification.

Farm Name	No of egg samples		Egg shell			Egg inner mass			Microscopic features
	Initial	Tested	XLD	SS	TSI	XLD	SS	TSI	
Farm 1	12	6	4	3	3	2	3	2	Gram-negative, pink colored, small rod
Farm 2	12	6	0	2	1	1	2	1	
Farm 3	12	6	2	3	2	3	3	3	
Farm 4	12	6	4	3	3	3	1	1	
Farm 5	12	6	3	4	3	2	3	2	
Farm 6	12	6	2	2	2	2	1	2	
Total	72	36	15	16	14	10	13	11	

Farm1= Jafar poultry, Farm 2= Hoque poultry, Farm 3= Kachuya poultry, farm 4= Udayan poultry, farm 5 = Islam poultry and Farm 6 = Liza poultry

Colonies were isolated as positive on the basis of characteristic colony color and morphology cultured on XLD and SS agar. Among the six studied farm, a total number of 14 eggs shell and 11(n=36) egg inner content found positive to the *Salmonella spp.* In individual farm, the highest egg shell contamination by *Salmonella* recorded in Farm 1 and 4 (n=4) and none from farm 2. In case of inner content of egg Farm 3 and 4 (n=3) showing highest number of positive in XLD and lowest from farm 2. In case of SS agar highest egg shell contamination was recorded in farm 5 (n=4) and lowest (n=2) in farm 2 and 6. In case of inner content, farm 1,3 and 5 showing highest (n=3) while other lower (n=1) positive for *Salmonella* spp. in farm 6. On SS agar *Salmonella* colonies were blackish growth, whereas XLD agar, the colonies appeared as black centered because of H_2S production. Table 6: Individual and overall Salmonella positive percentage in Egg of studied farm on the basis of biochemical test.

Farm name	Total population	Daily average egg production	No of collected egg from fresh sample	No of tested egg from collected egg	Apparent prevalence (Egg shell)%	Apparent prevalence (egg content)%	True prevalence (Egg shell)%	True prevalence (egg content)%	Average true prevalence (Egg shell)%	Average true prevalence (Egg content)%
Jafar poultry farm	3811	3650	12	6	50%	33.33%	0.079 %	0.052%	0.093%	0.068%
Liza Poultry farm	4700	4500	12	6	16.66%	16.66%	0.02%	0.021%		
Kachuya Poulty	2382	2000	12	6	33.33%	50%	0.08%	0.126%		
Haque Poultry	1846	1750	12	6	50%	16.66%	0.16%	0.054%		
Udayan Poultry	1603	1500	12	6	50%	33.33%	0.19%	0.125%		
Islam Poultry	6635	5800	12	6	33.33%	33.33%	0.03%	0.03%		

Farm1= Jafar poultry, Farm 2= Hoque poultry, Farm 3= Kachuya poultry, farm 4= Udayan poultry, farm 5 = Islam poultry and Farm 6 = Liza poultry

Table 2: Table for calculating true and apparent prevalence of Salmonella spp in egg samples

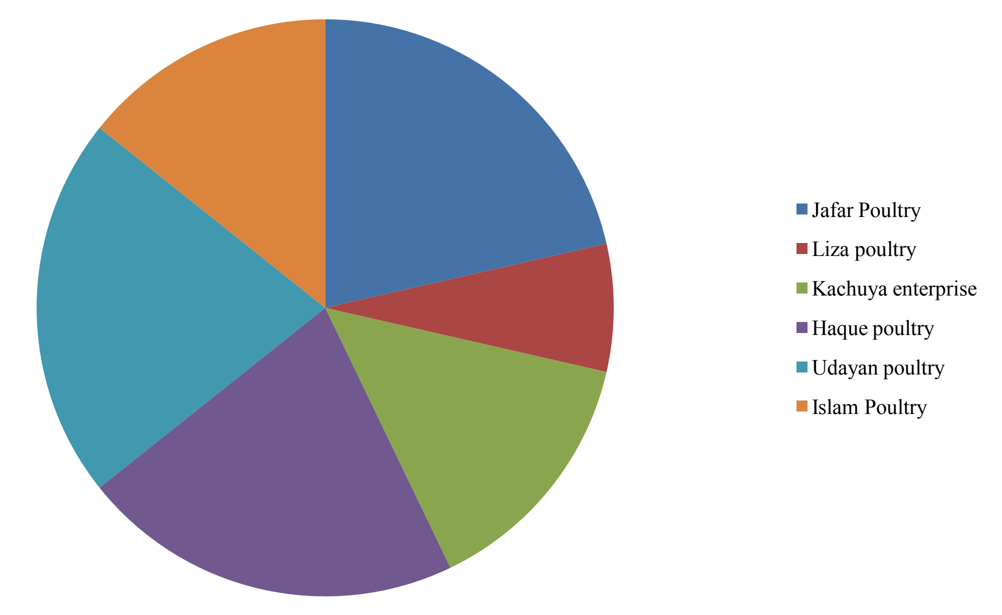

Figure: Presence of Salmonella spp in different studied farm.

The overall apparent prevalence of *Salmonella* in table egg collected from study area is 38.8% in egg shell and 30.5% in egg inner content while in individual farm level prevalence highest is in 50% and lowest of 16.67%. The highest prevalence of Salmonellosis is observed in Jafar poultry, Islam poultry and Udayan poultry farm while lowest prevalence in Hoque poultry of 16.66%. Others, 33.3% prevalence observed in both Liza poultry and Kachwya poultry. For the strain variation, Isa brown is showing lower prevalence of Salmonella contamination and Hisex brown showing higher of 44.45% Salmonella prevalence in egg sample.

Table: 3 Salmonella positive% within different strain

	Positive percentage in egg shell	*p*-value	Positive percentage in egg content	*p*-*value*
Isa brown	2 (66.67%)	0.273*	2 (66.67%)	0.406*
Hisex brown	3 (100%)		1(33.33%)	

* NS= non significant

Within the positive sample of different strain, hisex brown showing 100% contamination in egg shell while 33.33% in egg content. On the other hand, Isa brown is sowing same 66.67%

contamination in both egg shell and inner content of the egg. There is no statistically significant variation among the strain prevalence.

Microscopic study by Gram's staining method

Gram-negative, pink colored small rod shaped bacteria were found from suspected black centered colony. Based on the characteristic growth and colony color, it assumed that organisms are *Salmonella spp.*

Table: 4 associated factor for infection Salmonella spp in farm level

Farm management factor						
Traits	F1	F2	F3	F4	F5	F6
Pullets reared on floor	Y	N	Y	N	N	N
Feed contains animal products	Y	Y	Y	Y	Y	Y
Water chlorinated	N	Y	N	N	N	Y
Visitors allowed (no business)	Y	N	N	Y	N	N
Proper Manure handling	N	Y	N	Y	Y	Y
Cleaning and disinfecting between flocks						
Cages, walls, ceiling	ST	Y	Y	N	ST	Y
Wash and fumigate	Y	Y	Y	Y	Y	Y
Egg collection and marketing						
Reuse of egg trey	Y	Y	Y	Y	Y	Y
Washing and disinfecting the egg trey	N	Y	ST	N	Y	Y
Vehicle disinfected	N	Y	N	ST	N	ST
Storage room disinfection	N	Y	N	N	N	N

Farm1= Jafar poultry, Farm 2= Hoque poultry, Farm 3= Kachuya poultry, farm 4= Udayan poultry, farm 5 = Islam poultry and Farm 6 = Liza poultry and Y=yes, N= No and ST= Some times.

The table showing that, the overall management system of Liza poultry farm is better than the other as they follow the strict bio-security measurement. However, the management system of other farm is more or less same while comparatively Islam poultry is better than the rest farms except Liza farm. For collection and transportation of egg all poultry farm are using the reusable trey but only Liza farm an Islam poultry farm disinfect the trey regularly. The vehicle of egg transportation is usually used without any disinfection except Liza Poultry.

Table 2: overall prevalence of *Salmonella* spp in egg of commercial layer farm

CHAPTER V

DISCUSSION

Eggs and egg products considered as an important human diet in the world specifically in developing country due to less cost (Steinfeld *et al.*, 2006). However consuming eggs has been associated with negative health impacts. Eggs and egg products that are improperly handled can be a source of food-borne diseases, such as Salmonellosis. Salmonellosis is a leading food-borne disease distributed world-wide and a wide range of foods has been implicated in this disease (Newell *et al.*, 2010). Foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human Salmonellosis (Braden, 2006). There are large numbers of study referring that Salmonella causing contamination in egg resulting human cases of salmonella. However, the contamination of Salmonella in the internal content of chicken eggs can be due to infection in the ovary of birds while surface contamination of eggs can be through feces, feed, insects, or through handling, transport or storage material (Sing *et al.*, 2010).

The aim of this study was to determine the presence of *Salmonella* spp. in eggs in selected local egg production farms in Bangladesh. We have collected egg samples from six different farm of the study area. Initially 12 eggs were collected from each farm, and then randomly 6 eggs were selected from each farm for laboratory evaluation. We have investigated the true and apparent prevalence of Salmonella in table egg from both egg shell and inner contents. In this study the true prevalence of egg shell contamination by Salmonellosis is 0.093% and egg inner content is 0.068%. The incidence levels of S. Enteritidis in eggshell reported earlier were variable. In Spain, Perales and Audicana, (1989) reported that around 0.8 to 1% Salmonella contamination. In the United Kingdom prevalence levels were reported to be varying from zero (Mawer *et al*., 1989; Little *et al*.2007; Little *et al*., 2008) to 2%(Humphrey, 1994a, b, Evans *et al*., 1998, Elson *et al*., 2005).The prevalence of Salmonella in egg shell and egg content from bulk egg processing plant is also reported 0.5-3.7% in United states of America (Musgrove *et al*., 2006; Braden *et al*., 2006; Ohtuska *et al*., 2005). In a recent study conducted in France, 150 eggs were collected from the one day production of each of 28 randomly selected large scale layer flocks. One of the 28 flocks (39.3%) had at least one positive eggshell. Of the total of eggs tested, the prevalence of Salmonella in the eggshells was 0.3 -1.05% (Chemaly *et al*., 2009). The findings of current study findings are more or less consistent with previously stated study. Howver, there are some contrary in India and other country recorded higher prevelence of Salmonellosis in market egg content and shell.

A study was conducted by Suresh *et al*., (2006) in Coimbatore of South India found overall 3-7% Salmonella infection in egg shell and 2-45 in egg inner content. This study is higher than our true prevalence but correspondence to our apparent prevalence. The variation may be due to individual sampling in comparison to pooled survey sampling (Murchie *et al*., 2008). Other study in Belgium, New Zealand, Australia, and Canada reported a range of 2-13% Salmonella infection in large sampling frame as part of public health surveillance system (Namata *et al*., 2009; Murchie *et al*., 2008; Gould *et al*., 2004).

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*. The study is very close to our apparent prevalence in farm level of 30-35%.

Our study found higher contamination in egg shell than the inner content by Salmonella spp. This result is also supported by other study. A study in uttrprodesh of India find 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. Another study in north India by Sing *et al*., (2010) reported lower frequency in egg shell (1-2%) but higher frequency in egg content (8%). The collected samples were from retail and wholesale market of North India. The author reported overall prevalence of 3-5% salmonella in egg samples. 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. Our study is also correspondent to these studies of India.

In our study, individual farm level true prevalence shows highest in farm 1 of both in egg shell (0.079%) and egg inner content (0.052%) and lowest 0.03% in farm 6 ranges from 0.23-1.5%. The result is little lower than other study (Hoorebeke *et al*., 2010; Chamely *et al*., 2010) wile correspondence to Gracia *et al*., (2011) But the apparent prevalent shows highest in farm 1 and farm 4. 50%. The apparent prevalence is higher than study in Pakistan by (Sahazad *et al*., 2012), India by (Sing *et al*., 2010) of 35% and 28% respectively.
This variation may be Causes due to small sampling strategy of our study or sensitivity of diagnosing tools used for the detection of Salmonella (Chen *et al*., 2010). Alternatively it

could be higher for the farm level prevalence of Salmonellosis in birds. A study shows that prevalence of Salmonella in egg sample is more in known infected flock. The prevalence of Salmonellosis

Eggs from known infected flocks of commercial layer farm could be expected to more prevalence of Salmonella contamination. (Ref) The overall prevalence of Salmonellosis in commercial layer farm in Bangladesh is ranged from 8-24% (haider *et al*., 2004, Borua *et al*., 2012). So the current study findings of 33% are more or less consistent to the farm level infection.

5.3 Discussion on associated risk factor of Salmonellosis in egg:

Salmonella can contaminate the egg either from the ovary of hen or mishandling during processing stage. The inner content of Salmonella often related to farm level infection in laying bird. The genital tract of laying hens is responsible for inner content contamination of egg. The previously reported Salmonellosis in a farm or known infected farm's egg show higher prevalence in Salmonella contamination (Namata *et al*., 2005). In our study, the farm 3 have a history of Salmonella infection in rearing flock. For this reason, during laboratory evaluation the farm shows highest level of contamination in inner mass. Various study on epidemiological risk factor in egg contamination suggested that regular vaccination could decrease the level of contamination in farm by Salmonella spp. (Vandepalus *et al*., 2010; Berriman *et al*., 2013). Our studied farm 2 and farm 6 use to vaccinate regularly their bird against salmonella. So the prevalence is lower than other in egg content contamination.

Small scale layer farm always have minimum concern about biosecurity measurement in Bangladesh (Borua *et al*., 2012). This low biosecurity measurement often causes higher incidence of Salmonella infection in farm level resulting higher rate of egg contamination (Holt *et al*; 2011). In our study, low biosecured farm show higher frequency of egg contamination. The Jafar poultry, where the visitors are allowed and improper handling of manure is practices have shows highest rate of egg contamination than others.

The farmers in the study area buy fish meal or other feed ingredients from the local markets where birds and eggs of different farms are also sold. The same vehicles are used for transportation of birds, eggs and feeds between the farms and the markets, and in most cases, these vehicles remain contaminated with faeces, and non-disinfected. The use of the same vehicles between farms and markets for transportation of birds, eggs and feeds, and the access of the products of the farms to the same local markets were two practice commonalities. Different degrees of faecal contaminations of vehicles and frequencies of market visits have role in higher frequency of Salmonella infection in egg (Baruya *et al*., 2012). In our study, higher frequency of egg shell contamination shows in farm 1 and farm 4 where same vehicle used for transportation of feed as well as egg marketing. The farms are not concern about disinfecting there vehicle during transportation of egg in the market.

Reuse of egg trey for collection and transportation of egg in developing like Bangladesh have an importance role in contamination of egg (Sahazad *et al*., 2012). But regular disinfection of egg colleting trey can reduce the risk of contamination (Utrarachkij *et al*., 2012). Our studied all farms reuse the egg trey for collection and distribution of egg in the market. But only Liza poultry use to disinfect the egg trey. The presence of egg shell contamination is also lower in this farm.

CHAPTER VI
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Salmonellosis is an important food borne disease worldwide implicated through various types of food. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human Salmonellosis.

The present study focused on the isolation of *Salmonella* spp. in 36 samples from different farm level of Chittagong and Noakhali districts of Bangladesh. The overall prevalence of egg inner content contamination is 38.8%. The egg is considered as ideal food and major source of protein in Bangladesh. But the contamination of *Salmonella spp* can causes major public health burden by consuming raw or under cooked the eggs and egg products. In addition, the contamination of egg by salmonella involves major health expenses in developing country. So it is necessary to monitor the infection level in marketing channel of egg and egg products.

The following conclusions are drawn from the results of the present study:

1. The overall apparent prevalence in egg shell is 38.8% while the true prevalence is 0.093% in egg shell and 0 .068% in egg inner content.
2. The egg from hisex brown is more susceptible to Salmonella contamination than Isa brown. (While other factors are constant)
3. Lower or minimum biosecurity practices in farm level increase the rate of contamination in inner egg content.
4. Reuse of egg trey after disinfection can reduce the contamination.

6.2 Recommendations

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. To be safe, eggs must be properly handled, refrigerated, and cooked

CHAPTER VII

REFERENCES

Barua, H., Biswas, P. K., Olsen, K. E., & Christensen, J. P. (2012). Prevalence and characterization of motile *Salmonella* in commercial layer poultry farms in Bangladesh. *PloS one*, *7*(4), e35914.

Berriman, A. D., Clancy, D., Clough, H. E., Armstrong, D., & Christley, R. M. (2013). Effectiveness of Simulated Interventions in Reducing the Estimated Prevalence of *Salmonella* in UK Pig Herds. *PloS one*, *8*(6), e66054.

Braden, C. R. (2006). *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. *Clinical Infectious Diseases*, *43*(4), 512-517.

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). *Salmonella* nomenclature. *Journal of Clinical Microbiology*, *38*(7), 2465-2467.

Capita, R., Álvarez-Astorga, M., Alonso-Calleja, C., Moreno, B., & del Camino García - Fernández, M. (2003). Occurrence of *Salmonella* e in retail chicken carcasses and their products in Spain. *International journal of food microbiology*, *81*(2), 169-173.

Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M. (2012). Cross-contamination and recontamination by *Salmonella* in foods: A review: *Food Research International*, *45*(2), 545-556.

Centers for Disease Control and Prevention (CDC. (2000). Outbreaks of *Salmonella* serotype enteritidis infection associated with eating raw or undercooked shell eggs--United States, 1996-1998. *MMWR. Morbidity and mortality weekly report*, *49*(4), 73.

Chemaly, M., Huneau-Salaün, A., Labbe, A., Houdayer, C., Petetin, I., & Fravalo, P. (2009). Isolation of *Salmonella enterica* in laying-hen flocks and assessment of eggshell contamination in France. *Journal of Food Protection*,*72*(10), 2071-2077.

Chen, J., Zhang, L., Paoli, G. C., Shi, C., Tu, S. I., & Shi, X. (2010). A real-time PCR method for the detection of *Salmonella* enteric from food using a target sequence identified by comparative genomic analysis. *International journal of food microbiology*, *137*(2), 168-174.

Davies, R., & Breslin, M. (2004). Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar *enteritidis* had been used. *Avian Pathology*, *33*(2), 133-144.

De Jong, B., & Ekdahl, K. (2006). The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. *BMC Public Health*, *6*(1), 4.

De Reu, K. (2006). *Bacteriological contamination and infection of shell eggs in the production chain*. Ghent University.

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A. K., Kumar, A., Tiwari, R., & Kapoor, S. (2013). Food-borne Pathogens of Animal Origin-Diagnosis, Prevention and Control and Their Zoonotic Significance: A Review. *Pakistan Journal of Biological Sciences*, *16*(20).

Elson, R., Little, C. L., & Mitchell, R. T. (2005). *Salmonella* and raw shell eggs: results of a cross-sectional study of contamination rates and egg safety practices in the United Kingdom catering sector in 2003. *Journal of Food Protection*, *68*(2), 256-264.

Food and Drug Administration. (1997)- Food safety from farm to table: A national food safety Initiative. Avialable at: (www.foodsafety.gov/~dms /fsreport.html). (Last accessed on 23.3.2014).

Frenzen, P. D., Riggs, T. L., Buzby, J. C., Breuer, T., Roberts, T., Voetsch, D., & Reddy, S. (1999). *Salmonella* cost estimate updated using FoodNet data.*FoodReview*.

García, C., Soriano, J. M., Benítez, V., & Catalá-Gregori, P. (2011). Assessment of *Salmonella* spp. in feces, cloacal swabs, and eggs (eggshell and content separately) from a laying hen farm. *Poultry science*, *90*(7), 1581-1585.

Gould, D., Kraa, E., Dalton, C. B., Givney, R., Gregory, J., Stafford, R. J., & Kirk, M. D. (2004). Foodborne disease outbreaks in Australia, 1995 to 2000.*Communicable diseases intelligence quarterly report*, *28*(2), 211.

Gras, L., Enserink, R., Friesema, I., Heck, M., van Duynhoven, Y., & van Pelt, W. (2014). Risk Factors for Human Salmonellosis Originating from Pigs, Cattle, Broiler Chickens and Egg Laying Hens: A Combined Case-Control and Source Attribution Analysis. *PloS one*, *9*(2), e87933.

Guard-Petter, J. (2001). The chicken, the egg and *Salmonella* enteritidis.*Environmental microbiology*, *3*(7), 421-430.

Guo, C., Hoekstra, R. M., Schroeder, C. M., Pires, S. M., Ong, K. L., Hartnett, E & Cole, D. (2011). Application of Bayesian techniques to model the burden of human salmonellosis attributable to US food commodities at the point of processing: adaptation of a Danish model. *Foodborne pathogens and disease*, *8*(4), 509-516.

Hasan, M. N., Ara, N. A. R. G. I. S., Mamun, S. A., Rahman, M. M., & Rahman, M. H. (2009). Prevalence of *Salmonella* spp. in chicken egg from Khulna city. *Journal of Innovation and Development Strategy*, *3*(3), 1-6.

Herikstad, H., Motarjemi, Y., & Tauxe, R. V. (2002). *Salmonella* surveillance: a global survey of public health serotyping. *Epidemiology and Infection*, *129*(01), 1-8.

Holt, P. S., Davies, R. H., Dewulf, J., Gast, R. K., Huwe, J. K., Jones, D. R., & Willian, K. R. (2011). The impact of different housing systems on egg safety and quality. *Poultry science*, *90*(1), 251-262.

Hope, B. K., Baker, A. R., Edel, E. D., Hogue, A. T., Schlosser, W. D., Whiting, R., & Morales, R. A. (2002). An overview of the *Salmonella* Enteritidis risk assessment for shell eggs and egg products. *Risk Analysis*,*22*(2), 203-218.

Howard, Z. R., Moore, R. W., Zabala-Diaz, I. B., Landers, K. L., Byrd, J. A., Kubena, L. F., & Ricke, S. C. (2005). Ovarian laying hen follicular maturation and in vitro *Salmonella* internalization. *Veterinary microbiology*, *108*(1), 95-100.

Hugas, M., Tsigarida, E., Robinson, T., & Calistri, P. (2007). Risk assessment of biological hazards in the European Union. *International journal of food microbiology*, *120*(1), 131-135.

Hugas, M., Tsigarida, E., Robinson, T., & Calistri, P. (2009). The EFSA Scientific Panel on Biological Hazards first mandate: May 2003–May 2006. Insight into foodborne zoonoses. *Trends in food science & technology*, *20*(5), 188-193.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology*. Springer.

Jørgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R. A., Bolton, F. J & Humphrey, T. J. (2002). Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *International journal of food microbiology*, *76*(1), 151-164.

Kariuki, S., Revathi, G., Gakuya, F., Yamo, V., Muyodi, J., & Hart, C. A. (2002). Lack of clonal relationship between non-typhi *Salmonella* strain types from humans and those isolated from animals living in close contact. *FEMS Immunology & Medical Microbiology*, *33*(3), 165-171.

Kassis, N. M., Beamer, S. K., Matak, K. E., Tou, J. C., & Jaczynski, J. (2010). Nutritional composition of novel nutraceutical egg products developed with omega-3-rich oils. *LWT-Food Science and Technology*, *43*(8), 1204-1212.

Keller, L. H., Benson, C. E., Krotec, K., & Eckroade, R. J. (1995). *Salmonella* enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. *Infection and immunity*, *63*(7), 2443-2449.

King, D., & Strynadka, N. (2011). Crystal structure of New Delhi metallo-β-lactamase reveals molecular basis for antibiotic resistance. *Protein Science*,*20*(9), 1484-1491.

Limawongpranee, S., Hayashidani, H., Okatani, A. T., Ono, K., Hirota, C., Kaneko, K., & Ogawa, M. (1999). Prevalence and persistence of *Salmonella* in broiler chicken flocks. *The Journal of veterinary medical science/the Japanese Society of Veterinary Science*, *61*(3), 255.

Little, C. L., Rhoades, J. R., Hucklesby, L., Greenwood, M., Surman-Lee, S., Bolton, F. J & Chan, C. H. (2008). Survey of *Salmonella* contamination of raw shell eggs used in food service premises in the United Kingdom, 2005 through 2006. *Journal of Food Protection*, *71*(1), 19-26.

Little, C. L., Surman-Lee, S., Greenwood, M., Bolton, F. J., Elson, R., Mitchell, R. T. & O'Brien, S. (2007). Public health investigations of *Salmonella* Enteritidis in catering raw shell eggs, 2002–2004. *Letters in applied microbiology*, *44*(6), 595-601.

Little, C. L., Walsh, S., Hucklesby, L., Surman-Lee, S., Pathak, K., Gatty, Y. & Chan, C. H. (2007). Survey of *Salmonella* Contamination of Non–United KingdomProduced Raw Shell Eggs on Retail Sale in the Northwest of England and London, 2005 to 2006. *Journal of Food Protection*, *70*(10), 2259-2265.

Louis, M. E. S., Morse, D. L., Potter, M. E., DeMelfi, T. M., Guzewich, J. J., Tauxe, R. V., & Hargrett-Bean, N. (1988). The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections: new implications for the control of salmonellosis. *JAmA*, *259*(14), 2103-2107.

Malorny, B., Tassios, P. T., Rådström, P., Cook, N., Wagner, M., & Hoorfar, J. (2003). Standardization of diagnostic PCR for the detection of foodborne pathogens. *International Journal of Food Microbiology*, *83*(1), 39-48.

Martelli, F., & Davies, R. H. (2012). *Salmonella* serovars isolated from table eggs: An overview. *Food Research International*, *45*(2), 745-754.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C. & Tauxe, R. V. (1999). Food-related illness and death in the United States.*Emerging infectious diseases*, *5*(5), 607.

Middleton, D., Savage, R., Tighe, M. K., Vrbova, L., Walton, R., Whitfield, Y. & Crowcroft, N. S. (2013). Risk factors for sporadic domestically acquired *Salmonella* serovar Enteritidis infections: a case-control study in Ontario, Canada, 2011. *Epidemiology and infection*, 1-11.

Mims, C. A., Playfair, J. H. L., Roitt, I. M., Wakelin, D., & Williams, R. (1993).*Medical microbiology*. Mosby Europe Limited.

Mizumoto, N., Sasai, K., Tani, H., & Baba, E. (2005). Specific adhesion and invasion of *Salmonella enteritidis* in the vagina of laying hens.*Veterinary microbiology*, *111*(1), 99-105.

Morse, D. L., Birkhead, G. S., Guardino, J., Kondracki, S. F., & Guzewich, J. J. (1994). Outbreak and sporadic egg-associated cases of *Salmonella* enteritidis: New York's experience. *American journal of public health*, *84*(5), 859-860.

Murchie, L., Xia, B., Madden, R. H., Whyte, P., & Kelly, L. (2008). Qualitative exposure assessment for *Salmonella spp*. in shell eggs produced on the island of Ireland. *International journal of food microbiology*, *125*(3), 308-319.

Musgrove, M. T., Jones, D. R., Northcutt, J. K., Cox, N. A., Harrison, M. A., Fedorka-Cray, P. J., & Ladely, S. R. (2006). Antimicrobial resistance in *Salmonella* and Escherichia coli isolated from commercial shell eggs. *Poultry science*, *85*(9), 1665-1669.

Namata, H., Welby, S., Aerts, M., Faes, C., Abrahantes, J. C., Imberechts, H., & Mintiens, K. (2009). Identification of risk factors for the prevalence and persistence of*Salmonella* in Belgian broiler chicken flocks. *Preventive veterinary medicine*, *90*(3), 211-222.

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H & Kruse, H. (2010). Food-borne diseases—the challenges of 20years ago still persist while new ones continue to emerge. *International journal of food microbiology*, *139*, S3-S15.

Ohtsuka, K., Yanagawa, K., Takatori, K., & Hara-Kudo, Y. (2005). Detection of *Salmonella enterica* in naturally contaminated liquid eggs by loop-mediated isothermal amplification, and characterization of *Salmonella* isolates. *Applied and environmental microbiology*, *71*(11), 6730-6735.

Panisello, P. J., Rooney, R., Quantick, P. C., & Stanwell-Smith, R. (2000). Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*,*59*(3), 221-234.

Pignato, S., Giammanco, G., Santangelo, C., & Giammanco, G. M. (1998). Endemic presence of *Salmonella* bongori48: z 35 causing enteritis in children in Sicily. *Research in microbiology*, *149*(6), 429-431.

Piknová, L., Štefanovičová, A., Drahovská, H., Sásik, M., & Kuchta, T. (2002). Detection of *Salmonella* in food, equivalent to ISO 6579, by a three-days polymerase chain reaction-based method. *Food Control*, *13*(3), 191-194.

Popoff, M. Y., Bockemühl, J., & Gheesling, L. L. (2003). Supplement 2001 (no. 45) to the Kauffmann–White scheme. *Research in microbiology*, *154*(3), 173-174.

Rabsch, W., Tschäpe, H., & Bäumler, A. J. (2001). Non-typhoidal salmonellosis: emerging problems. *Microbes and infection*, *3*(3), 237-247.

Radkowski, M. (2001). Occurrence of *Salmonella* spp. in consumption eggs in Poland. *International journal of food microbiology*, *64*(1), 189-191.

Romo, L. A. R. (2004). *Control of Salmonella enterica serovar enteritidis in shell eggs by ozone, ultraviolet radiation, and heat* (Doctoral dissertation, The Ohio State University).

Rusul, G., Khair, J., Radu, S., Cheah, C. T., & Yassin, R. M. (1996). Prevalence of*Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. *International journal of food microbiology*, *33*(2), 183-194.

Safaei, H. G., Jalali, M., Hosseini, A., Narimani, T., Sharifzadeh, A., & Raheimi, E. (2011). The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella* spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. *Jundishapur Journal of Microbiology*,*4*(4).

Shahzad, A., Mahmood, M. S., Hussain, I., Siddique, F., & Abbas, R. Z. (2012). Prevalence of *Salmonella* species in hen eggs and egg storing-trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. *Pak J Agric Sci*, *49*, 565-568.

Singh, S., Yadav, A. S., Singh, S. M., & Bharti, P. (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International*, *43*(8), 2027-2030.

Steinfeld, H., Wassenaar, T., & Jutzi, S. (2006). Livestock production systems in developing countries: status, drivers, trends. *Rev. sci. tech. Off. int. Epiz*,*25*(2), 505-516.

Steve Yan, S., Pendrak, M. L., Abela-Ridder, B., Punderson, J. W., Fedorko, D. P., & Foley, S. L. (2004). An overview of *Salmonella* typing: Public health perspectives. *Clinical and Applied Immunology Reviews*, *4*(3), 189-204.

Suresh, T., Hatha, A. A. M., Sreenivasan, D., Sangeetha, N., & Lashmanaperumalsamy, P. (2006). Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other

Salmonella s in the eggs and egg-storing trays from retails markets of Coimbatore, South India. *Food microbiology*, *23*(3), 294-299.

Tindall, B. J., Grimont, P. A. D., Garrity, G. M., & Euzeby, J. P. (2005). Nomenclature and taxonomy of the genus *Salmonella. International journal of systematic and evolutionary microbiology*, *55*(1), 521-524.

Tsolis, R. M., Xavier, M. N., Santos, R. L., & Bäumler, A. J. (2011). How to become a top model: impact of animal experimentation on human *Salmonella* disease research. *Infection and immunity*, *79*(5), 1806-1814.

Utrarachkij, F., Pornraungwong, S., Siripanichgon, K., Nakajima, C., Suzuki, Y., & Suthienkul, O. (2012). Possible horizontal transmission of *Salmonella* via reusable egg trays in Thailand. *International journal of food microbiology*, *154*(1), 73-78.

Uyttendaele, M. R., Debevere, J. M., Lips, R. M., & Neyts, K. D. (1998). Prevalence of*Salmonella* in poultry carcasses and their products in Belgium. *International Journal of Food Microbiology*, *40*(1), 1-8.

Vaclavik, V. A., & Christian, E. W. (2014). Eggs and Egg Products. In *Essentials of Food Science* (pp. 173-199). Springer New York.

Van Hoorebeke, S., Van Immerseel, F., Schulz, J., Hartung, J., Harisberger, M., Barco, L & Dewulf, J. (2010). Determination of the within and between flock prevalence and identification of risk factors for *Salmonella* infections in laying hen flocks housed in conventional and alternative systems. *Preventive veterinary medicine*, *94*(1), 94-100.

Vandeplas, S., Dubois Dauphin, R., Beckers, Y., Thonart, P., & Thewis, A. (2010). *Salmonella* in chicken: current and developing strategies to reduce contamination at farm level. *Journal of Food Protection*, *73*(4), 774-785.

Velge, P., Cloeckaert, A., & Barrow, P. (2005). Emergence of *Salmonella* epidemics: The problems related to *Salmonella enterica* serotyp Enteritidis and multiple antibiotic resistance in other major serotypes. *Veterinary research*, *36*(3), 267-288.

Wang, S. J., & Yeh, D. B. (2002). Designing of polymerase chain reaction primers for the detection of *Salmonella* enteritidis in foods and faecal samples.*Letters in applied microbiology*, *34*(6), 422-427.

Wilson, J. S., Hazel, S. M., Williams, N. J., Phiri, A., French, N. P., & Hart, C. A. (2003). Nontyphoidal *Salmonella* in United Kingdom badgers: prevalence and spatial distribution. *Applied and environmental microbiology*, *69*(7), 4312-4315.

World Health Organization. (2002). *Risk assessments of Salmonella in eggs and broiler chickens* (Vol. 2). Food & Agriculture Org.

APPENDIX

Table 1 : Reference table (Natural data)

Farm name	Strain	Total population	Age of birds
Jafar poultry farm	Isa brown	4000	72 week
Liza Poultry farm	Isa brown	5000	68 week
Kachuya Poulty	Isa brown	2500	56 week
Haque Poultry	Hi-sex brown	2000	68 week
Udayan Poultry	Hi-sex brown	1800	55 week
Islam Poultry	Hi-sex brown	7000	60 week
		Total- 22300	

Table 2 : Sample table (Epidemiological data)

Farm name	Strain	Total population	No. of dead birds	Remarks
Jafar poultry farm	Isa brown	4000	189	
Liza Poultry farm	Isa brown	5000	300	
Kachuya Poulty	Isa brown	2500	118	
Haque Poultry	Hi-sex brown	2000	154	
Udayan Poultry	Hi-sex brown	1800	197	
Islam Poultry	Hi-sex brown	7000	365	