**INTRODUCTION**

Poultry industries play an important role in poverty alleviation and economic development of Bangladesh. Poultry meat contributes approximately 37% of total animal protein supplied in the country (Rahman and Rahman, 1998). Government of the People's Republic of Bangladesh has recently given priority in potential poultry sector. There are 89.48 million poultry population in Bangladesh (Samad, 1996).

There are several constraints in poultry industries in Bangladesh. Among them pullorum disease (caused by *S. pullorum*, recently used nomenclature) is one of the major constraints of poultry industries in Bangladesh (Hossain *et al*., 2006).Salmonella infection is one of the most important bacterial diseases in poultry causing heavy economic loss through mortality and reduced production (Haider *et al*., 2003; Talha *et al*., 2001).There are mainly two types of non motile avian *Salmonella sp.* namely *Salmonella gallinarum* and *Salmonella pullorum* that cause fowl typhoid and pullorum disease, respectively (Hossain *et al*., 2006). Salmonella are Gram negative, short plump shaped rods, non-sporeforming, non- capsulated, aerobicand facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE Manual, 2006). More than 2300 serotypes of *Salmonella* have been identified, only about 10% of these have beenisolated from poultry (Gast, 1997). Pullorum disease is usually confined to the first 2-3 weeks of age and occasionallyoccurs in adults (Shivaprashad, 1997). Fowl typhoid is frequently referred to as a disease of adult birdsand there are also reports of high mortality in young chicks (Christensen *et al.,* 1992).

The epidemiologyof fowl typhoid and pullorum disease in poultry, particularly with regard to transmission from onegeneration to the next are known to be closely associated with infected eggs (Wigley *et al.,* 2001).Contaminated eggs produced by infected laying hens are thought to be one of the main sources of human infection with *Salmonella* Enteritidis (Humphrey *et al*., 1989). Eggs may become contaminatedwith *Salmonella* in two main ways: (i) *Salmonella* may silently infect the ovaries of apparently healthyhens and contaminate the eggs before the shells are formed. (ii) *Salmonella* infected bird droppingscontain *Salmonella* that can contaminate the outer egg shells and may penetrate when crack the shell (Deryck and Pattron, 2004).

Pathogenesis and sequential pathology of Pullorum Disease (PD) is an important factor to understand the disease mechanism. For the detection of *Salmonella* organism many of technologies have been developed. Immunohistochemistry is a latest technique for that purpose (Christine *et al*., 1999). A few researches have been completed on *Salmonella pullorum* infections using the conventional methods like necropsy, histopathology and isolation of bacteria by culture, stain and sugar fermentation tests (Islam *et al*., 2006; Haider *et al*., 2003).

Selection procedures for detection of Salmonella infection in poultry is the aim of many studies (Seran *et al*., 2010). In vitro culture is the predominant means for isolating and identifying salmonella species from fecal samples. This is time consuming usually require 72 to 96 hours for the organism to be defined by its cultural, biochemical and serological properties (Pomeroy B. S. and Nagaraja K. V. 1991). A number of serological test have been developed for detecting invasive serotypes, the most successful being slide agglutination using either serum or whole blood for the detection of poultry flocks infected with Salmonella gallinarum or its biovarpullorum (Snoeyenbos G. S., 1991 and Nagaraja *et al*., 1991). This test has been applied for successfully for more than 50 years and has contributed considerably to the control of pullorum disease and fowl typhoid from flocks in several countries. This test is however crude and has been found to be too unreliable and insensitive for use with other serotypes (Barrow P. A. 1992). Tube and micro agglutination test and the more sensitive micro antiglobulin tests have been applied to experimental and field infection with serogroups with B, C and D. However these tests are cumbersome and do not lend themselves readily to extensive use for large scale flock screening (Feberwee *et al*., 2001).

Salmonella serogroups B and D represents a high percentage of salmonella strains isolated from chicken meat (Seran *et al*., 2010) and among layer farm chickens (Ibrahim H. M., 2011). These serogroups are nearly most of the strains causing disease in Egypt, so these tests was directed against serogroups. In Egypt S. pullorum antigen (for tube and slide agglutination assays) and S. typhimurium for tube agglutination test are the only prepared antigen in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (Mona *et al*., 2012).

Knowledge of the prevalence of the disease with confirmatory diagnosis is of paramount importance to embark on a control or prevention program as clinical signs, post-mortem findings and flock history are of limited value in arriving at a diagnosis because of the similarity of the diseases to a number of other diseases (Snoeyenbos G.S., 1978). Therefore, this study was conducted to estimate the prevalence of S. Gallinarum/Pullorum in apparently healthy chickens and to measure the relative importance of fowl typhoid and pullorum disease in morbidity/ mortality of intensively managed chickens in Kazi layer farm.

**REVIEW OF LITERATURE**

***2*.*1. Overall prevalence of Salmonella infection***

Large increases in the number of cases of human food-poisoning caused by salmonella have occurred in several countries in recent years. Diagnosis and control of avian Salmonellosis depend upon the use of effective antigens in the used serological test (OIE, 2010).

Dominant serotypes from clinical cases vary with geographical region: for example, *S. enteritidis*is the most common in Europe (Hassanein *et al*., 2011). Ten strains of *S. typhimurium*, 9 strains of serogroup B, 4 strains of *Salmonella* serogroup D and 3 strains of *S. pullorum* were detected (Sadek, 1996). The seroprevalence of Salmonella infection in layer chickens was studied and*S. Pullorum* and *S. gallinarum* is the most predominant in salmonella infection (Islam *et al*., 2006). While serovar identified by Hassanein *et al*. (2011) was *Salmonella enterica* subsp. Enterica serovar Enteritidis using PCR method.These serogroups are nearly most of the strains causing disease in Egypt, so the test was directed against these serogroups. Because the ELISAs for avian Salmonellas are indirect, detecting primarily specific IgG in serum or IgG in egg yolk, the assay can also be adapted to detect specific IgM produced early in infection (Hassan *et al*., 1991) and the polyvalent antigens are more reliable for detection of salmonellosis than monovalent antigen (Sadek, 2005).

The overall seroprevalence of Salmonellosis was recorded as 43.4% (Islam *et al*., 2006). Yang *et al*. (1996) reported relatively similar findings (39.02%). Ashenafi *et al*. (2003) and Habib-ur-Rahman *et al*. (2003) reported 64.2% and 63.5 % respectively, that was much higher than that of the present study. The variation of seroprevalence might be speculated due to geographical variation or difference of management. But the present finding (43.4%) in commercial farms was higher than the seroprevalence (23.46%) recorded by Sikder *et al*. (2005) in local chickens. The difference with Sikder *et al*. (2005) was corresponded with the findings of Jha *et al*. (1995) and Robinson *et al*. (2000), who recorded seroprevalence rate higher in commercial flock than local chickens.

A total of 605 sera samples were collected from 121 commercial layer farms and were subjected to RPA test. Out of these, 85 (14.1%) were found positive for single Salmonella infection. The overall prevalence ofSalmonellainfection was 25.3% (14.1+11.2) (Hossain *et al*., 2010). Similar reports have been described by Alam *et al*. (2003) who reported 23.8% seropositive chickens for Salmonellainfection in Dinajpur district of Bangladesh. Bouzoubaa *et al*. (1992) recorded 23.5% seropositive chickens for salmonellosis from Morocco. Besides Minga *et al*. (1987) and Bhattacharya *et al*. (2001) reported 33.8% and 37.7% seropositive chickens for Salmonellainfection in Tanzania and India, respectively. Whereas, Terzolo *et al*. (1977), Prukner (1987), Ghosh (1988), Muneer *et al*. (1988), Waltman and Home (1993), Yang *et al*. (1996), Hasegawa *et al*. (1999) reported 9.0%, 13.9%, 19.6%, 7.5%, 15.0%, 10.0% and 16.0% prevalence of Salmonellainfection in chickens, respectively.

A total of 6 samples collected from 5 birds were positive as the bacteria were isolated from both liver and cecum in one of the chickens. Four (12.9%) and 1 (3.2%) of 31 sick/dead birds examined had *S. Gallinarum* and *S. Pullorum* respectively. Five of the 6 isolates recovered were *S. Gallinarum*while the rest 1 was *S. Pullorum*. Of the recovered 6 isolates, 3 (50%) were from liver, 2 (33.3%) from cecum and 1 (16. 7%) from spleen. *Salmonella Gallinarum* was isolated from all the organs tested (liver, spleen and cecum) while the only isolate of S. Pullorum was recovered from liver. *Salmonella Gallinarum/Pullorum* was isolated from dead chickens originated from 2 of the 3 farms investigated in the autopsy study. Four of 20 (20%) sick/dead birds from one of the farms were positive for *S. Pullorum/Gallinarum*, while 1 out of 10 chickens (10%) was positive in the other farm. Interestingly, 207cloacal swab samples were negative while *S. Gallinarum/Pullorum* was isolated from 20% of sick/dead chicken from the same farm.*Salmonella Pullorum* was isolated from a 7 day old chick, while *S. Gallinarum* was isolated from three 7 day old chicks and a year old chicken (Kassaye *et al*., 2010).

In order to determine seroprevalence of Salmonella a total of 364 sera samples were collected from six Model Breeder Poultry Farm during winter and rainy season. All sera samples were tested by Serum Plate Agglutination (SPA) test. The results of seroprevalence of Salmonella infection in flock no. 1 was found to be 13.33% at first sampling but it increased to 14.70% at second sampling (5 months after first sampling). The prevalence of Salmonellainfection also 21.87% to 24% in flock no. 2, 19.44% to 27.58% in flock no. 3, 14.28% to 25% in flock no. 4 and 28.57% to 30% in flock no. 5 and 30.25% to 30.76% in flock no.6. The prevalence rate was recorded the highest (30.76%) in flock no. 6, during second sampling at the age of 39 lowest (13.33%) in flock no. 1, during first sampling at the age of 32 weeks (Sikder *et al*., 2005).

***2*.*2. Prevalence of Salmonella infection in different ages***

Concerning to the prevalence depending on the ages, the highest prevalence of Salmonella was 37.6% (27.2+10.4) at 64 weeks and above age group whereas the lowest prevalence was 16.6% (3.3+13.3) at 16-23 weeks age group (Hossain *et al*., 2010). Similar report was demonstrated by Sikder *et al*. (2005) who reported the highest Salmonella infection was 30.8% at 39 weeks of age and the lowest was 13.3% at 32 weeks of age. Truong *et al*. (2003) reported that the prevalence of Salmonellainfection increased with the increase of age.

***2*.*3. Seasonal incidence of Salmonella infection***

The prevalence of Salmonalla infection was the highest (18.5+11.9=30.4%) in summer followed by winter (11.6+12.1=23.7%), rainy (14.2+10.8=25.0%) and autumn (13.3+10.0=23.3%) (Hossain *et al*., 2010). Similar report was demonstrated by Rahman *et al*. (2004) who reported 48.1% prevalence of Salmonellainfection in summer in comparison to 23.7% in winter. Sikder *et al*. (2005) also recorded the highest (25.0%) prevalence of Salmonellainfection in rainy season than in winter (21.9%). Bhattacharjee *et al*. (1996) reported the highest prevalence of salmonellosis during pre-monsoon (13.1%) in comparison to winter (10.4%), monsoon (6.8%) and post-monsoon (6.8%) period. The highest rate of Salmonellainfection in summer season is probably due to the high growth rate of bacteria and the influence of hot weather that might reduce the immune status of the birds against infection (Hossain *et al*., 2010).

***2.4. Prevalence of Salmonella infection with regard to flock size***

Serological investigation showed the highest (17.1+17.1=34.2%) Salmonellainfection in large flocks (≥5001 birds) in comparison to small (≤1000 birds) flocks (12.5+8.8=21.3%) (Hossain *et al*., 2010). The present data were higher than those in the report of Skov *et al*. (1999) who recorded 16.8% *Salmonella* infection in a flock containing 30-40 thousand chickens in comparison to 11.9% in a flock containing 10-20 thousand and 9.7% in a flock containing less than 10 thousand chickens. Mdegela *et al*. (2000) recorded higher prevalence of *Salmonella* infection in commercial flocks (18.4%) than in scavenging chickens (6.3%) and reported that infection rate increased with the increase of flock size.

***2.5. Detection of salmonella by necropsy findings***

The prevalence of salmonella infection in liver, ovary and intestinal swabs of dead poultry was 11.42%. The findings revealed that prevalence was higher in liver and ovarian samples than intestinal samples (Hossain *et al*., 2006).

Some sequential gross pathological lesions were observed in the study of Shahinuzzaman *et al*., 2011.Chicks were sacrificed at day 1 (D1), day 3 (D3), day 5 (D5), day 7 (D7) and day 9 (D9) of PostInfection (PI) and observed the remarkable gross lesions in liver, lung, heart and cecum. Grossly, liver foundfragile (40%) at D7 and D9. Cheesy materials in cecum (20%) showed at D9. The highest reisolation of *S.pullorum* demonstrated in cecum (68%).To get the complete information caused by inoculated *Salmonella pullorum* bacteria, reisolation procedures were performed by some routine methods. Cecum (68%) was the prominent organ for reisolation of *Salmonella pullorum* and then liver (52%), lung (48%), crop (44%), spleen (12%) and heart (4%) respectively from D1 to D9. It was confirmed by observing the colony characters of *Salmonella pullorum*on Brilliant Green Agar (BGA), *Salmonella-Shigella*(SS) agar and TripleSugar Iron (TSI) agar. Out of 150 samples **57** gave positive colony characters of *Salmonella pullorum*. Formore confirmation of *Salmonella pullorum* reisolation, carbohydrate fermentation test of some basic sugars and biochemical test was performed. 5 Isolates were selected for this purpose (Shahinuzzaman *et al*., 2011).Enlarged and congested liver with focal necrosis; haemorrhagic and discoloured ovary with stalk formation and mild haemorrhagic to catarrhal enteritis in intestine and caecum were recorded during necropsy(Islam *et al*., 2006). These types of necropsy findings were supported by Calnek *et al*. (1991), Chauhan and Roy (1996), Syed-Habib-ur-Rahman *et al*. (2004), Hossain *et al*. (2003) and Khan *et al*. (1998)

**Materials and method**

This study was conducted on Hisex brown stain (layer) at Kazi poultry farm in Chittagong district. Samples were collected from three flocks in the period of January to May, 2013.

***3*.*1.Sampling procedure***

From each flocks chickens were randomly selected for blood collection. 2-2.5 ml of blood was collected aseptically from wing vein using sterile syringe and needle. After that syringe with blood was kept in a cool box in a standing position for 6 hours, serum was harvested by decanting. The harvested sera were transferred to 1.5 ml micro centrifuge tubes and were kept in a cool box before shipping to the laboratory. Sera samples were stored at -20°C in the laboratory until use for RPA test (OIE, 2002).

***3*.*2.Preparation of antigens***

Antigens are the killed and colored *Salmonella* organisms. *Salmonella pullorum* antigens from standard (O: 1, 9,121 and 123) and variant (O: 1, 9,121 and 122) strains were used in this surveillance program for pullorum disease and fowl typhoid (Proux *et al*.*,* 2002). The *Salmonella* antigen (Nobilis® SP) used in this study were purchased from the Intervet International B.V. Boxmeer-Holland.

***3*.*3. Rapid plate agglutination (RPA) test***

The RPA test was conducted according to the instructions of OIE Manual (2002). For this test 0.02 ml of antigen and 0.02 ml of chicken serum were placed side by side with micropipettes on a glass plate. Then antigen and serum sample were mixed thoroughly by stirring with a small tooth pick. The glass plate was illuminated from below so as to facilitate observing the reaction, avoiding excessive heat from the light source. Positive reaction was characterized by the formation of definite clumps within 2 minutes after mixing the test serum with antigen (Fig.3.and 4.). The clumps usually started appearing and became concentrated at the periphery of the mixture. Negative reaction was judged by the absence of agglutination reaction. Care was taken so that the natural granulation of the antigen showed not to be taken as a positive reaction.

***3.4.Pathological study***

A total number of 25 dead layer chickens were examined to detect Salmonella infections. At necropsy, gross tissue changes were observed and recorded carefully and representative tissue samples (liver, lungs, spleen & intestine) containing lesions were preserved in 10 % buffered formalin for histopathological studies.

**Results and discussion**

***4.1. Overall prevalence of Salmonella infection***

A total of 20 sera samples were collected from commercial layer farms and were subjected to Rapid Serum Agglutination test. Out of these, 12 (60%) were found positive for single *Salmonella* infection. The overall seroprevalence of Salmonellosis was recorded as 43.4% (Islam *et al*., 2006). Yang *et al*. (1996) reported relatively similar findings (39.02%) which are lower than that of the present study. Ashenafi *et al*. (2003) and Habib-ur-Rahman *et al*. (2003) reported 64.2% and 63.5 % respectively, that was almost similar than that of the present study. The variation of seroprevalence might be speculated due to geographical variation or difference of management. But the present finding (43.4%) in commercial farms was higher than the seroprevalence (23.46%) recorded by Sikder *et al*. (2005) in local chickens. The difference with Sikder *et al*. (2005) was corresponded with the findings of Jha *et al*. (1995) and Robinson *et al*. (2000), who recorded seroprevalence rate higher in commercial flock than local chickens.

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| **Fig.1. Pouring of antigen from bottle and serum from eppendorf tube in glass slide.** | **Fig.2. Mixing of antigen with test serum.** |
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| **Fig.3. Medium size clumps show moderately positive reaction.** | **Fig.4. Large clumps, almost complete background clearing show highly positive reaction.** |

23.8% seropositive chickens for Salmonellainfection were found in Dinajpur district of Bangladesh (Alam *et al*., 2003). Bouzoubaa *et al*. (1992) recorded 23.5% seropositive chickens for salmonellosis from Morocco. Besides Minga *et al*. (1987) and Bhattacharya *et al*. (2001) reported 33.8% and 37.7% seropositive chickens for Salmonella infection in Tanzania and India, respectively. Whereas, Terzolo *et al*. (1977), Prukner (1987), Ghosh (1988), Muneer *et al*. (1988), Waltman and Home (1993), Yang *et al*. (1996), Hasegawa *et al*. (1999) reported 9.0%, 13.9%, 19.6%, 7.5%, 15.0%, 10.0% and 16.0% prevalence of Salmonella infection in chickens, respectively.

***4.2. Prevalence of Salmonella infection in different ages***

Table.1: Seroprevalence of salmonella at the age of 48 weeks in Kazi layer farm.

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| **Total no. of sample** | **Positive sample** | **Prevalence (%) of salmonella at the age of 48 weeks in Kazi layer farm** |
| **20** | **12** | **60** |

The prevalence found in the farm was 60% at the age of 48 weeks which is higher than the studies of Hossain *et al*. (2010) and Sikder *et al*. (2005). Concerning to the prevalence depending on the ages, the highest prevalence of Salmonella was 37.6% (27.2+10.4) at 64 weeks and above age group whereas the lowest prevalence was 16.6% (3.3+13.3) at 16-23 weeks age group (Hossain *et al*., 2010). Similar report was demonstrated by Sikder *et al*. (2005) who reported the highest Salmonella infection was 30.8% at 39 weeks of age and the lowest was 13.3% at 32 weeks of age. Truong *et al*. (2003) reported that the prevalence of Salmonella infection increased with the increase of age.

***4*.*3. Seasonal incidence of Salmonella infection***

The prevalence of Salmonalla infection in this study was 60% in summer season. The prevalence of Salmonalla infection was the highest (18.5+11.9=30.4%) in summer followed by winter (11.6+12.1=23.7%), rainy (14.2+10.8=25.0%) and autumn (13.3+10.0=23.3%) (Hossain *et al*., 2010). Similar report was demonstrated by Rahman *et al*. (2004) who reported 48.1% prevalence of Salmonella infection in summer in comparison to 23.7% in winter. Sikder *et al*. (2005) also recorded the highest (25.0%) prevalence of Salmonella infection in rainy season than in winter (21.9%). Bhattacharjee *et al*. (1996) reported the highest prevalence of salmonellosis during pre-monsoon (13.1%) in comparison to winter (10.4%), monsoon (6.8%) and post-monsoon (6.8%) period. The highest rate of Salmonella infection in summer season is probably due to the high growth rate of bacteria and the influence of hot weather that might reduce the immune status of the birds against infection (Hossain *et al*., 2010).

***4.4. Prevalence of Salmonella infection with regard to flock size***

Serological investigation showed 60% salmonella infection in 4500 birds that is comparatively higher than the study of Hossain et al. 2010. Serological investigation showed the highest (17.1+17.1=34.2%) Salmonella infection in large flocks (≥5001 birds) in comparison to small (≤1000 birds) flocks (12.5+8.8=21.3%) (Hossain *et al*., 2010). The present data were higher than those in the report of Skov *et al*. (1999) who recorded 16.8% *Salmonella* infection in a flock containing 30-40 thousand chickens in comparison to 11.9% in a flock containing 10-20 thousand and 9.7% in a flock containing less than 10 thousand chickens. Mdegela *et al*. (2000) recorded higher prevalence of *Salmonella* infection in commercial flocks (18.4%) than in scavenging chickens (6.3%) and reported that infection rate increased with the increase of flock size.

***4.5. Pathological study***

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| **Fig.5. Salmonella infected chicken shows congestion, discoloration and misshaped ova formation.** | **Fig.6. The liver shows friable congestion and bronze discoloration with focal necrosis.** |

In this study enlarged and congested liver with focal necrosis; haemorrhagic and discoloured ovary with stalk formation and mild haemorrhagic to catarrhal enteritis in intestine and caecum were recorded during necropsy. These types of necropsy findings were supported by Calnek *et al*. (1991), Chauhan and Roy (1996), Syed-Habib-ur-Rahman *et al*. (2004), Hossain *et al*. (2003) and Khan *et al*. (1998).

**CONCLUSION**

Although the sample size was small, an effort was made to conclude the seroprevalence of salmonella infection which was 60% in respect to age, season and flock size. This may conform that a higher level of salmonella was present in the farm. Besides friable congestion and bronze discoloration of liver with focal necrosis; hemorrhagic, discolored and misshaped ovary with mild hemorrhagic to catarrhal enteritis in intestine and caecum were recorded during necropsy.

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