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Prevalence and Antibiogram of *Salmonella* in Hisex Brown Strain at Commercial Poultry Farm in Chittagong

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Abstract

The present study was conducted to determine the sero-prevalence of *Salmonella* infections in poultry and their antibiogram profile. This study also covered necropsy and histopathological findings in different organs produced by *Salmonella*. Blood samples and visceral organs (liver, lung and intestine) from birds were collected from commercial layer farm of Chittagong district during January-May 2013. Rapid serum agglutination test was done by commercially available *Salmonella* antigen kit and postmortem examination was performed. Cultural properties on different selective media, biochemical test and staining technique were used to confirm *Salmonella* isolates. Finally cultural sensitivity test of the isolates were done against 6 commonly used antibiotics in poultry practice. The overall sero-prevalence of Salmonellosis was 60% in birds and the rate of sero-prevalence was higher at the age of 48 weeks in birds. *Salmonella* isolates were found highest resistant against Amoxicillin (100%) and Tetracycline (100%) followed by Ciprofloxacin (87%), Enrofloxacin (87%), Doxycycline (50%). Highest sensitive was observed against Gentamycin (100%). Pathologically friable, bronze color liver with focal necrosis, various grades of enteritis, and hemorrhagic and congested egg follicles with stalk formation were found in postmortem.

Keywords: Commercial layer, rapid serum agglutination, *Salmonella*, sero-prevalence.

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 *Salmonella* 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).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's negative, short plump shaped rods, non-spore forming, non-capsulated, aerobic or 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 been isolated from poultry. Pullorum disease is usually confined to the first 2-3 weeks of age and occasionally occurs in adults. Fowl typhoid is frequently referred to as a disease of adult birds and there are also reports of high mortality in young chicks.

The epidemiology of fowl typhoid and pullorum disease in poultry, particularly with regard to transmission from one generation 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. Eggs may become contaminated with *Salmonella* in two main ways: (i) *Salmonella* may silently infect the ovaries of apparently healthy hens and contaminate the eggs before the shells are formed, (ii) *Salmonella* infected bird droppings contain *Salmonella* that can contaminate the outer egg shells and may penetrate when crack the shell. 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. Immuno-histochemistry is a latest technique for that purpose. 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).

Selection procedures for detection of *Salmonella* infection in poultry is the aim of many studies. 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. 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 biovar Pullorum. 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. 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).

Materials and Methods

This study was conducted on Hisex brown stain (layer) at poultry farm in Chittagong district. Samples were collected from three flocks in the period of January to May, 2013. A total of 60 serum samples were collected during the study period.

Sampling:

From each flocks chickens were randomly selected for blood collection. About 2 to 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. Serum samples were stored at -20°C in the laboratory until use for RPA test (OIE, 2002).

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. The *Salmonella* antigen (Nobilis® SP) used in this study were purchased from the Intervet International B.V. Boxmeer-Holland.

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. 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.

Histopathological study:

A total number of 60 layer chickens were examined to detect *Salmonella* infections. At necropsy, gross tissue changes were observed and recorded carefully and representative tissue samples (liver, egg follicle & intestine) containing lesions were preserved in 10 % buffered formalin for histopathological studies.

Isolation and Identification of *Salmonella sp* from samples:

Staining Characteristic:

Gram's staining technique of the slide smear revealed small, uniform rod shaped Gram's negative organisms arranged singly and sometimes in pair form indicating *Salmonella sp*.

Growth of on selective media:

Pre-enrichment was done using BPW after incubation at 37˚C for 16 hours. One ml of inoculums was transferred into selenite-cysteine broth (SCB). After incubation a loop-full inoculum plated onto XLD agar and subjected for incubation at 37˚C for 24 hours. Culture plate revealed red colonies and some colonies were black centered. Those isolates which showed black centered colonies were considered as positive for *Salmonella sp* and indicator of H_2S production. Black centered colonies from XLD agar were plated onto BGA agar and after incubation the results were recorded. The positive isolates from XLD agar produced red or pink color colonies surrounded by brilliant red zones in BGA agar were recorded as positive for *Salmonella sp.*

Carbohydrate test:

Bacteriological culture positive isolates of *Salmonella sp* were subjected for biochemical characterization and those isolates produced positive reaction in carbohydrate fermentation tests were considered as Salmonella sp. Carbohydrate fermentation test was performed for the detection of *Salmonella sp* and five sugars were used like dextrose, lactose, sucrose, maltose and mannitol and incubated for 24 hour at 37ºC. The isolated *Salmonella sp* fermented dextrose and mannitol and produced acid and slight gas in the inverted Durham's tube.

Antimicrobial Susceptibility Profiling of *Salmonella sp*:

All biochemical test positive bacterial isolates (*Salmonella sp*) were investigated for their diversity in antimicrobial susceptibility profiles by disk diffusion method on Mueller-Hinton agar (Oxoid ltd, Basingstoke, Hampshire, UK) according to the Clinical Laboratory Standards Institute. Bauer-Kirby disk-diffusion procedure was used on Mueller-Hinton (MH) agar, prepared according to the manufacturer's instructions (Oxoid ltd, Basingstoke, Hampshire, UK). The following antibiotics and disc potencies were used: GEN: Gentamicin (10µg), DO: Doxycycline (30µg), CIP: Ciprofloxacin (5µg), ENR: Enrofloxacin (5µg), AMC: Amoxycillin (10µg), TE: Tetracycline (30µg) (Oxoid Ltd. Basingstoke, Hampshire, England). Interpretation was done based on the recommendations from Clinical and Laboratory Standards Institute (CLSI, 2007).

Results

Overall Prevalence of *Salmonella* Infection:

A total of 60 sera samples were collected from commercial layer farms and were subjected to Rapid Serum Agglutination test. Out of these, 36 (60%) were found positive for single *Salmonella* infection. The overall sero-prevalence of Salmonellosis was recorded as 43.4%. The present findings are 60% sero-prevalence in commercial farms. 23.8% seropositive chickens for *Salmonella* infection were found in Dinajpur district of Bangladesh (Alam *et al.*, 2003).

Prevalence of *Salmonella* Infection According to Age:

The prevalence found in the farm was 60% at the age of 48 weeks. 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.

Histopathological Study:

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 as histopathological findings.

Antimicrobial susceptibility profile testing of *Salmonella sp*

A resistance of *Salmonella* isolates was observed against Amoxicillin and Tetracycline to 100%, followed by Ciprofloxacin (87%), Enrofloxacin (87%), Doxycycline (50%). Drug sensitivity pattern of *Salmonella* showed highly sensitive to Gentamycin. Antimicrobial resistance among *Salmonella* isolates is increasing worldwide and is likely due to the wide spread use of antimicrobial agents for the empiric treatment and as growth enhancers in animal production. The increasing rate of resistance to amipicillin, tetracycline and chlorampenical since 1996 among isolates from human, pigs, and chickens can be attributed to the emergence of multi resistance serovar *Salmonella* Typhimurium.

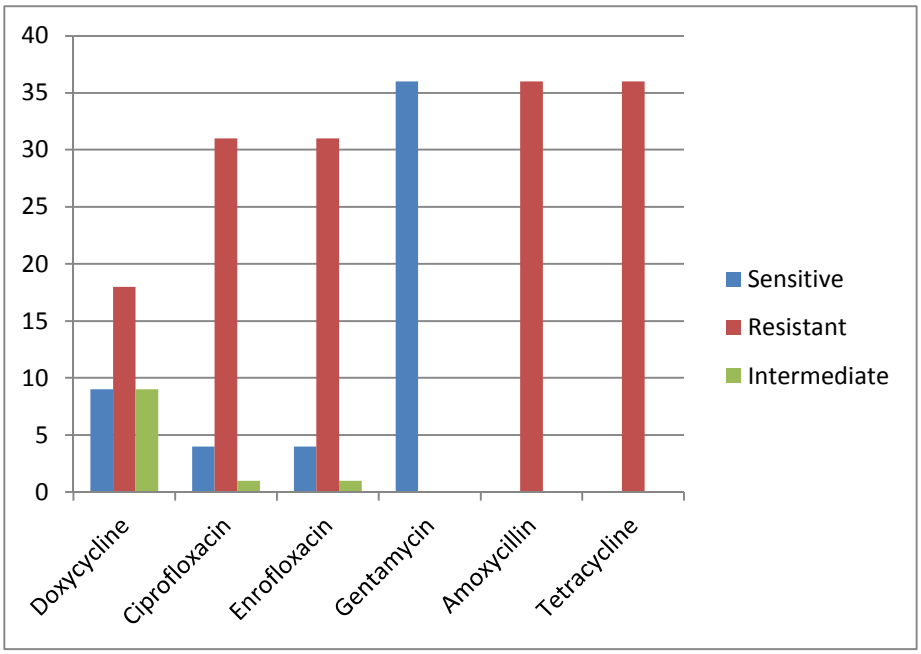

Fig. Graph showing percentage of resistance to different antibiotic disk.

Discussion

Overall prevalence of *Salmonella* infection

The overall sero-prevalence 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 present finding (60%) 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) recorded seroprevalence rate higher in commercial flock than local chickens. 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 Bhattacharya *et al.*, (2001) reported 33.8% and 37.7% seropositive chickens for Salmonella infection in Tanzania and India respectively.

Prevalence of *Salmonella* Infection According to Age

The 60% prevalence was found at the age of 48 weeks which is higher than the studies of Hossain *et al.*, (2010) and Sikder et al. (2005). The highest prevalence of *Salmonella* was 37.6% (27.2+10.4) at 64 weeks and the lowest prevalence was 16.6% (3.3+13.3) at 16-23 weeks age group. 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.

Histopathological Study:

The findings of necropsy were supported by Calnek *et al.*, (1991), Chauhan and Roy (1996), Habib-ur-Rahman *et al.*, (2003), Hossain *et al.*, (2003) and Khan *et al.*, (1998).

Antimicrobial Susceptibility Profiling of *Salmonella sp*:

Salmonella isolates are most sensitive to Gentamycin among all antibiotics. The present study showed close agreement with Rahman *et al.*, (2009). On the other hand, highest resistance was found against Amoxicillin and Tetracycline. Similar type of finding was observed by Hemen *et al.*, (2012).

Conclusion

Although the sample size was small, an effort was made to conclude the sero-prevalence of salmonella infection which was 60% in respect to age. This may confirm 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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