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Prevalence of *Salmonella* in Apparently Healthy Chickens in Mymensingh, Bangladesh

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A B S T R A C T

Salmonella spp. are the most frequently reported cause of food-borne illnesses worldwide that are closely associated with the consumption of contaminated poultry and egg products. This study was designed to isolate and identify *Salmonella*, and evaluate its prevalence in chickens of different lines and ages during summer and rainy seasons. Cloacal swab samples (n=200) of apparently healthy chickens were collected. Isolated *Salmonella* were characterized using cultural, biochemical and serological examinations. A total of 104 samples (52%) were found to be positive for *Salmonella* spp. The prevalence of *Salmonella* spp. was significantly higher (p<0.01) during summer (67.78%) as opposed to rainy season (39.09%). Prevalence of *Salmonella* was 71.11% in broiler, 38.89% in layer and 25% in indigenous chicken. Broilers showed significantly higher prevalence of *Salmonella* as compared to layer and indigenous chickens (p<0.01). Among the five age groups, the highest prevalence was observed in chickens of 18-week of age (65%) and the lowest was in chickens of 2-week age (16.67%). The data of this study showed higher prevalence of *Salmonella* in broilers and underscored the need for detail epidemiological investigations as well as strict hygienic practices in farm and live bird markets all over Bangladesh.

Keywords: *Salmonella*, Prevalence, Seasons, Chickens

Introduction

Salmonellosis is a disease condition caused by a large group of bacteria of the genus *Salmonella* that can affect all species of domestic animal including cattle, sheep, goat, pigs and poultry and ultimately human beings throughout the world (OIE, 2010). It is an important cause of enteric illness. A wide range of foods has been implicated in food-borne salmonellosis. However, as the disease is primarily zoonotic, foods of animal origin have been consistently implicated as the main sources of human salmonellosis (FAO/WHO, 2002). *Salmonella* infection in human causes several clinical conditions such as: enteric fever, enterocolitis and systemic infections (Piyush and Anju, 2008).

The most common source of human salmonellosis is food of poultry origin since poultry products are the most important reservoirs of *Salmonella*. Poultry and poultry products are often implicated in sporadic cases and in outbreaks of human salmonellosis (Bryan and Doyle, 1995; Humphrey, 2000). As *Salmonella* is a pathogen present in the gut of poultry, there is chance of transmission of *Salmonella* infection from poultry to human through food chain. It might happen if proper hygienic measure is not maintained during processing of poultry meat preparation. Cross-contamination occurs especially at scalding, defeathering, evisceration and giblet operations (Bryan and Doyle, 1995). Unfortunately, no effective barriers that would control *Salmonella* during processing exist (Fries, 2002). However, differences in hygiene practices between slaughterhouses along with resulting differences in carcass contamination suggest that improved hygiene management could significantly reduce the risk of *Salmonella* contamination of broiler meat (Heyndrickx *et al*., 2002). In this study, isolation and identification of *Salmonella* have been conducted in order to know its prevalence in the apparently healthy chickens on farms, live bird market and village in Mymensingh.

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Materials and Methods

Sample collection

Cloacal swab samples (n=200) were collected from apparently healthy broilers (n=90), layers (n=90) and indigenous chickens (n=20) in different areas of Mymensingh, namely Bangladesh Agricultural University (BAU) Poultry Farm, Boira Poultry Farm, Boira market and Kamal Ranjit (KR) Live bird market during summer (March-May) and rainy (July-September) seasons. Swab samples were collected from chickens belonged to 2-week (n=30),

5-14 weeks (n=95), 18-week (n=20), 28-32 weeks (n=25) and 52-week (n = 30) of age.

Isolation of *Salmonella*

After aseptic collection, the swab samples were enriched in nutrient broths at 37^{0}C for 24 hours. Then the broths were streaked on Salmonella-Shigella agars (SS) and incubated at 37^{0}C for 24 hours. Finally, black centered colonies grown on SS agar were sub-cultured on nutrient agar, blood agar, MacConkey agar, eosin methylene blue agar (EMB), triple sugar iron agar (TSI) and brilliant green agar (BG) and incubated at 37^{0}C for 24 hours for further characterization to identify *Salmonella.*

Identification of bacteria

Salmonella was identified by cultural characteristics, Gram staining, motility test, biochemical and serological tests. Colony morphology such as shape, size, surface texture, edge, elevation, color and opacity observed after 24 hours of incubation were also recorded. The representative *Salmonella* colonies were stained using Gram staining method (Merchant and Packer, 1967). The motility test was performed according to the previously described method (Cheesbrough, 1985) to know the motility characteristics of *Salmonella*. Isolated bacteria showing characteristic colony morphology of *Salmonella* on culture media were subjected to various biochemical tests such as; sugar (Dextrose, Sucrose, Lactose, Maltose and Mannitol) fermentation, Indole and, Methyl Red-Voges Proskauer (MR-VP) tests (Cowan, 1985). Serological identification of *Salmonella* was performed by rapid slide agglutination test (Andrews *et al*., 2005) using polyvalent "O" (Poly ''O'') antisera (S & A reagents Lab, Bangkok, Thailand) following the manufacturer's protocol.

Statistical analysis

Prevalence of *Salmonella* in chickens during summer and rainy seasons as well as in various chicken lines and ages was compared for statistical significance by Chi-square test (SPSS 11.5, UK) where a p-value of ≤ 0.05 was considered to be statistically significant.

Results

Cultural findings

Bacteria isolated from the cloacal swabs of broilers, layers and indigenous chickens produced black centered, smooth, small round colonies on SS agar. On BGA, colonies were translucent pink surrounded by a pink zone. On MacConkey agar, colonies were colorless, smooth, transparent and raised. On TSI agar, colonies were black in color against a yello-wish background and on EMB agar they were pink in color, circular and smooth.

Staining and motility tests

Isolates of chicken were Gram negative, rod shaped and motile with swinging movement.

Biochemical tests

All of the isolates fermented dextrose, maltose and mannitol with the production of acid and gas but did not ferment lactose and sucrose. The isolates were negative to Voges-Proskauer test and indole test but were positive to methyl red test.

Serological test

All culturally and biochemically positive *Salmonella* spp. showed agglutination reactions in rapid slide agglutination tests against polyvalent "O" (Poly ''O'') antisera.

Prevalence of *Salmonella* in summer and rainy season

In summer, 61 samples out of 90 were *Salmonella* positive. On the contrary, 43 of 110 were positive for *Salmonella* in rainy season. The overall prevalence of *Salmonella* was 52% in two seasons. Statistical analysis revealed that the prevalence of *Salmonella* in chicken was statistically higher in summer as compared to rainy season (p<0.01). Seasonal prevalence of *Salmonella* in chickens is presented in Fig.1.

Prevalence of *Salmonella* in broiler, layer and indigenous chicken

The prevalence of *Salmonella* in broiler, layer and indigenous chickens is presented in Fig.2. Prevalence of *Salmonella* was significantly higher in broiler when compared to layer and indigenous chicken ($p < 0.01$).

Prevalence of *Salmonella* according to ages

The highest prevalence was 65% in chickens of 18-week of age whereas chickens at 2-week age showed the lowest (16.67%) prevalence (Fig. 3).

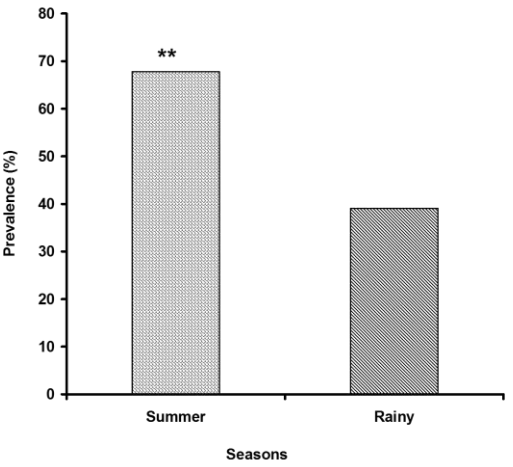

Fig. 1. Prevalence of *Salmonella* in chicken during summer and rainy seasons. Prevalence of *Salmonella* was significantly higher in summer as compared to rainy season which is indicated by asterisks (**, $p < 0.01$).

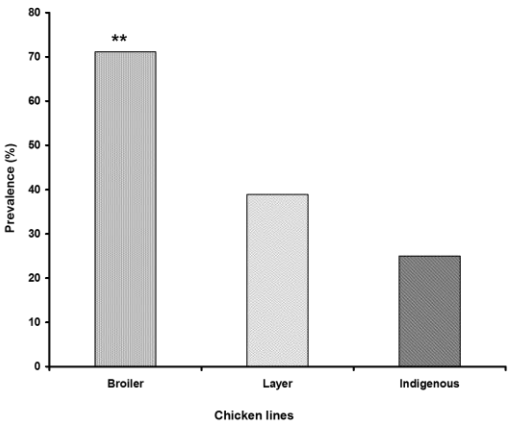

Fig. 2. Prevalence of *Salmonella* according to chicken lines. Prevalence of *Salmonella* was significantly higher in broilers as compared to layers and indigenous chickens which is indicated by asterisks (**, $p<$ 0.01).

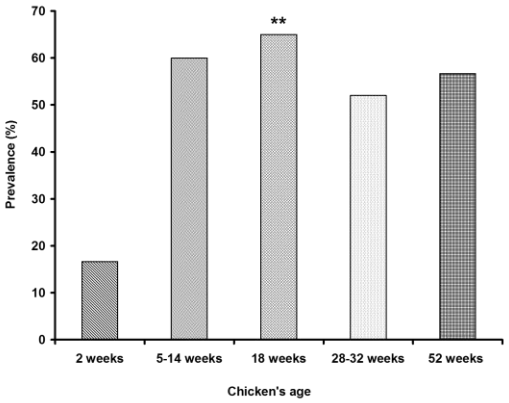

Fig. 3. Prevalence of *Salmonella* in chicken at different age. Prevalence of *Salmonella* was significantly higher in chickens at 18-week of age compared to other age groups which is indicated by asterisks (**, $p < 0.01$).

Discussion

This piece of research showed differences in the prevalence of *Salmonella* spp. in different seasons, chicken lines and ages in the Mymensingh area. This study recorded significantly higher prevalence of *Salmonella* in summer as compared to rainy season. Similarly, a study conducted in Nepal recorded the highest prevalence of *Salmonella* during summer in chicken raw meat (Maharjan *et al*., 2006). Haque (2011) also reported a higher prevalence of *Salmonella* in apparently healthy geese in the Mymensingh area during summer. Temperature may be a major factor for the survival and proliferations of *Salmonella*; warm temperatures provide suitable environment for the growth of *Salmonella* (Guthrie, 1992; Latimer, 1999). The prevalence of *Salmonella* recorded in this study is in agreement with several other studies (Dallal *et al.,* 2009; Meldrum and Wilson, 2007; Molla *et al.,* 2003; Carraminana *et al.,* 2004; Mehrabian and Jaberi, 2007).

The prevalence of *Salmonella* recorded in broilers, layers and indigenous chickens in the present study showed similarity with the findings of other researchers (Limawongpranee *et al*., 1999; Snow *et al.,* 2007). The highest prevalence of *Salmonella* in broiler chickens recorded in the current study might be due to overcrowding and improper sanitary measures of the farms. The results of this study indicated that broilers could be an important reservoir of *Salmonella* spp. Contrarily, indigenous chickens showed the lowest prevalence which might be due to free range management system with less or no crowding or presence of inherent resistance against *Salmonella*. The highest prevalence was recorded in 18-week old chickens which satisfies the findings of Li *et al*. (2007).

The overall prevalence of *Salmonella* (52%) as recorded in this study showed similarities and differences with findings of other researchers working on the prevalence of *Salmonella* in poultry. For instance, Haque (2011) isolated 48.07% *Salmonella* from cloacal samples of apparently healthy ducks, whereas, Aktar (2011) found 59% prevalence of *Salmonella* in quails, and Sarkar (2009) recorded 20.83 and 75% prevalence of *Salmonella* in feces and cloacal swabs of apparently healthy water birds, respectively.

Conversely, Chiu *et al.* (2010) found 0.3% prevalence in cloacal swabs of breeder broiler and 11.3% in broiler, and Mondal (2007) recorded 13.07% prevalence of *Salmonella* in healthy and diarrheic ducks. The differences of *Salmonella* prevalence among different lines of chickens might be due to the species resistance, hygienic practices during rearing as well as various environmental and geographic factors.

Infection by *Salmonella* is a common cause of food poisoning in human (Hobbs and Robert, 1993). Human may get *Salmonella* infection during processing of poultry carcasses and close contact with poultry in live bird markets and farms.

Conclusion

Data from this study suggest that chickens at farms and live bird markets are the major reservoirs of *Salmonella* spp. As a result, this might cause food poisoning if proper hygienic measures are not undertaken during rearing, handling and processing of poultry and poultry byproducts. This study will create public awareness about *Salmonella* in healthy poultry and would be helpful for controlling *Salmonella* associated food-borne infections originating from chickens.

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