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Research article

A cross-sectional survey on the prevalence and antimicrobial resistance of *Salmonella* in commercial and backyard chicken farms in Bangladesh

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A B S T R A C T

A cross-sectional survey was conducted to find out the prevalence of *Salmonella* in chicken farms in Chittagong by analyzing the fecal samples from May to November 2009. Isolation and identification of *Salmonella* from fecal samples were done according to the conventional methods and all the isolates of *Salmonella* were tested for their sensitivity patterns to 9 antimicrobials. For farm prevalence of *Salmonella*, three categories of chicken farms were targeted: commercial layer, commercial broiler and backyard. Seroprevalence of *Salmonella* Pullorum was also done, having retrieved blood serum samples from the serum bank maintained at the Department of Microbiology, Chittagong Veterinary and Animal Sciences University (CVASU). This serum bank was made, having collected serum samples from chickens of the Participatory Livestock Development Project (PLDP) and Smallholder Livestock Development Project-2 (SLDP-2) areas. The results showed that the farm-prevalence of *Salmonella* in backyard and commercial layer farms were almost identical- 28 vs 23% (P=0.517), but a significant lower prevalence of *Salmonella* was observed in commercial broiler farms compared with backyard (P= 0.008) and layer (P=0.062) farms. Of the 288 serum samples tested 37 (9.5%) were seropositive for *Salmonella* Pullorum. However, there was no significant difference between seroprevalence of *S*P in the backyard chickens in two different areas (P=0.980). All the isolates were resistant to 4 antimicrobials tested, namely colistin sulphate, co-trimoxazole, oxytetracycline and penicillin. The antimicrobial sensitivities of the isolates of different production categories varied widely.

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INTRODUCTION

Poultry sector plays an important role in the national economy of Bangladesh. Several constraints among which occurrence of diseases seriously affect the optimal performance of poultry industry in Bangladesh (Haque *et al*., 1991). Fowl typhoid, caused by *Salmonella* Gallinarum is one of the predominant poultry diseases in Bangladesh which can cause significant economic loss to a farm (Begum *et al*., 1993; Hoque *et al*., 1992). The

incidence of salmonellosis in Bangladesh was found to be 9.3% (Bhattacharjee *et al*., 1996). The epidemiology of fowl typhoid and pullorum disease in poultry, particularly with view of transmission from one generation to the next is known to be closely associated with infected eggs (Wigley *et al*., 2001). The birds that survive from clinical disease when infected at a young stage may show few signs of infection but can become carriers (Berchieri *et al*., 2001). The genus *Salmonella* includes a large group of serologically and biologically related bacilli and as a rule they are motile by means of peritrichous flagella with the exception of serovars *S.* Pullorum and *S.* Gallinarum. *Salmonellae* are Gram negative, short plump shaped rods, non-spore forming, non-capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae. More than 2500 serovars exist. All motile serovars of *Salmonella* are zoonotic and poultry is considered one of the major sources to infect humans. Reports on prevalence of *Salmonella* at farm level belonging to any kind of poultry production in Bangladesh is scanty, if not absent. This is important to know to predict the threat being posed from *Salmonella* of poultry origin to the public health and as well as to the overall poultry production.

The aims of the study were to elucidate a comparative scenario of its prevalence among the three kinds of production- commercial layer, commercial broiler and backyard chickens, seroprevalence of one poultry specific serovar – *S.* Pullorum in backyard chickens and the resistance spectrum being acquired in the circulating strains against commonly used antimicrobials in the veterinary field.

MATERIAL AND METHODS

Sample size determination

The three production categories – commercial layer, commercial broiler and smallholders' backyard chickens were regarded as three individual units of sampling. The required sample size was 100 per unit based on the formula $n=4pq/l^2$ (Permin and Hansen, 1998). Because the farm prevalence of *Salmonella* in any of the three production categories was not known in Bangladesh, a prevalence of 50% was considered with a precision of 5% to estimate the required sample size.

Sampling strategy

The study was carried out between May and November 2009, both the months inclusive. Having collected samples both fecal and serum, all the investigations were done at the Laboratory of the Department of Microbiology, Chittagong Veterinary and Animal Sciences University (CVASU). Randomly selected 100 chicken farms for each category of production in Chittagong district were visited and pooled fecal samples were collected from each selected farm along with bird-population statistics and farm management data by filling out a prototype sample collection sheet. Each sample was given a unique code number, e.g. B1, B2, …, B100 for commercial broiler farms; L1, L2, …, L100 for commercial layer farms and S1, S2, …, S100 for small holders' backyard chicken farms, and immediately transported to the CVASU Microbiology Laboratory in a cool box. Two hundred gram pooled feces was collected from each commercial poultry farm and 50 gram from each backyard chicken smallholding. After collection, each sample was kept frozen at -84˚C in the Department of Microbiology, CVASU until investigated.

Isolation and identification procedures

The study was conducted utilizing the conventional methods for the detection of *Salmonella* following the standard guidelines. There were four definite sequential steps: (1) Non-selective pre-enrichment, (2) Selective enrichment, (3) Plating out and identification, and (4) Confirmation by following standard bacteriological procedures. Buffered peptone water (Oxoid) was used as non-selective pre-enrichment broth. Rappaport-Vassiliadis medium (RVS broth) was used as selective enrichment. For plating out and identification either MacConkey agar or *Salmonella*-Shigella (SS) agar or both were used.

Collection and analysis of serum samples

Three hundred and eighty-eight serum samples investigated in this study were collected from the rural chicken households in the 6 northern- and 4 southern upazilas in Bangladesh under the so called Participatory Livestock Development Project (PLDP) and the Smallholder Livestock Development Project-2 (SLDP-2), respectively. One of the important features of these two projects was to supply genetically improved birds particularly Sonali, a crossbred (male Rhode Island Red x female

Fayoumi). These developmental projects were financed by the Danish International Development Agency (DANIDA) during the period of 2002-2007. Of the 388 serum samples investigated in this study 209 and 179 samples were collected from the PLDP and the SLDP-2 areas, respectively. A serum sample either from the PLDP or from the SLDP-2 area was collected in a sterile Eppendorf tube (1.5 ml) to which merthiolate with a concentration of 1:10,000 was added and initially stored at 4°C in a refrigerator kept in the Upazila Livestock Office concerned. Eventually, all the samples collected from the PLDP and the SLDP-2 areas were transferred to the department of Microbiology, CVASU, and kept further frozen at -84°C, constituting a serum bank for smallholders' chickens.

Standard *Salmonella* (Nobilis® SP) antigen manufactured by Intervet International, Holland was used for serum plate agglutination test (SPA) test for the detection of *Salmonella* Pullorum (SP) antibodies in the serum samples following the instructions of the manufacturer. For negative control SPF chicken serum (produced by the Veterinary Laboratories Agency, UK) was used. A positive reaction was indicated by agglutination within 2 minutes after mixing of antigen and the serum.

Antimicrobial sensitivity testing

The antimicrobial sensitivity testing was done by the disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI). The isolates were tested against nine antimicrobials: amoxicillin, ampicillin, ciprofloxacin, colistin sulphate, co-trimoxazole, gentamicin, neomycin, penicillin and oxytetracycline.

Data analysis

All breed, sex, production, management and disease data relating to prevalence of *Salmonella* at farm level was entered into a spreadsheet (Microsoft® Office Excel 2003) and transferred to STATA-9.2 for data management and summary.

RESULTS

Population statistics of the farms surveyed for the prevalence of *Salmonella*

Only non-descriptive indigenous chickens were kept in the backyard farms investigated. Multi-age small size flocks were sustained in the farms with the median number of birds 8 (minimum 2, maximum 20). Hybrid layer and broiler strains were reared in the layer and broiler farms. The age of the birds housed in a farm at any one time was homogenous. All the layer and broiler farms investigated in this study belonged to the FAO defined system 3 (Small Scale Commercial Production System) with the provision of minimum or no biosecurity for the birds. The median flock size of layer and broiler farms were 1500 (minimum 200, maximum 35000) and 1200 (minimum 23, maximum 7000), respectively (Table 1). None of the farm had a history of vaccination against any serotype of *Salmonella*. By scavenging backyard chickens collected bulk of their feed requirement on their own; however, owners of the farms occasionally provided some additional feed in the forms of broken rice, maize, rice polish, pulses etc., but not any of commercial ready-made feed. On the contrary, commercial chickens in both layer and broiler farms were fed with commercially available ready-made feed or with farmers' self-formulated feed. Precisely, 48% and 28% commercial farms used feed that had the ingredient fish meal and meat and bone meal, respectively.

Table 1. An overview of population statistics of farms investigated for farm prevalence of *Salmonella* in Chittagong

Production category	n	Bird population number			Production system*
		Min	Med	Max	
Backyard	100	2	8	20	4
Layer	100	200	1500	35000	3
Broiler	100	23	1200	7000	3

*FAO defined (2005)

Prevalence of *Salmonella* at farm levels

Figure 1 portrays the overall farm prevalence of *Salmonella* observed in the backyard, commercial layer and broiler farms. The prevalence of *Salmonella* in backyard and commercial layer farms was almost similar- 28% and 23%, respectively (P= 0.517), but a significant lower prevalence was observed in commercial broiler farms compared with backyard (P=0.008) and layer (P=0.062) farms. The prevalence of *Salmonella* was almost similar (P= 0.784) in layer farms using or not using fish meal in feed. Two layer farms positive with *Salmonella* had

≤1 months' old chicks. Of the 16 farms investigated, that had chicks of ≤2 weeks' old, 4 (25%) were *Salmonella* positive. The highest rate of *Salmonella* isolation was with the farms which had histories of using enrofloxacin (35%) followed by chlortetracycline (33%) and sulfur drugs plus trimethoprim (31%).

Seroprevalence of SP in chickens on backyard farms

Of the 288 serum samples collected from backyard chickens in the PLDP and SLDP-2 areas 37 (9.5%) were seropositive for *S.* Pullorum (Table 2). However, there was no significant difference between seroprevalence of SP in the backyard chickens in the PLDP and SLDP-2 areas (P=0.980).

Table 2. Seroprevalence of *Salmonella* Pullorum (SP) in Backyard chicken flocks in Bangladesh

Study area	No. of Sample	No. Positive	*SP* Seroprevalence (%) (95% CI*)
PLDP	209	20	9.5 (6.2-14.3)
SLDP-2	179	17	9.5 (5.9-14.7)
Total	388	37	9.5 (7.0-12.9)

*CI = Confidence interval; PLDP: Participatory Livestock Development Project, operated in northern 17 districts during 2002-2005; SLDP-2: Smallholder Livestock Development Project-2, operated in 6 southern districts during 2004-2007; P = 0.980.

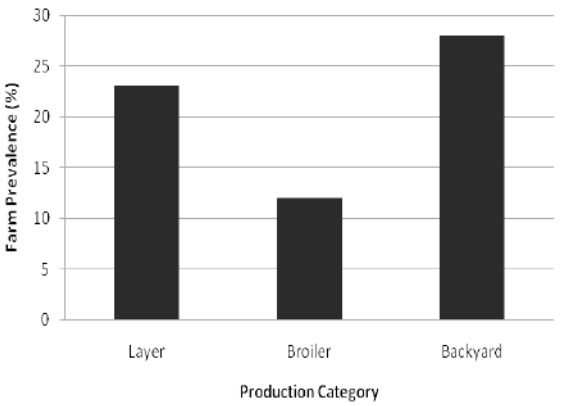

Figure 1: Rates of *Salmonella* isolation in three different production types (Layer, Broiler and Backyard)

Antimicrobial sensitivity patterns of the *Salmonella* isolates

All the isolates were resistant to 4 antimicrobials tested, namely colistin sulphate, co-trimoxazole, oxytetracycline and penicillin. The sensitivities of the isolates belonging to the 3 chicken production categories varied widely: 71% isolates from backyard chickens were sensitive to gentamicin, the highest proportion of the isolates of any of the chicken production tested, 44% isolates from layer chickens were sensitive to this antibiotics while only about 17% isolates of broiler origin were sensitive to it. About 22% isolates of backyard and layer chickens each were sensitive to neomycin. Only 7% isolates of backyard chicken origin were sensitive to ampicillin and only 4% isolates of layer chicken origin were sensitive to ciprofloxacin, but the other isolates were resistant to these antibiotics (Table 3).

DISCUSSION

The prevalence of *Salmonella* including all three categories (Layer, Broiler and Backyard) was 21%, whereas its prevalence in layer farms was 23%, in broiler 12% and in the backyard chickens 28%. An overall prevalence of 14.7% in live poultry birds was earlier reported from India (Murungkar *et al*., 2005); some reports indicated negligible prevalence in poultry carcasses (Vaidya *et al*., 2005). In broiler, Oliveira *et al*. (2006) found the prevalence 11.8%. The result is closely related to that of ours. Mehrabian *et al*. (2007) isolated *Salmonella* from meat products in Tehran and found an overall prevalence of 20% in broiler. Willayat *et al*. (2006) isolated *Salmonella* from 2.4% and 4.0% samples of fresh- and frozen chicken, respectively. In 1996 Bhattacharjee *et al*. conducted a study in Bangladesh and the overall prevalence of salmonellosis was found to be 9.28% in poultry, lower than that was observed in this study. Bouzoubaa *et al*. (1992) revealed that, up to 58% of the village chickens had antibodies against *S.* Gallinarum and *S.* Pullorum. Similar findings were reported by Adesiyun *et al*. (1984) from Nigeria. Chrysostome *et al*. (1995) reported that 10% of the village chickens had antibodies against *S*. Pullorum. In Mauritania, Bell *et al*. (1990) found that 17.5% of the birds had antibodies against *S.* Pullorum. The present study reveals that, overall prevalence of *Salmonella* in backyard chickens could be 28%. In both layer and broiler farms, *Salmonella* was isolated from young birds (≤2 weeks in case of broiler and ≤1 month in case of layer), indicating that vertical transmission from breeder farms might have occurred. *Salmonella* is probably going to be resistant to almost all commonly used antibiotics, such as, enrofloxacin, sulfa drugs, sulfa drugs plus trimethoprim, etc.

Table 3. Antimicrobial susceptibility of 63 *Salmonella* isolates of chicken origin (Broiler, Layer and Backyard) to 9 selected antimicrobial agents. Test was performed by micro disc diffusion technique of Kirby-Bauer on Mueller-Hinton Agar

Antimicrobial Agent (Disc Potency)	Antimicrobial Sensitivity Result (Number/%)								
	Sensitive: No./%			Intermediate: No./%			Resistant: No./%		
	ILO	IBO	IBaO	ILO	IBO	IBaO	ILO	IBO	IBaO
Amoxycillin (10µg)	0/0	0/0	0/0	0/0	0/0	1/3.6	23/100	12/100	27/96.4
Break Point (mm)*	(≥15)			(12-14)			(≤11)		
Ampicillin (10µg)	0/0	0/0	2/7.1	0/0	0/0	0/0	23/100	12/100	26/92.9
Break Point (mm)*	(≥15)			(12-14)			(≤11)		
Ciprofloxacin (5µg)	1/4.3	0/0	0/0	0/0	0/0	0/0	22/95.7	12/100	28/100
Break Point (mm)*	(≥20)			(17-19)			(≤16)		
Colistin sulphate (25µg)	0/0	0/0	0/0	0/0	0/0	0/0	23/100	12/100	28100
Break Point (mm)*	(≥15)			(-)			(≤14)		
Co-trimoxazole (25µg)	0/0	0/0	0/0	0/0	0/0	0/0	23/100	12/100	28/100
Break Point (mm)*	(≥16)			(-)			(≤15)		
Gentamicin (120µg)	10/44	2/16.7	20/71.4	4/17.4	2/16.7	4/14.3	9/39.1	8/66.7	4/14.3
Break Point (mm)*	(≥20)			(17-19)			(≤16)		
Neomycin (30µg)	5/21.7	0/0	6/21.4	10/43.5	6/50	11/39.3	8/34.8	6/50	11/39.3
Break Point (mm)*	(≥15)			(12-14)			(≤11)		
Oxytetracycline (30µg)	0/0	0/0	0/0	0/0	0/0	0/0	23/100	12/100	28/100
Break Point (mm)*	(≥15)			(12-14)			(≤11)		
Penicillin G (10 unit)	0/0	0/0	0/0	0/0	0/0	0/0	23/100	12/100	28/100
Break Point (mm)*	(≥15)			(12-14)			(≤11)		

ILO = Isolates of Layer Origin; IBO = Isolates of broiler origin; IBaO = Isolates of backyard origin; *Diameter of the zone of inhibition indicates the defined criterion; n for isolates of layer, broiler and backyard chickens' origin were 23, 12 and 28, respectively

In commercial farms, antibiotics are used indiscriminately which might be responsible for acquiring resistance in *Salmonella*. In the study it was found that, more antibiotics were used in the broiler farms. Moreover, gentamicin was injected subcutaneously in almost all day-old-chicks at the hatcheries. *Salmonella* strains of backyard chicken origin were found to be mostly susceptible to gentamicin (71%) whereas lowest susceptibility (17%) and highest resistance (67%) were found in the isolates of broiler origin. In some studies, a changing pattern of the multi-drug resistant *Salmonella* isolates was noted (Madhulika *et al*., 2004; Das and Bhattacharya, 2006). A high resistance of *Salmonella* strains of broiler and layer chickens origins, obtained from this study to major commonly used antimicrobials should warrant the veterinarians and farmers towards a rational use of them.

CONCLUSION

The prevalence of *Salmonella* at commercial layer or smallholder farm level would be ~25%; however, its prevalence at commercial broiler farm level might be~12%. About 10% backyard chickens are seropositive for *Salmonella* Pullorum. The circulating *Salmonella* strains in the commercial productions are highly resistant to most antimicrobials tested for this study, indicating the need for rational use of them in poultry practice and production in the country.

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