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Isolation and identification of *Escherichia coli* and *Salmonella sp.* from apparently healthy Turkey

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Abstract

A study was designed with the aim of isolation and identification of *Escherichia coli* and *Salmonella sp*. in order to identify the prevalence of these bacteria in a turkey flock. For this, 120 (n) cloacal swab samples were collected from the apparently healthy turkey of a farm at Foujdarhat Cadet College Campus, Chittagong. The study was performed in between April and May 2016 where 120 swab samples were tested by using standard laboratory protocol in Poultry Research and Training Centre, Chittagong. Among biochemical tests, Indole test was done for the confirmation of *E. coli* where TSI agar slant was done for ensuring the positive *Salmonella sp.* Here, 90 (75%) samples were found positive for *E. coli* and 42 (35%) were for *Salmonella sp*. However, 21 (17.5%) samples had mixed infection with both *E. coli* and *Salmonella sp*. In this study, the prevalence of *Salmonella* infection according to sex and laying, the non-laying category the result was found statistically significant ($p \leq 0.05$); similarly, in case of *E. coli* the prevalence between flock-1 and flock-2 also showed significant ($p \leq 0.05$) findings. It was observed that Turkey generally acts as a carrier of a vast range of organisms including *E. coli* and *Salmonella sp*. This is first time studied to investigate *E. coli* and *Salmonella sp*. from Turkey in Chittagong, Bangladesh. So, further study is highly recommended.

Keywords: Prevalence, diarrhea, *Escherichia coli*, Salmonella spp.

Introduction

In Bangladesh, poultry production system is grouped into the traditional and commercial system (Haque *et al*., 1991). Commercial rearing system of poultry for meat and egg has created a new era in the present time. Poultry, namely domestic chickens, turkeys, geese, and guinea fowls are kept throughout the world. Infections with bacteria of the genus *Salmonella sp.* and *E. coli* bring about a variety of acute and chronic diseases of poultry in Bangladesh (Bhattacharjee *et al*., 1996 and Kamal *et al*., 1988). Thereby those are responsible for mortality, reduced egg production, and hatchability as well (Barnes *et al*., 1997). The intestinal tract of poultry consists of complex and dynamic microbial community comprising primarily of bacteria (Zhao *et al*., 2001). The large family Enterobacteriaceae includes Gram (-ve) bacteria along with many harmful symbiotics such as *Salmonella spp*., *E. coli*, *Yersinia pestis*, *Klebsiella*, and *Shigella*.

Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter* (Garrity *et al.,* 2004). *Escherichia coli* is a Gram (-ve), rod-shaped, facultatively anaerobic bacteria. This pathogenic *E. coli* can be categorized based on serogroups, pathogenic mechanisms, variation in epidemiology and different interaction with the intestinal mucosa, clinical symptoms or virulence factors (Kaper, 2005). Mainly they are-(1). Enterotoxigenic *E. coli*, (2). Enteroinvasive *E. coli*, (3). Enteropathogenic *E. coli*, (4). Enterohemorrhagic *E. coli* and (5). Enteroaggregative *E. coli*. Colibacillosis refers any localized or systemic infection caused entirely or partly by Avian Pathogenic *Escherichia coli* (APEC) including coli septicemia, coli granuloma, cellulitis, swollen head syndrome, peritonitis, salpingitis, osteomyelitis, synovitis, panophthalmitis, and omphalitis (Barnes *et al.,* 1997). It is one of the principal causes of morbidity and mortality in chickens and turkeys (Blanco *et al*., 1998). The majority of the economic loss results from the mortality of the affected bird (Otaki, 1995). Environmental contamination, poor ventilation, contaminated water; improper hygiene practices favor the *E. coli* infection (Samad, 2005). Infection in young chicken and omphalitis may due to the entry of organisms through penetration prior to incubation of eggs (Weinak *et al*., 1981).

Poultry is the natural hosts for both of this organisms Salmonella *pullorum* and *Salmonella gallinarum* (Snoeyenbos *et al*., 1991). Salmonellosis is the causative agent of pullorum disease, fowl typhoid and fowl paratyphoid (Gomis *et al*., 1997). Pullorum disease is usually confined to the first 2-3 weeks of bird age and occasionally occurs in adults (Shivaprashad *et al.,* 1997). Fowl typhoid is an acute and chronic disease which is caused by *Salmonella gallinarum* and causes a serious problem such as mortality, lowered egg production, and reduced hatchability by affecting the mature birds (Christensen *et al*., 1997). The vertical transmission has occurred via infected eggs and the horizontal through contaminated utensils and others infected materials (Shivaprashad *et al*., 1997 and Snoeyenbos *et al*., 1991). The incidence of salmonellosis in poultry has been well established in many countries eg. United States, Belgium, U. K., Malaysia, Spain and Japan; and the level of contamination by *Salmonella* ranged between 20-89% from total poultry population (Capita *et al*; 2003). The Center for disease control and prevention estimates that *Salmonella* in food is responsible for 1.3 million illness; 15000 hospitalizations and over 500 deaths a year. Once the infection has occurred many of the birds excrete *Salmonella* organisms and contaminate the environment (Poppe, 1996)..

Materials and Methods

During UVH placement, one turkey flock owner, from nearby Foujdarhat Cadet College Campus, Salimpur, Chittagong; came to our reputed SAQTVH hospital unit for getting treatment of three turkeys having severe diarrhea. He also mentioned that he had total 24 turkeys affected with diarrhea among total 120 turkeys in the flock. In this regard, considering the above facts, the present study was undertaken to find out actual causal agent of such types of illness to the birds, and thereby, the prevalence of underlying pathogen.

Study area and study duration

The study was conducted from April to June 2016 on 120 turkey samples at the nearby Foujdarhat Cadet College Campus, Salimpur, Chittagong.

Sample collection

The samples were collected from cloacae of turkey through pre-sterilized cotton swab and immediately transferred into screw capped test tubes containing buffered peptone water. Thermo flask containing ice was used to transport the samples from the collection site to Poultry Research and Training Centre (PRTC) Laboratory for analysis. Collected samples were preserved in a refrigerator at 4ºC until screening out the bacteria.

Data collection

All the data including flock size, flock number, sex, age, duration of illness etc. of the individual birds were noted in a structured record keeping questionnaire.

Media used for isolation

Peptone water (Oxoid Ltd; pH 6.2±0.0) was used as primary enrichment media for *E. coli* and *Salmonella sp*. 4 selective media were used for the isolation of these bacteria. The Mac-Conkey agar (Oxoid Ltd, pH 7.4±0.2), EMB agar (Merck, pH 7.1±0.2) were used for *E. coli* where XLD agar (Oxoid Ltd, pH 7.4±0.2), and SS agar (Merck, pH 6.9±0.2) were used for *Salmonella sp*.

Culture procedures for both *E. coli* and *salmonella sp*

1ml of swab suspension was inoculated in a screw cap test tube containing 10ml of nutrient broth and incubated at 37ºC for 24 hours. Then for *E. coli*, samples were streaked on Mac-Conkey agar and incubated overnight. The growth of *E. coli* produces large, pink colonies on an agar plate. Subculture was then performed on EMB agar at 37ºC for 24 hours. The characteristic metallic sheen colonies were suggestive for *E. coli* positive. Then for further confirmation, biochemical test (Indole test) was done. For *Salmonella sp*., after inoculation of the sample on nutrient broth, one loop-full of the colony from broth was streaked on XLD agar plate and incubated at 37ºC for 24 hours. Then the positive samples were further inoculated on SS agar and incubated overnight at 37ºC for 24 hours. After incubation, colonies were observed. The colony with a black center in XLD and blackish growth in SS agar were considered as presumptive *Salmonella* positive.

Biochemical tests

Tests for *Escherichia coli*

E. coli produces strong acids and usually gas forms by the fermentation of d-glucose (positive in the methyl red test) and do not produce acetyl-methyl carbinols (acetoin) (negative in the Voges-Proskauer test). Lysine is decarboxylated by the majority of the strains. Indole test is also positive in case of *E. coli*.

Indole test

Indole test was performed to determine the ability of an organism to form indole compound by splitting of amino-acid tryptophan. The positive result was noted by pink colored ring formation after addition of reagent.

Table1: Different biochemical tests for *E. coli*

Test result	Indole test
Positive	Pink ring
Negative	No ring

Biochemical tests for *Salmonella spp.*

TSI slant for identification of *Salmonella spp*

A straight inoculating needle was used to take isolated colony from culture. The TSI slant was inoculated by stabbing the butt down to the bottom and then streaked over the surface of the slant. Then, the TSI slant was incubated overnight at 37ºC.

Table2: Biochemical test (TSI agar slant) for identification of *Salmonella sp*

Test result	TSI slant (Triple Sugar Iron test)
Salmonella positive	Production of H_2S; blackening of the medium.

Preservation of culture

All the positive isolates were inoculated into Trypticase Soy Broth (TSB) (Oxoid, England), incubated overnight at 37ºC and then stored at -80ºC adding 15% glycerol in 1.5 ml Eppendorf tubes for further research.

Data analysis

Obtained data was imported to the Microsoft Office Excell-2007 and transferred to the software STATA/IC-11 for analysis. Descriptive analysis was done. An association was regarded as significant if the *p* value was ≤0.05.

Results

In this study, 120 cloacal swab samples were studied where 90 samples were positive for *E. coli* and given a prevalence of 75%. The positive samples were confirmed *E. coli* by Indole test which showed 100% sensitivity. In case of *Salmonella sp.*, from 120 cloacal swab samples, it was observed that 42 samples were positive which given a prevalence of 35%. Biochemical test of positive samples of *Salmonella sp.* was performed by TSI agar slant that also showed 100% sensitivity.

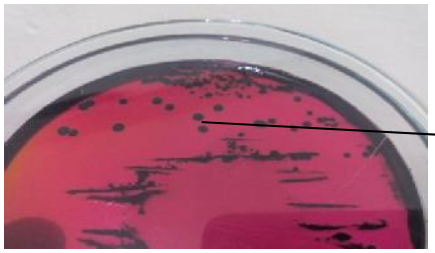

Fig. 1: *Salmonella* colonies on XLD agar

Fig. 2: *Salmonella* colonies on S-S agar

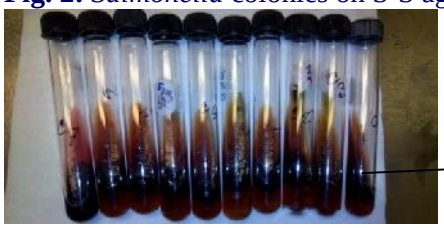

Fig. 3: *Salmonella* on TSI agar slant

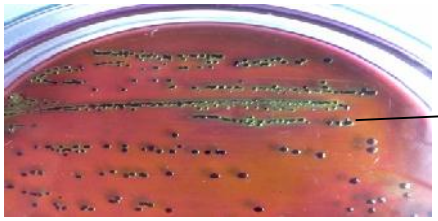

Fig. 3: *E. coli* in EMB agar

Fig. 3: *E. coli* in MacConkey agar

Table3: The prevalence of *E. coli* and *Salmonella sp.* according to various traits

Trait	Identification for	Variables	Negative	Positive	Total	p value
Sex	*E. coli*	Female	18 (60%)	33 (36.7%)	51(42.5%)	0.196
		Male	12 (40%)	57 (63.3%)	69 (57.5%)	
		Total	30	90	120	
	Salmonella spp.	Female	24 (30.77%)	27 (64.3%)	51 (42.5%)	0.041
		Male	54 (69.23%)	15 (35.7%)	69 (57.5%)	
		Total	78	42	120	
Age (m)	*E. coli*	3-5	6 (20%)	54 (60%)	60 (50%)	0.615
		≤ 2.5	15 (50%)	15 (16.7%)	30 (25%)	
		≥ 6.5	9 (30%)	21 (23.3%)	30 (25%)	
		Total	30	90	120	
	Salmonella spp.	3-5	51 (65.38%)	9 (21.43%)	60(50%)	0.83
		≤ 2.5	15(19.23%)	15 (35.7%)	30(25%)	
		≥ 6.5	12 (15.38%)	18 (42.7%)	30(25%)	
		Total	78	42	120	
Layer/ Non-layer	*E. coli*	Laying	9(30%)	21 (23.33%)	30(25%)	0.673
		Non laying	21(70%)	69(76.7%)	90(75%)	
		Total	30	90	120	
	Salmonella spp.	Laying	12 (15.4%)	18(42.9%)	30(25%)	0.05
		Non laying	66(84.6%)	24(57.1%)	90(75%)	
		Total	78	42	120	
Flock	*E. coli*	Flock-1	15(50%)	75 (83.3%)	90 (75%)	0.04
		Flock-2	15(50%)	15(16.7%)	30(25%)	
		Total	30	90	120	
	Salmonella spp.	Flock-1	63(80.7%)	27(64.3%)	90(75%)	0.251
		Flock-2	15(19.23%)	15(35.7%)	30(25%)	
		Total	78	42	120	

*Flock-1 (3-5 month and ≥ 6.5 month aged) and Flock-2 (≤ 2.5 month aged).

Here, according to male and female Turkey their present no significant ($p>0.05$) variation in case of *E. coli*. But, in the case of *Salmonella sp.* there had a significant ($p \leq 0.05$) variation between male and female turkey. In this study, it was observed that 60 % turkey, aged 3-5 months were positive for *E. coli* whereas, 42.7% turkey, aged ≥6.5 months were positive for *Salmonella sp.* Though; there have no significant variation among the aged birds. This is interesting that 76.7 % non-laying birds were positive for *E. coli* and 42.9% laying birds were positive for *Salmonella sp.* There were significant variations between laying and non-laying birds in case of *Salmonella sp.* Although, the prevalence of *E. coli* between flock-1 and flock-2 showed statistically significant variation ($p \leq 0.05$) where 83.3% turkey of flock-1 were positive, but in case of *Salmonella sp.* either flock-1 or flock-2 there had no significant findings.

Discussion

The aim of this study was to focus on isolation and identification of the *E. coli* and *Salmonella sp.* from cloacal swabs and find out the prevalence of these infections on a turkey flock affected with severe diarrhea. Earlier, Cuiwei Zhao *et al.*, 2001 found 11.9% *E. coli* infection in a commercial turkey flock where, Mohammad Jahantigh *et al*., 2015 found 14.8% *Salmonella sp.* The CSPI (Center for Science in the Public Interest) under Washington-US Department of Agriculture (USDA) showed that 13% of turkeys were contaminated with *Salmonella sp*., which was slightly lower than this study. However, in the study, overall prevalence of *E. coli* and *Salmonella sp*. were 75% and 35% respectively and prevalence of *E. coli*

were comparatively very much higher than the prevalence of *Salmonella sp.* In an experimental study of Narayan Rath *et al*., 1999 and G. R. Huff *et al.,* 2015 observed comparatively higher mortalities and air-sacculitis in males (significant with p≤0.05) than females in a 5-week old turkey flock. Here, according to male and female Turkey there present no significant (p>0.05) variation in case of *E. coli* prevalence but, in case of *Salmonella sp.* there had a significant (p≤0.05) variation between male and female turkey with higher prevalence in females. In a previous study by the European Union (EU) on 2006-2007, showed 30.7% *Salmonella*-positive on non-laying 4-5 months aged turkeys but in this study it was observed as 57.1% which have a significant variation (p ≤0.05). According to EU, in layer turkey aged ≥6.5 months, *Salmonella* prevalence was 13.6% which is found 42.7% in the study. Islam *et al*., 2006 reported as the prevalence of *Salmonella* infection increased with the increase of age which also similar to my findings. Between the two flocks (Flock-1 and Flock-2) there had a significant (p ≤0.05) variation in *E. coli* infection. In the case of *Salmonella sp.*, Hossain *et al*., 2010 found 34.2% *Salmonella* infection in large flocks (≥5001 birds) in comparison to 21.3% in the small (≤1000 birds) flocks. It is quite similar to the study. It might be due to a study on the non-commercial and experimental farm having smaller birds population where 17 female birds among the total of 40 birds, geographical variation and strains of the organisms.

Conclusion

The current study revealed the presence of *E. coli* and *Salmonella sp.* in the cloacal swab of a turkey flock. The prevalence of *E. coli* and *Salmonella sp.* in the flock were 75% and 35% respectively. The *E. coli* infection was simply higher than the *Salmonella* infection. The high prevalence of *Salmonella* and *E. coli* in Turkey could be a threat to public health. Hence, it is an alarming issue for turkey farms. Finally, the comprehensive planned study should be needed to characterize the various serotypes of *E. coli* and *Salmonella sp.* and identify the prevalence pattern with antimicrobial resistance to tackling this alarming point.

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