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Prevalence of *Salmonella* spp. in Poultry and Poultry Products in Dhaka, Bangladesh

Mohammed Rezaul Karim, Mohammed Giasuddin*, Mohammed Abdus Samad, Mohammad Showkot Mahmud, Mohammed Rafiqul Islam, Mohammed Hafizur Rahman, Mohammed Abu Yousuf

Animal Health Research Division, Bangladesh Livestock Research Institute, Dhaka, Bangladesh

Abstract

The study was carried out for determination of prevalence of Salmonella spp. in poultry and poultry products during July 2014 to June 2015. A total of 355 samples comprising 150 Cloacal swab samples of poultry, 50 Egg shells, 50 Egg contents, 30 Intestinal contents, 30 Liver swabs, 30 Broiler Meat and 15 Swabs of slaughter house were collected and processed for isolation of Salmonella spp. Out of 355 samples, 90(25.35%) samples were identified as positive for Salmonella. Out of the overall positive samples, 32% were cloacal swab samples of poultry, 28% were egg shells, 0% was egg content, 36.66% were intestinal contents, 23.33% were liver swabs, 20% were broiler meat and 26.66% were swabs of slaughter house were found positive for salmonella based on cultural properties, biochemical reactions and serum agglutination tests. In biochemical tests, all the isolates fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment lactose but a few isolates partially fermented sucrose. Acid production was marked by the color change from reddish to yellow and the gas production was noted by the presence of gas bubbles in the inverted Durham's tubes kept inside each of the test tubes containing sugar media. All isolated Salmonellae were positive for MR test, negative for V-P test and negative for indole test. For the slide agglutination test, *Salmonella* agglutinating antiserum (poly 'O' and poly 'H') was used which agglutinated all the isolates and thereby identified the organism as *Salmonella* spp.

Keywords

Prevalence, *Salmonella* spp, Poultry and Poultry Products

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1. Introduction

Salmonellosis is a food-borne bacterial disease having a zoonotic importance in global perspective. Salmonella are intracellular pathogens found both in cold- and warm- blooded animals. The genus Salmonella contains 2463 serotypes which are currently divided into two species: Salmonella enterica *(2443 serotypes)* and Salmonella bongori *(20 serotypes) due* to the difference in 16S rRNA sequence [1]. More than 99% of *Salmonella* strains causing human infections belong to *Salmonellaenterica* subspecies *enterica* [2]. *Salmonella enterica* is one of the most common bacterial causes of food-borne illness [3]. Salmonellosis is a direct occupational anthropozoonotic disease of great economic and public health concern [4]. It is estimated that approx. 80.3 of 93.8 million of global human *Salmonella*-related gastroenteritis cases are food-borne [5]. Some serotypes causing human infections such as Typhimurium are isolated from many different potential exposure sources, whereas others tend to be associated with certain food, environmental, or animal sources [6-9]. Among these, serotypes

* Corresponding author
E-mail address: mgias04@yahoo.com (M. Giasuddin)

Enteritidis and Typhimurium are the two most prevalent serotypes causing salmonellosis in humans as well as in livestock [10, 11]

Foodborne diseases caused by non-typhoid salmonella represent an important public health problem worldwide. Nearly 1.4 million cases of salmonellosis occur each year in the United States [12]. Salmonella accounted for 28% of outbreaks of known etiology and 35% of foodborne disease cases associated with outbreaks of known etiology during 1973 to 1987 in the United States [13]. Foods of animal origin, especially poultry and poultry products, and raw eggs have been implicated in the outbreak of human salmonellosis. Broilers are widely acknowledged to be a considerable reservoir for Salmonella infections in man due to the ability of Salmonella to proliferate in the gastrointestinal tract of chickens and subsequently survive on commercially processed broiler carcasses and edible giblets [14, 15]. In many different countries the rate of Salmonella contamination of broiler carcasses, either from processing plants or retail markets, has been reported to vary from 5 to 100% [15-19]. The reported level of Salmonella contamination of retail chicken in the United Kingdom has apparently declined in recent years from 79% in 1979 and 1980 to 48% in 1990 [20]. Many studies have established that Salmonella Enteritidis contaminates eggs when the organism is passed from the infected reproductive tissue of hens, rather than the shell, to the contents of contaminated eggs [21-24].

A wide range of foods has been implicated in food-borne illness attributable to *Salmonella enterica.* Foods of animal origin, especially poultry, poultry products and raw eggs, are often implicated in sporadic cases and outbreaks of human salmonellosis [25]. *Salmonella* spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals [25]. Recent years have seen increases in salmonellosis associated with contaminated fruits and vegetables. Other sources of exposure include water, handling of farm animals and pets, and human person-to-person when hand-mouth contact occurs without proper washing of hands.

Salmonella infection is one of the major constraints of poultry farming that hinders its development in Bangladesh [27-29]. In recent days, the prevalence of salmonellosis in breeder flock, commercial broiler, and layer flocks is increasing day by day. However, very limited research works had been carried out in Bangladesh concerning salmonellosis in poultry. Therefore, salmonellosis status of a farm needs to be determined for its proper control and management [30].

Objectives

i) To determine the prevalence of *Salmonella* spp. in poultry and poultry products

ii) Isolation and identification of *Salmonella* spp.

2. Materials and Methods

2.1. Sample Collection

Cloacal swab, Egg shell and content, Intestinal content, liver swab, Broiler Meat and Swab of slaughter house were collected. Sterile cotton swab sticks were used for sample collection and collected samples were directly brought to the laboratory in an insulated ice box with minimum delay and bacteriologically examined immediately. Samples were collected from different areas (Shimulia, Ashulia, Savar Paurashova, Pathalia and Birulia) of Savar Upazila during July 2014 to June 2015.

2.2. Cultivation of the Sample

Isolation and identification of *Salmonella* were done according to the procedure described by OIE [31], Merchant and Packer [32], and Cowan [33].

The collected swab containing samples were grown into Selenite cysteine (SC) broth and Rappaport Vassiliadis (RV) broth (Oxoid Ltd.) at 37°C for 18–24 hours. SC broth and RV broth cultures were grown in Brilliant green, Xylose Lysine Dextrose agar and Bismith Selenite, Salmonella– Shigella agar to get pure and putative Salmonella culture. Frozen whole broilers were thawed at 4°C overnight and 25 g of chicken meat were weighed and put into a stomacher bag containing 225 ml of buffered peptone water (BPW). After stomaching in a stomacher (Pro media SH-001, Tokyo, Japan), the sample and BPW were incubated together in the bag for 18 to 20 h at 35°C. After incubation, 0.1 ml of samples were inoculated into 10 ml of Selenite cysteine (SC) broth and incubated at 43°C for 24 h. One loop of sample from a sterile transfer loop was transferred from each tube to xylose lysine desoxycholate agar and was streaked for isolation. The agar plates were incubated upside down for 24 h at 35°C. Presumptive Salmonella colonies were transferred to triple sugar iron agar and lysine iron agar slants using a sterile inoculation needle and were incubated at 35°C for 24 h before being inspected. At least one presumptive Salmonella colony was chosen from every plate, and these colonies were grown on trypticase soy agar plate at 37°C prior to confirmation. Isolates were screened using Gram stain, indole test, Voges–Proskauer test, and methyl red test (Difco). Salmonella spp. were confirmed and identified by serotyping and antisera, purchased from S & A Reagent Lab, were used according to the manufacturer's instructions. Slide and tube agglutination tests were used for serotyping. Shell

eggs were soaked in 200 ppm chlorine solution containing 0.1% sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, Mo.) for 30 min. Chlorine solution was prepared by adding 8 ml of commercial bleach (5.25% sodium hypochlorite) to 992 ml distilled water containing 1 g sodium dodecyl sulfate. Egg yolk was obtained by cracking eggs aseptically, and 25 g egg yolk was combined with 225 ml tryptic soy broth (Difco). After mixing by stomacher, sample and broth were incubated at 35°C for 24 h. Salmonella isolation from egg shells was performed by pouring 20 ml of tryptic soy broth in the stomacher bag over the egg and agitating the bag by hand for 1 min. After agitation, the broth was transferred into a labeled sterile bottle and incubated at 35°C for 24 h. The organisms were further characterized as Salmonella species according to their morphology, Gram staining, motility, and biochemical properties [31-33].

2.3. Biochemical Tests

Isolated organisms with supporting growth characteristics of Salmonella on various media were maintained on XLD agar & BG agar and were subjected to biochemical tests (sugar fermentation test, MR-VP reaction and Indole reaction).

2.4. Slide Agglutination Test

Slide agglutination test was performed with Salmonella agglutinating antiserum (poly 'O' and poly 'H') to identify *Salmonella* spp. The test was performed according to the protocol supplied by the manufacturer (S & A Reagent Lab).

3. Results

A total of 355 samples comprising 150 Cloacal swab samples of poultry, 50 Eggs (Egg shell and Egg content), 30 Intestinal contents, 30 Liver swabs, 30 Broiler Meat and 15 Swabs of slaughter house were collected and processed for isolation and identification of Salmonella spp. Out of 355 samples, 25.35% were over all positive samples, Out of the positive samples 32% Cloacal swab samples of poultry, 28% Egg shells, 0% Egg content, 36.66% Intestinal contents, 23.33% Liver swabs, 20% Broiler Meat and 26.66% Swabs of slaughter house were found for positive Salmonella based on cultural properties, biochemical reactions and serum agglutination tests (Table 1).

By Gram's staining under microscope, the organism's revealed gram negative, pink color, small rod shaped appearance. Biochemical tests revealed that all the isolates fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment lactose but a few isolates partially fermented sucrose. Acid production was marked by the color change from reddish to yellow and the gas production was noted by the presence of gas bubbles in the inverted Durham's tubes kept inside each of the test tubes containing sugar media. All isolated Salmonellaewere positive for MR test, negative for V-P test and negative for indole test.

For the slide agglutination test, Salmonella agglutinating antiserum (poly 'O' and poly 'H') was used which agglutinated all the isolates and thereby identified the organism as *Salmonella spp*.

4. Discussion

Salmonella is a leading cause of food-borne illness in many countries with eggs and poultry being important vehicles of transmission. During the past two decades S. Enteritidis has become a leading serotype causing human infections, with hen eggs being a principal source of the pathogen. Egg and egg products have been associated with the occurrence of different Salmonella enteritidis and non-enteriditis mediated food borne outbreaks. The emergence of S. enteritidis as the leading cause of human Salmonellosis in many countries was attributed to this serotypes unusual ability to colonize the ovarian tissue of hens and be present within the contents of intact shell eggs [34]. The present study was conducted to determine the prevalenceof Salmonella spp. in the poultry and poultry products. In this present study, 355 samples comprising 150 Cloacal swab samples of poultry, 50 Eggs (Egg shell and Egg content), 30 Intestinal contents, 30 Liver swabs, 30 Broiler meat and 15 Swabs of slaughter house were examined for the isolation andidentification of Salmonella. Out of 355 samples, 25.35% were over all positive samples, Out of the positive samples 32% Cloacal swab samples of poultry, 28% Egg shells, 0% Egg content, 36.66% Intestinal contents, 23.33% Liver swabs, 20% Broiler Meat and 26.66% Swabs of slaughter house were found for positive Salmonella. Eggsare considered to be the major sources of confirmedsalmonellosis [35]. Infected ovaries and oviductsof the hen are the major sources of contamination [36]. Eggs can become contaminatedalso on the surface, either from faeces or the environment. Cracked eggs are those that could not be sold at the marketbecause of being damaged by the end of laying or during transportation. In egg contents, the low and sporadic incidence of eggcontamination was probably because of the protective effectof the egg's complex system of membrane barriers and theantibacterial components of the albumen. Penetration of theshell by Salmonella Enteritidis has been suggested [37, 38] and theSalmonella Enteritidis, Salmonella Typhimurium, or Salmonella Heidelberg present in feces could penetrate to theinterior of eggs and grow during storage. It wasreported

the shells and contents of 2,090 packs of sixraw eggs from shops in Northern Ireland were examined andnine isolates of Salmonella were detected from separate packsof eggs (0.43 %) [39]. One of the isolates wasfrom egg contents (0.05 %) and eight of the isolates weredetected on the shell of eggs. In 2002, from five countries ofEuropean Union (Denmark, Finland, Ireland, Sweden and Norway), the prevalence of Salmonella spp. in laying hensproducing table eggs was from 0-25% and S. Enteritidis from 0-1.9%, have been reported [40]. The findings of thepresent work are to some degree consistent with the resultsobtained by others. It is assumed that in the USA one in 10,000eggs is infected with Salmonella spp., in Great Britain one in15,000 eggs [41]. In a survey done in NewZealand by Environmental Science and Research Limited [42] in 1994 found that no Salmonella weredetected on the shells of 341 samples of 6 eggs (2,046 eggs intotal) or in the contents of 339 samples of 6 eggs (2,037 eggsin total). It was reported Salmonella spp. was detected from13.3% and 0.6% of eggs samples that were produced in Spain and France, respectively [43]. Animal based foods, especially chicken, have beenassociated with the occurrence of Salmonella in humans forthe consumption of egg and egg product.

Table 1. Prevalence of *Salmonella spp*. in Poultry and Poultry Products.

Types of Sample	Total samples	Positive sample	% of *Salmonella* spp.
Cloacal swab	150	48	32%
Egg shell	50	14	28%
Egg content	50	0	0
Intestinal content	30	11	36.66%
Liver swab	30	7	23.33%
Broiler Meat	30	6	20%
Swab of slaughter house	15	4	26.66%
Total= 355		Total= 90	Total= 25.35%

5. Conclusion

The overall prevalence rate of*Salmonella*spp. in the poultry and poultry products was 25.35% and specially 20% in broiler meat. The present results indicated that poultry and poultry products could be considered a potential source of human salmonellosis in Bangladesh. Continuous monitoring and improvement of biosecurity in poultry farms is needed to reduce the prevalence of *Salmonella* spp. in poultry and poultry products. Consumers-awareness efforts would protect public health from Salmonellosis. Poultry in a clean environment, slaughtering food animals in hygienic spaces, and a greater adherence to food processing standards are also required to prevent the intra- and interspecies transmission of salmonellosis.

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