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Prevalence of Salmonella in Eggs Collected from Local Markets and Poultry Farms in Mekelle, Ethiopia

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Abstract: Salmonellosis is an egg associated infection involving either the consumption of raw eggs or of products made with raw eggs. The present study was conducted to determine the prevalence of *Salmonella* in hens' eggs collected purposely from retail market, poultry farms and back yards in Mekelle. Based on expected prevalence of Salmonellosis in Ethiopia at 95% confidence interval and 5% absolute precision, egg shell and yolk of 156 eggs were examined for S*almonellae* as per standard procedures. The prevalence of *Salmonella* in egg shells and yolks was 15.38% and 8.33% respectively. Source-wise prevalence of Salmonellosis in eggs collected from retailers, poultry farms and backyards was 18.18%(22/121), 4%(1/25) and 10%(1/10) in eggs shells and 9% (11/121), 4%(1/25) and 10% (1/10) in yolks respectively and it had significant difference. The present study reports the contamination of eggs by salmonellae that can be a risk of food borne disease due to eating raw eggs or the products having such eggs in the study area.

Key words: Egg Shell · Egg Yolk · Prevalence · Salmonella · Mekelle · Ethiopia

INTRODUCTION

Salmonella has a widespread occurrence in animals, especially in poultry and swine. Environmental sources of this organism include water, soil, animal feces, raw meats, raw poultry including eggs and raw sea foods [1]. There are about 2500 known serotypes of *Salmonellae* and many of these are well documented human pathogens [2]. It is one of the important food borne pathogens and is an egg associated infection involving either the consumption of raw eggs or of products made with raw eggs. *Salmonella* contamination of eggs at farms and market outlets may arise by vertical transmission or horizontal means [3]. Since the poultry eggs and their products are the commonest vehicles of *Salmonella* to humans [4], outbreaks and sporadic cases of Salmonellosis are frequently associated with the intake of infected hen eggs. Keeping in view the eggs as a food of the people of this region and food borne salmonellosis has public health significance, this study was undertaken to know the prevalence of salmonella in eggs available in Mekelle city of Tigray region, Ethiopia.

MATERIALS AND METHODS

Study Area: The study was carried at Mekelle which is an ancient city in northern Ethiopia and the capital city of Tigray regional state. It is located 783km^2 north of Addis Ababa, at an altitude of 2000-2200 meters above sea level (m.a.s.l) and longitude of 13°29' North39°28' East coordinates respectively. The total area of the city is estimated to be above 53km^2, the average rain fall ranges from 150-250mm and the average temperature is 19°C. Its rainy season occurs mainly between June and September, although a short rainy season do occur mainly between March to April. The city has a total population of 215,546; and is one of largest cities in Tigray region [5].

Study Design and Sampling: A cross-sectional survey was under taken for the isolation of Salmonella from eggs collected from poultry farms, retailers and back yards at Mekelle city in Tigray region of Ethiopia during the period from October 2013 to April 2014. The sample size was determined based on the expected prevalence of 11.5% *Salmonellae* that was previously reported from Kombolcha and expected prevalence was calculated at

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95% confidence interval and 5% absolute precision [6]. Accordingly, a total 156 eggs from retailers and farms including back yard farms were collected by random sampling technique. Eggs were individually packed with in sterile plastic bag and brought to the laboratory.

Isolation of Salmonella: Egg shell and yolk of each of 156 eggs were examined for *Salmonellae* by culturing them in pre-enrichment (buffered peptone water) and enrichment media (Rappaport Vasilidias Broth and Tetrathionate Broth) followed by their growth on selective medium (Salmonella Shigella Agar) [7]. Based on the characteristic growth of *Salmonella* on selective medium, the isolates were characterized as presumptive *Salmonella* which were identified biochemically as per the methods described by Edwards and Ewing [8].

Non selective Pre-Enrichment: Shell of each egg and its yolk were immersed separately in 225 ml of buffered peptone water (pH 7.2) and incubated at 37°C for 24 hours.

Selective Enrichment: Rappaport Vasilidias Broth (RVB) and Tetrathionate Broths (TTB) were used to selectively enrich Salmonellae. For this, 0.1 ml of pre-enriched sample was cultured in 9.9 ml of RVB followed by incubation at 42°C for 24 hrs while 1 ml sample was inoculated into 9 ml TTB and incubated at 37°C for 24 hrs.

Streaking on Selective Agar Medium: The enriched cultures in RVB and TTB were streaked onto the Salmonella Shigella Agar (SSA) and incubated the plates at 37°C for 24 hours. Plates were examined for characteristic colonies of *Salmonella* which appeared as transparent having black coloration on it and were considered as presumptive salmonellae.

Purification of Culture: The presumptive *Salmonella* colony was purified thrice by growing each time in nutrient broth followed by streaking on nutrient agar. The purified culture was preserved as presumptive *Salmonella* in nutrient agar slants at 4°C and sub-cultured from time to time.

Identification and Biochemical Characterization of the Presumptive Isolates of Salmonella: Presumptive isolates of *Salmonella* were identified as per the methods described by Edwards and Ewing [8]. The cultures were subjected to primary identification reactions like Gram's stain, morphology and catalase test. These were further subjected to indole test, oxidase test, methyl red test, VP test, growth on Simmon's citrate and acid production from lactose and glucose and lysine decarboxylation as per the procedure described by MacFaddin [9]. Cultures as Gram-negative, non-sporing, non-capsulated, non-lactose and non-sucrose fermenters, catalase positive and indole negative, methyl red positive, VP negative, citrate positive and produce yellow butt and red slant with hydrogen sulphide gas production on triple sugar iron agar medium were considered as *Salmonella*.

Statistical Analysis: Data were analyzed using the SPSS version 18 statistic software. Prevalence of Salmonella by source of samples and sample types were expressed as percentages. Computation of descriptive statistics such as, frequency and percentage were applied to compute the questionnaires data. The prevalence was defined as the number of *Salmonella* positives per the number of samples examined. Pearson's chi-square test was used to compare the association of prevalence of *Salmonella* between different sample sources and sample type.

RESULTS AND DISCUSSION

Egg quality is influenced by the eggs' contamination due to microbes of public health significance. *Salmonella* is an important food borne pathogen of public health significance. Since the poultry eggs and their products are the commonest vehicles of *Salmonella* to humans [4], this study was undertaken. Out of the total 156 chicken eggs examined for isolation of Salmonella, 15.38% and 8.33% of egg shells and egg contents, respectively were positive (Tables 1 & 2). The results indicated that egg shell contamination with the *Salmonella* was significantly higher (15.38%) than that of egg contents (8.33%). Prevalence of *Salmonella* from egg shells and egg contents has been 40% and 8.33% respectively recorded by Akhtar *et al*. [10] and 11.5% and 4.69% in eggs from Kombolcha [11] and Addis Ababa [12] respectively. Egg contamination can occur through egg contact with fecal material, insects and feed or even through transportation, storage or during handling. The variations in these factors of contamination may be the cause of variation in the prevalence of *Salmonella* in eggs. As the hygienic standards in handling, transportation and storage improves, the *Salmonella* prevalence may lower down. This is evident from several reports of

Table 1: Source-wise prevalence of Salmonella in egg shell

Source of egg	No. of shell examined	No. of shell positive	P-value
Retailer	121	22(18.18%)	0.03
Farms	25	1(4%)	
Back yard	10	1(10%)	
Total	156	24(15.38%)	

Table 2: Source wise prevalence of Salmonella in egg-yolk

Source of egg	No. of yolk examined	No of yolk positive	P-value
Retailer	121	11(9%)	0.04
Farms	25	1(4%)	
Back yard	10	1(10%)	
Total	156	13(8.33)	

Salmonellosis in eggs from developed countries like zero to 7% in the United Kingdom [13], around 1% in Spain [14] and 0.4% in Republic of Ireland [15].

Source-wise prevalence of *Salmonellae* of eggs collected from retailers, poultry farms, backyards was 22(18.18%), 1(4%), 1(10%) and 11(9%), 1(4%), 1(10%) in eggshells and yolks respectively and they had a significant difference in prevalence (Tables 1& 2). Similarly it was recorded to be 6.1% in egg shells and 1.8% in egg contents of retail eggs in South India [16]. In contrary to this finding, prevalence of *Salmonella* in egg content of the poultry farm (10.5%) was significantly higher than the prevalence of *Salmonella* in egg content of open market (3.0%) ($p = 0.003$) as reported by Minte *et al.* [11]. The higher prevalence of *Salmonella spp.* in eggs collected from market outlets (retailers) could be explained due to contamination of eggshells during transportation or poor handling and storage.

This study reports the contamination of shell and yolk of eggs by Salmonella but quantification of such contamination should further be investigated. Salmonella is deleterious for egg quality and they are hazardous for consumers' health. This fact suggest the importance of establishing good animal health practice in poultry farms and a refrigeration chain throughout egg transportation, storage and commercialization, as practiced in other countries in an attempt to prevent the contamination of chicken eggs. Horizontal transmission of salmonellae is usually derived from fecal contamination on the egg shell which may be able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage by Salmonella but can be effectively reduced by cleaning and disinfection of the environment [17] and by good production and handling practices.

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