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The Prevalence and Public Health Importance of *Salmonella* from Chicken Table Eggs, Ethiopia

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Abstract: A study was conducted on 400 chicken table eggs from Kombolcha poultry multiplication and breeding farm and market at Kombolcha, Ethiopia, from October 2009 to April 2010. The objectives were to determine prevalence and public health importance of *Salmonella* from chicken table eggs. The samples were examined for the presence of *Salmonella* following standard techniques and procedures outlined by the International Organization for Standardization. From the total of 400 eggs examined for *Salmonella,* 46 (11.5%) were positive, from which 25 (6.3%) and 27 (6.8%) were found from egg shell and egg content, respectively. The 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%) ($p = 0.003$). The difference in prevalence observed between farm egg shell (7.0%) and market egg shell (5.5%) was not statistically significant ($p = 0.535$). In addition, the prevalence of *Salmonella* in egg shells were 14% and 0% before and after fumigation, respectively and the difference in these prevalence was statistically significant ($p = 0.000$). Questionnaire survey was also carried out on 130 consumers (house holds of Kombolcha). Based on the result the majority of the respondents used to store eggs in open containers, while 10% and 23% were used refrigerators and cool places, respectively. Their preference on eating raw eggs indicated that 6.2% egg consumers showed their preference for raw egg eating, while 93.8% donot prefer eating raw eggs. Six (4.6%) respondants that practiced eating raw eggs were faced a problem, while 95.4% were not facing the problem after eating raw eggs.

Key words: Egg Shells · Egg Contents · Salmonella · Prevalence · Kombolcha · Ethiopia

INTRODUCTION

Eggs are considered to be an "excellent" source of choline and selenium and a "good" source of vitamin B12, phosphorus and riboflavin. Furthermore, they are considered to be one of the highest quality proteins available and are used as the standard to compare protein quality in other foods. The protein found in eggs is highly digestible with a biological value of 94%. The yolk contains vitamins A, D, E and K as well as folic acid, pantothenic acid and zinc [1]. Eggs are also popular as ingredients in dishes, they are multifunctional and are used for coagulation, foaming, emulsifying and for contributing color and flavor to dishes [2]. The albumen or white account for 66% of an egg's total liquid weight and contains the majority of the egg's proteins [2].

Both *Salmonella* Enteritidis and *Salmonella* Heidelberg are deposited inside eggs after invading to the reproductive tissues of infected hens. Infected hens can deposit *Salmonella* in either the yolk or albumen of developing eggs because of the colonization of different regions of the reproductive tract [3,5]. Although *Salmonella* can survive or slowly multiply in egg albumen [6-8], rapid multiplication occurs in egg yolk [9-11]. Accordingly, egg refrigeration must achieve growth-inhibiting internal temperatures more rapidly when bacteria are present in the yolk than when they are found in the albumen. Because high levels of contaminants have seldom been reported in freshly laid eggs, initial *Salmonella* deposition inside nutrient-rich yolks appears to be relatively uncommon [8]. Moreover, freshly laid eggs are typically reported to contain no more than a few hundred *Salmonella* cells [4,12], so prompt refrigeration to internal temperatures of 7.2°C or lower protects consumers by preventing bacterial multiplication to more dangerous levels inside eggs during storage [12].

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Despite some attempts to study prevalence of *Salmonella* in Ethiopia, mainly in pig, cattle, poultry meat, minced beef [13-15] and humans [13], but the status of the problem in chicken table egg is still unknown. However, studies made elsewhere indicated that chicken eggs are important sources of *Salmonella* particularly among those raw consumers [16]. Moreover, none of the previous studies in Ethiopia on chicken table eggs determined the occurrence, magnitude and distribution of *Salmonella* in both exotic as well as local chicken table eggs. Therefore, this study was undertaken at Kombolch poultry farm and open markets with the objectives of determine the prevalence of *Salmonella* in chicken table eggs and to assess the public health importance of S*almonelal.*

MATERIAL AND METHODS

Study Design and Sampling: A cross-sectional survey of *Salmonella* in chicken table eggs in Kombolcha poultry multiplication center and markets were undertaken from October 2009 to April 2010. Questionnaire survey was also adminsterd to know the storage, preparation and utilization pattern of chicken table eggs.

The sample size required for this study was determined based on the expected prevalence of salmonellosis and the desired absolute precision [17]. There is no previous study on *Salmonella* in chicken eggs. Therefore, 50% expected prevalence with 95% desired confidence interval and 5% absolute precision was used. Accordingly, a total of 400 eggs; 200 eggs from kombolcha poultry farm (100 eggs before fumigation and 100 eggs after fumigation using formaldehyde gas) and 200 eggs from kombolcha market were collected. In addition, 8 eggs from market (one egg from one egg saler) were collected once per week using simple random sampling techenique. Eggs were individually packed with steril plastic bag and shipped to the laboratory.

Sample Processing: The sterile plastic bags containing selected eggs were opened with scissors and the samples processed immedatelly. Swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped in sterile buffered peptone water (BPW) were used to swab the entire surface area of the eggshell. The swabs were directly inoculated into 10 ml BPW in screwcapped bottles [18].

The same eggs from which shell sample was collected were used for interior (egg content) sampling. The eggs surface were sterilized by immersing in 70% alcohol for 2 min, air dried in a sterile chamber for 10 min and then cracked with a sterile knife. Each egg's content was mixed thoroughly and 25ml of the mixed egg content was inoculated into 225 ml of BPW and homogenized for two minutes with shaker.

Isolation and Identification of *Salmonella*: The isolation was conducted utilizing the conventional methods for the detection of *Salmonella* following the standard guidelines from ISO 6579: [19] (Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella* spp.). .

Non-Selective Pre-Enrichment: The swabs were directly inoculated into 10 ml BPW in screw capped bottles and incubated at 37°C for 16-18 hrs. Each egg's content was mixed thoroughly and 25 ml of the mixed egg content was inoculated into 225 ml of BPW and incubated at 37°C for 16-18 hrs [18].

Selective Enrichment: The pre-enrichment broth after incubation was mixed and 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth). Another 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Muller-Kauffmann tetrathionate broth (MKTT broth). The inoculated RV broth was incubated at 41.5 °C ±1°C for 24 ± 3 hours and the inoculated MKTT broth at 37 °C±1 °Cfor 24 ± 3 hours [18].

Plating out and Identification: Xylose lysine desoxycholate (XLD) agar (Titan Biotech Ltd., Bhiwadi, India) and *Salmonella* - *Shigella* (SS) agar (Titan Biotech Ltd., Bhiwadi, India) plates were used for plating out and identification purpose. A loop-full of inoculums from the RV broth and MKTT was transferred and streaked separately onto the surface of xylose lysine deoxycholate agar (XLD agar) and *salmonella-shigella* agar (SSA) separately. The plates were incubated at 37°C ± 1°C for 24 ± 3 hours. After proper incubation, the plates were examined for the presence of suspected *Salmonella* colonies which XLD agar is pink with a darker pink center whereas lactose-positive salmonellae are yellow with or without blackening.

Confirmation was done by using biochemical test according to ISO 6579 [19].

Data Analysis: All data were entered into a Microsoft Excel spreadsheet and transferred to SPSS version 15 for analysis. Prevalences of *Salmonella* by source of samples and sample types were expressed as percentages.

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 and computation of descriptive statistics such as, frequency and percentage were applied to compute the questionnaires data.

RESULTS

Prevalence of *Salmonella:* Out of the total 400 chicken table eggs examined for bacteriological status of *Salmonella,* an overall 11.5% prevalence of *Salmonella* was found. From this, 6.3% and 6.8% were from egg shell and in egg content, respectively. Moreover, the prevalence of *Salmonella* in the poultry farm was 15% (7.0% on egg shell and 10.5% in egg content), while 8.0% (5.5% on egg shell and 3.0% in egg content) was found in the market (Table 1). Six (1.5%) eggs (5 samples from farm and 1 sample from market) were found culture positive for both egg shell and egg content samples.

The total prevalence of *Salmonella* varied among the sampling sites. The prevalence of *Salmonella* in the poultry farm (15%) was significantly higher than the prevalence of *Salmonella* in the in open market (8%) (p = 0.028). The prevalence of *Salmonella* in egg content of the poultry farm (10.5%) was also significantly higher than the prevalence of *Salmonella* in egg content of open market (3.0%) (p = 0.003). The difference in prevalence observed between farm egg shell (7.0%) and market egg shell (5.5%) was not statistically significant (p = 0.535) as shown in Table 2.

Of the total 200 samples examined from the poultry farm, 100 eggs were fumigated with 37.5% formalin before swab sampl was taken, the rest 100 samples was taken without fumigation. Prevalence of *Salmonella* in egg shell were 14% and 0% before and after fumigation, respectively and the difference in these prevalence was statistically significant (p = 0.000). In contrary, the prevalence of *Salmonella* in egg content before (12%) and after fumigation (9%) was not statistically different (p = 0.489) as shown in Table 3.

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Table 1: Prevalence of *Salmonella* by sample types and source of samples examined

	Prevalence			
Source of sample	Egg shell	Egg content	Egg shell and content (both)	Total
Farm (n=200)	14 (7.0%)	21 (10.5%)	5 (2.5%)	30 (15%)
Market(n=200)	11 (5.5%)	6 (3.0%)	1 (0.5%)	16 (8.0%)
Total(n=400)	25 (6.3%)	27 (6.8%)	6 (1.5%)	46 (11.5%)

Table 2: The prevalence of *Salmonella* between farm and market on egg shells and egg contents

	Farm (n=200)	Market (n=200)	$\chi 2$	p-value
Egg shell	14 (7.0%)	11 (5.5%)	0.384	0.535
Egg content	21 (10.5%)	6 (3.0%)	8.937	0.003
Total	30 (15%)	16 (8%)	4.815	0.028

Table 3: Prevalence of *Salmonella* before and after fumigation of eggs in the poultry farm

	Prevalence			
Source of sample	Egg shell	Egg content	Egg shell and content (both)	Total
Farm (n=200)	14 (7.0%)	21 (10.5%)	5 (2.5%)	30 (15%)
Market(n=200)	11 (5.5%)	6 (3.0%)	1 (0.5%)	16 (8.0%)
Total(n=400)	25 (6.3%)	27 (6.8%)	6 (1.5%)	46 (11.5%)

Table 4: The prevalence of *Salmonella* in egg shell and egg contents

Source of sample	No. positive	Prevalence (%)	$\chi 2$	p-value
Egg shell (n = 400)	25	6.3	12.606	0.000
Egg content (n = 400)	27	6.8		

The overall prevalence of *Salmonella* in egg content (6.8%) was statistically significant (6.3%) with the prevalence in egg shell (p = 0.000) as shown in Table 4.

Questionnaire Survey Result: To know the storage, preparation and utilization pattern of chicken table eggs, a survey based study was designed and pre-tested questionnaire was prepared. The interviews were taken from 130 egg consumers of Kombolcha town during the study periods.

The consumers were subjected to enquire for their preference/liking from where they purchase eggs. It was noted that 45% of consumers preferd to purchase from market, 42.3% were preferred to purchase from the near by poultry farm, 7.7 % from owen reared chicken and the rest 3.1 % and 1.5 % were preferd both market and poultry farm and all sources, respectively.To enquire for their preference in type of egg, it was noted that 62.3% consumers liked to consume local breed eggs, while 27.7% showed their preference for the improved breed eggs and only 10% of the consumer preferred to consume both types. This might be due to the taste difference between local and improved breed eggs.

Questionnaire were also forewarded to know their preference on eating raw eggs that 6.2% egg consumers showed their preference for raw egg eating, while 93.8% dislike eating raw eggs. This is might be due to their feeding habit. The reasons given by the respondants eating raw eggs were due to the benfit of health care and for the purpose of medicine 3.8% and 2.3%, respectively. Six (4.6%) respondants that practiced eating raw eggs were faced a problem, while 95.4% were not facing the problem after eating raw eggs. To show their preference on type of dish they used to prepare from eggs, 1.5% preferred cooked omelet, 35.4% liked eggs as mixed preparation, 23.1% of the consumers liked fried eggs and 40% consumer were prefer to boiled eggs. The habit of washing eggs before subjecting for consumption showed that 26.2% were did not havehabit of washing eggs and the rest 73.8 % had a habit of wasing eggs. The types of materials in which the eggs was stored before prepation showed that 66.2% of the respondant used open containers, while 10% and 23% were used refrigerators and cool places, respectively. The majority of the respondants (91.5%) were used this storage practice to prevent spoilage and the rest 8.5 % were to protect egg breakage. The survey on storage of egg before preparation during hot season showed that 68.5% stored 1-7 days, 26.2% stored 7-15 days and only 5.4% stored 15-30 days. The egg consumers in Kombolcha town during survey were also asked to perceive their seasonal storage of eggs before preparation. According to their response, 13.1% were stored the eggs from 1-7 days, 70% were stored the eggs from 7-15 days during cool season before preparation and 16.9% stored the eggs from 15-30 days during cool season before preparation. Out of 130 respondants, 83.8% was practiced to stay prepared food at less than 30 minute of time, 9.2% were stay prepared for 30-60 minutes before consumption, while 3.8% and 3.1% were stay prepared food for 1-6 hrs and 6-24hrs, respectively.

DISCUSSION

In the present study, the prevalence of *Salmonella* from egg shells (exterior) and egg contents (interior) was 25 (6.3%) and 27 (6.8%), respectively. These findings are in agreement with the report of Suresh *et al.* [18] which indicated that prevalence of *Salmonella* was recorded in 6.1% of egg shells and 1.8% of egg contents in South India. In Spain, Perales and Audicana [20] reported around 1% *Salmonella* contamination in egg-shells. In the United Kingdom prevalence levels were reported to be varying from zero [21] to 7% [22]. In Faisalabad, Pakistan reported percentages of salmonellae recovered from egg-shells and egg interior were 40% and 8.33%, respectively [23]. A recent survey of retail eggs in the United Kingdom found an overall prevalence of *Salmonella* contamination of 0.34% [24] and a survey of 5,000 samples in Northern Ireland and the Republic of Ireland found only two positive samples [25]. A 2006/07 survey of non-UK produced eggs on retail sale in parts of England showed a prevalence of 3.3% [26].

The 7.7% *Salmo*nella prevalence in retail eggs recorded from South India [18] was inagreement with the 8% of the present study. The 8% [16] *Salmonella* prevalence in market eggs in this study was higher than that of 1.8% retail eggs samples in New Zealand. The overall *Salmonella* prevalence of 0.34% from retail egg samples reported in the United Kingdom [27] was also lower than that of the current study. The higher *Salmo*nella prevalence in retail eggs in this study when

compared to the above findings could probably be due to environmental contamination or it could be due to contamination during transportation and storage.

The 15% [30 *Salmonella* prevalence in farm eggs in this study was higher than the 0.3% farm egg samples in Bangladesh [27]. The higher *Salmonella* prevalence in farm eggs in the present study might be due to environmental contamination, ineffective cleaning and disinfecting of materials used as stated by different investigators [28]. Feed mills and hatcheries also remain potential sources of introduction of *Salmonella* infections [29,30]. The higher prevalence could also be due to rodent populations, which are involved in the carry-over of *Salmonella*, particularly *Salmonella enteritidis* between flocks and between houses [28,31,32]. The presence of arthropod pests [33], the housing system and flock size [34, 35] and the presence of birds of different ages on and in the farm [34] could be important reasons for higher prevalence in this study as all are present around the farm examined.

In this study, *Salmonella* prevalence on egg shells was 6.3% and this was inagreement with the work of Suresh *et al.* [18] who reported 6.1% of *Salmonella* prevalence on egg shells in India The prevalence of *Salmonella* on egg shellswas 5.4% in Trinidad and Tobago[36] which was comparable with the present finding. However, The prevalence was 0.04% in Ireland [25] While theprevalence of *Salmonella* on egg shells was 0.38%in United Kingdom [26]. Furthermore, 0.29% *Salmonella* egg shells prevalence was reported from United Kingdom and USA [27]. All these *Salmonella* egg shells prevalences were lower than that of the present finding and could be due to egg shells surface contamination. Eggs laid in wet, dirty nests or on the floor are more likely to be contaminated [37]. Main source of egg shells contamination is the feces, dust, litter and hands of egg collector can also contaminate the egg shells [37].

A 6.8% *Salmonella* prevalence from egg content (interior egg contents) obtained in this study was lower than the 9.2% prevalence of Trinidad and Tobago [36], but higher than the 0.06% prevalence recorded in United Kingdom [38]. Studies from Ireland, United Kingdom and USA showed negative for salmonella in egg content [25, 27].

Contamination of eggs with *Salmonella* was believed to occur when the organism passed from the shells into its inner contents [39]. However, transovarian *Salmonella* contamination of shelleggs from the *Salmonella* infected laying hens have further added to the problem. Studies showed that both naturally and experimentally infected chickens produce eggs containing *Salmonella enteritidis* in their liquid interior contents [4]. Freshly laid eggs are rarely reported to harbor more than a few hundred *Salmonella* cells [4, 12, 40]. Prompt refrigeration of the eggs to the internal temperature of 7.2°C or lower can inhibit the growth of *Salmonella* and reduce the chances of consumers being exposed to an infective pathogen dose. Location of *Salmonella* contaminants within eggs is one of the factors. *Salmonella* has the ability to colonize the reproductive tract of the hen and become entrapped in the egg albumen or yolk of the developing eggs [11, 40,41]. Gast and Holt [42] reported that the deposition of *Salmonella* is more often on the yolk membrane than in the interior contents of the yolk. *Salmonella* can survive in the albumen, but is inhibited from growing for an extended period of time due to high pH and inhibitory factors in the albumen [11]. On the contrary, yolk is a rich microbial medium with little capability of inhibiting *Salmonella*. Rapid growth of *Salmonella*, especially at storage temperature above 20°C can occur [9, 11].

In our study, the prevalence of *Salmonella* from fumigated exterior (egg shells) was 0% and this is different from the earlier observation Gholam [43] who reported 1.2% of s*almonella* prevalence in fumigated egg shells. Bacteria are present on the shells of the egg when it is collected from the nest and as the egg cools, the bacteria penetrate the shells. Once this has occurred, no subsequent fumigation will destroy these bacteria. To reduce penetration to a minimum, it is advisable to fumigate the eggs as soon as they are collected and while they are still warm [43]. To avoid recontamination of the egg after fumigation, the eggs should be packed in fumigated trys and cases as soon as possible. Placing the trys of eggs in polyethylene bags will provide the maximum protection from contamination even if they are held in in the egg room for several days [43].

In conclusion, in the present study respective of 15% and 8% prevalence of *Salmonella* from farm and market in Kombolcha, Ethiopia was obtained. Salmonellae were detected on egg shells and egg content with different frequencies of occurrence. There was high contamination of egg with *Salmonella* in the poultry farm indicating that poor management and biosecurity measures. On the questionnaire survy result, few of the consumers have practiced eating raw eggs for medicinal value and this has considerable negative effect on their health. This is, therefore, comprehensive educational programs for the consumer and food handler, both in commercial

establishments and in the home, correct cooking and refrigeration practices for foods of animal origin and personal and environmental hygiene are of paramount importance.

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