Antimicrobial Susceptibility and Public Health Importance of *Salmonella* Isolated from Raw Chicken Egg in MizanTeferi Town, South West Ethiopia

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Abstract

Background: *Salmonella* is a leading cause of food-borne illness in many countries with eggs and poultry being important vehicles of transmission and display high natural susceptibility levels to the most commonly used antibacterial agents. The study was carried out to estimate the prevalence and antibiotic susceptibility of *Salmonella spp.* isolated from fresh raw chicken eggs collected from MizanTeferi town, South Eastern, Ethiopia.

Results: From the total of 366 eggs examined for *Salmonella spp.*, 27(7.4%) were positive, of which 23 (6.3%) and 4 (1.1%) were isolated from egg shell and egg content, respectively. The prevalence of *Salmonella spp.* in egg and egg content from open market (7.6%), (1.6%) was significantly higher than the prevalence of *Salmonella spp.* in poultry farm (7.1%), (0.5%) ($P<0.05$) respectively. Ciprofloxacin (85.2%) showed maximum susceptibility, followed by tetracycline (77.7%) and gentamycin (66.6%). Clindamycin was the most resisted (51.8%), followed by ampicillin (44.4%), amoxicillin (40.7%) and erythromycin (33.3%). Most of the isolates are resistant to multiple antimicrobial agents tested. Questionnaire survey was also carried out on 200 consumers and egg sellers (100 from each group). Their preferred form of egg consumption revealed that 67.5% egg consumers preferred only cooked eggs while 32.5% egg consumers showed preference for raw eggs. Twenty nine (44.6%) respondents who practiced eating raw eggs faced problems like nausea and vomiting, abdominal discomfort and diarrhea, while thirty six (55.4%) faced no problems following raw eggs consumption.

Conclusion: To minimize the potential contamination of the egg by the pathogen, they should be properly handled, transported, cooked and kept appropriately. In general, public health measures such as improved food hygiene and health education are of paramount importance.

Background

Poultry, egg and egg products are among the most nutritious foods on earth and they share an important part of the human diet. However, they are perishable just like meat, fish and other food items, and consumption of improperly handled poultry and egg products are closely associated with negative health impacts causing a food-borne illness, such as salmonellosis [1]. Poultry gets infected by *Salmonella* serovars among which *S. Typhimurium*, *S. Enteritidis* and *S. Heidelberg* are known to infect many hosts. But, *Salmonella* Enteritidis, *Salmonella* Kentucky, *Salmonella* Heidelberg are serovars most commonly diagnosed associated with infection in chickens [2].

Salmonellosis is an important zoonotic disease caused by the genus *Salmonella* which constitutes a major public health burden and represents a significant cost in many countries [3]. Poultry eggs, meat and their products are the commonest vehicles of *Salmonella spp.* to humans [4]. Every year millions of human cases of salmonellosis are reported worldwide and which results in thousands of deaths [5].

Salmonella can be spread from infected hens to the yolk or albumen of developing eggs in different regions of the reproductive tract [6]. Although *Salmonella* can survive or slowly multiply in egg albumen [7, 8]; rapid multiplication occurs in egg yolk [9]. 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 [10]. Moreover, freshly laid eggs are typically reported to contain no more than a few hundred *Salmonella* cells [11],

Antimicrobial resistance is a major and increasing global healthcare problem [12]. Considerable debate surrounds the relationship between antimicrobial use in animals and the resistance problem in people [13]. The presence of resistant organisms in the poultry and poultry products for consumption is a safety concern to the population and therapeutic concern for the physicians which might pose prolonged treatment in cases of outbreaks, delayed recovery or treatment failure [14].

Salmonellosis is endemic in the country and there is a desire to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence. Based on the ubiquitous nature of *Salmonella spp.* and previous studies made in Ethiopia, it was hypothesized that eggs sold in local markets and poultry farm may serve as a source of *Salmonella* species particularly among those raw egg consumers and considerable proportion of them might have developed resistance to antimicrobials that are commonly used both in the veterinary and public health sectors. Therefore, the purpose of the present study was to isolate and determine the proportion of *Salmonella spp.* among two groups and antimicrobial susceptibility of *Salmonella spp.*

isolates from chicken eggs collected from the MizanTeferi local markets and poultry farm. It also aims at assessing the egg handling and consumption habits of farmers and egg consumers in the study area.

Methods

Description of Study area

The study was conducted in MizanTeferi town which is located in the Bench Maji Zone (BMZ), south western part of Ethiopia as indicated in Fig. 1. Agro ecologically BMZ consists of 52% lowland (500–1500 metres above sea level), 43% intermediate highland (1500–2300 metres above sea level) and 5% highland (> 2300 metres above sea level). The mean annual temperature and rainfall varies from 15.1°C-27.5°C and 400–2000 mm respectively [15].

Figure 1: Map of study area

Study Design And Sampling Strategies

Comparative cross-sectional study (between poultry farm and local markets) was conducted on raw chicken eggs destined for human consumption from MizanTeferi poultry farms and local markets (directly from the farmers in Mizan) was conducted from December, 2017 to April, 2018. The sample size required for this study was determined based on the prevalence of *Salmonella spp.* done by [16] on chicken table eggs at Kombolcha, Northern Ethiopia. The prevalence was 10.5% from poultry farm and 3% from local markets [16]. The estimate was desired to be with 5% sampling error and 95% confidence intervals. The sample size was calculated using the following formula for each sampling sites [17].

$$n = \frac{(1.96^2 \sqrt{2P_1(1-P_1)} - Z_{\beta} \sqrt{P_1(1-P_1) + P_2(1-P_2)})^2}{(P_2 - P_1)^2}$$

Where P1 and P2 are the expected proportions in each group, Z_{β} is power

n = No. of sample size.

P_1 = 10.5% (from farm); P_2 = 3% (from local market) [16].

Z_{β} = power (80%) = 0.8

Accordingly, a total of 366 eggs; 183 eggs from semi- intensive poultry farms in Mizan and 183 eggs from Mizan open market directly from the farmers was collected. Thirty eggs from market (one egg from one egg seller) and poultry farm (15 from each group) was collected once per week using systematic random sampling technique. Eggs were collected individually in separate sterile bags, placed on ice in an ice box and transported to the Mizan Regional Veterinary Laboratory. Samples were processed within four hours upon arrival. In cases where there were delays, samples were stored in a refrigerator at 4°C.

Sample Processing

The sterile plastic bags containing selected eggs were open with scissors and the samples processed immediately. Swabs were used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped into sterile buffered peptone water (Oxoid) (CM1049) was used to swab the entire surface area of the egg shell. The same eggs from which shell sample collected will be used for interior (egg contents) sampling. The egg's surface was sterilized using 70% ethanol followed by flaming and then cracked open with a sterile scalpel blade. The isolation was conducted utilizing the conventional methods for the detection of *Salmonella spp.* following the standard guidelines from ISO 6579 [18].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was done by the agar disk diffusion method as described by the Clinical and Laboratory Standards Institute [19]. The pure *Salmonella spp.* isolates confirmed by the biochemical testing procedure as described in ISO 6579:

2002 was tested for antimicrobial susceptibility. The antibiotics to be used were selected among the currently available and commonly used chemotherapeutic agents for treatment of *Salmonella* infection in human and animals such as amoxicillin 25 µg (AMX), ampicillin 10 µg (AM), chloramphenicol 30 µg (C), ciprofloxacin 5 µg (CIP), clindamycin 2 µg (CM), erythromycin 15 µg (E), gentamycin 10 µg, kanamycin 30 µg (K), spectinomycin 100 µg (SPT), tetracycline 10 µg (TE), penicillin 10 µg (P) and clindamycin 5 µg (CM).

Questionnaire Survey

Structured questionnaire was administered to farmers/ egg sellers/ and consumers to identify conditions of handling practice (storage), transportation, preparation, utilization patterns of chicken eggs, assessment about habits of consuming raw egg and related hazards in the study area by interviewing type. Sample size determination for questioner was based on [20].

$$N = \frac{0.25}{SE^2}$$

$$SE^2$$

where, N-sample size SE-standard error=5%

$$= 0.25/0.05^2=100$$

Accordingly, a total of 200 questionnaires; 100 to consumer and 100 to farmers or egg sellers was interviewed. Mizan town has five kebele which are subdivided in to sub kebeles (ketenas). A total 25 ketenas were found in the town and all ketenas were included. 4 (four) chicken egg sellers and 4 (four) consumers were selected from each ketenas. Each ketena has a responsible health extension worker. Random sampling of household was done by using lists of households visited by health extension workers. Once households were selected head of each household responsible for food preparation and purchaser were interviewed using structured questioners. Amharic language was used in the conditions where the language is spoken, whereas, in the areas where Amharic is not spoken interpreters were used.

Data Management and Analysis

Data management, entry and analysis were employed using Microsoft Office Excel 2007. Descriptive statistics such as percentage and proportion were used to describe samples detected positive to *Salmonella spp.* isolation from the total sample analysed by sources of samples and sample type. The Pearson's chi-square (χ^2) test was used to determine the difference in levels between study groups and the association between the prevalence of *Salmonella spp.* and associated factors (source and type of egg sample). P-value of less than 0.05 was considered to determine statistically significant differences. All statistical analysis was performed using STATA software package (version 12.1).

Results

Prevalence of *Salmonella* species

A total prevalence of 7.4 % *Salmonella spp.* infected eggs was found both in egg contents and egg shell of 366 samples. From this, 6.3% and 1.1% were from egg shell and from egg contents respectively. Moreover, the prevalence of *Salmonella spp.* in the poultry farm was 7.1%, while 7.6% was found in the market (Table 1).

The total prevalence of *Salmonella spp.* varied among the sampling sites. The prevalence of *Salmonella spp.* in egg shell of open market and the prevalence of *Salmonella spp.* in egg shell of poultry farm were not significantly different ($p > 0.05$). The prevalence of *Salmonella spp.* in egg contents of the open market was significantly higher than the prevalence of *Salmonella spp.* in egg contents of poultry farm (0.5%) ($p < 0.05$). The difference in prevalence observed between farm egg shell and market egg shell was not statistically significant ($p > 0.05$) as shown in Table 2.

The overall prevalence of *Salmonella spp.* in egg shell was statistically significantly different ($p < 0.05$) from prevalence in egg contents (Table 3). Egg shells are 6.1 (OR=6.1, 95% confidence interval 2.1-17.7) times more likely to be contaminated with *Salmonella* species compared to egg contents.

Farmers/egg sellers/ and Consumers Chicken Egg Use and Handling (Storage) Practice

A total of 200 questionnaires (100 to consumer and 100 to farmers or egg sellers) were interviewed. Mizan town has five kebeles which are subdivided into sub kebeles (ketenas). Ketenas were randomly selected and households were also being randomly selected in collaboration with the ketenas health extension workers. The interviewers were subjected to enquire for the source of egg that they used to consumption. It was noted that 26% of consumers preferred to purchase from market, 51.5% were preferred to consume from homemade and 22.5 % from both market and homemade. Questionnaire were also forwarded to know their preference on eating raw eggs that 32.5% egg consumers showed their preference for raw egg eating, while 67.5% dislike eating raw eggs. This might be due to their feeding habit. The reasons given by the respondents eating raw eggs were due to the nutritious purpose and egg for the purpose of medicine 29.3% and 70.7%, respectively and this could have some negative effects on health. Twenty nine (44.6%) respondents that practiced eating raw eggs were faced a problem like nausea and vomiting, abdominal discomfort and diarrhea, while thirty six (55.4%) were not facing the problem after eating raw eggs. These problems may be arise from contamination of egg by the pathogen in the study area. The respondents' family consuming raw egg habit were asked and shows 44.5% have consuming raw egg for medicinal and nutritious purposes. To show their preference on type of dish they used to prepare from eggs, 42.5% preferred boiling with shell, 52% liked scrambled egg and 5.5% of the consumers liked baked eggs. The habit of washing eggs before subjecting for consumption showed that 77.5% were did not have habit of washing eggs and the rest 22.5 % had a habit of washing eggs. The types of materials in which the eggs was stored before preparation showed that 65.8% of the respondent used open containers such as carton and box, while 28.3% and 5.9% were used together with crops and refrigerators respectively. The majority of the respondents (90%) were used this storage practice to protect from breakage and the rest 10 % were to prevent egg from spoilage. The survey on storage of egg before preparation showed that 72.2% stored 1-7 days, 24.4% stored 7-15 days and only 3.2% stored 15- 30 days. The egg consumers in MizanTeferi town during survey were also asked to what they do with cracked eggs and show that 22.5% were consuming and the rest respondents 77.5% were rejected when the egg cracked. Out of 200 respondents, only 27.5% was knowing about disease could be transmitted by consuming raw egg and the rest 72.5% were unknown (Table 4).

Antimicrobial Resistance Testing

Levels of antimicrobial resistance testing: All the 27 isolates of *Salmonella spp.* were tested for antimicrobial susceptibility testing on twelve different antimicrobials. Of these Ciprofloxacin (85.2) showed maximum susceptibility and followed by Tetracycline (77.7) and Gentamycin (66.6%). Clindamycin was the most resisted (51.8%), followed by Ampicillin (44.4%), Amoxicillin (40.7%) and Erythromycin (33.3%). While Penicillin (33.3%) and Spectinomycin (33.3%) were intermediate resistant. In general, in this study antimicrobial susceptibility test revealed that Ciprofloxacin, Tetracycline and Gentamycin were the drugs indicated more active against *Salmonella spp.* isolated from egg samples, while Clindamycin, ampicillin and amoxicillin were less effective against *Salmonella spp.* isolates (Table 5).

Resistance pattern of *Salmonella* species against 12 antimicrobial agents

All of the isolates were resistant to at least three antimicrobial agents tested, most commonly for clindamycin, amoxicillin, ampicillin, and streptomycin (Table 6).

Discussions

In the current study out of the total 366 chicken eggs examined, 6.3% and 1.1% were positive for *Salmonella spp.* isolated from egg shells and egg contents, respectively (Tables 1 & 2). The results showed that *Salmonella spp.* contaminate egg shell was significantly higher (6.3%) than that of egg contents (1.09%) ($P < 0.05$).

The current study is higher prevalence of Iran studied by [21] 1.33% in egg shell and 0% in egg contents [21], 5.3% and 0% in shell and contents respectively in Haramaya poultry farm recorded by [22] and 2.4% in shell and 0.5% in contents also in Haramaya poultry farm recorded by [23], and in South India [24], 6.1% and 1.8% reported in egg shells and egg contents of retail eggs, respectively. This variation is due to higher environmental contamination and loss of awareness about proper handling and storage of egg. But the current study prevalence was lower than the prevalence of 40% and 8.33% of *Salmonella spp.* were reported from egg shells and egg contents respectively by [25], 11.5% in eggs from Kombolcha [16], 7.7% in egg shell and 5.6% in contents from Alage done by [26],

15.38 in shell and 8.33 in contents from Mekelle done by [27] and 4.69% in eggs from Addis Ababa [28] in the current study. Contamination of egg occurs through different factors such as contact with fecal material, insects, and feed, transportation, storage and during handling. So, variation of the different factors leads to variation in the prevalence of *Salmonella spp.* in eggs.

If the hygienic standard in handling, transportation and storage improves, the *Salmonella spp.* prevalence may lower down. This is evident from several reports of salmonellosis in eggs from developed countries where it ranges from zero to 7% in the United Kingdom [29], 0.62% in US [30], 2.4% in Denmark [31] and 0.4% in Republic of Ireland [32].

Based on the source of egg collected, the prevalence of *Salmonella spp.* was 13 (7.1%) and 14(7.6%) in egg shells and contents of poultry farm and open markets respectively. The results showed that there was no significant difference in prevalence of poultry farm and open markets ($p>0.05$). Contrary to this finding, the study conducted in Kombolcha showed that prevalence of *Salmonella spp.* in contents of egg from poultry farm (10.5%) was significantly higher than that of open market reported by [16].

Salmonella is harmful pathogen that enters the food chain supply at any points in the food production and affects the quality of eggs and the consumer's health. *Salmonella* is horizontally transmitted usually from fecal contamination of egg shell and egg contents by migration through the egg shell and membranes. This route of transmission is facilitated by moist egg shells, storage at ambient temperature and shell damage by *Salmonella spp.* This fact suggests that good animal health practice in poultry farms and food safety intervention should be established to prevent contamination of chicken eggs [1]. The food handlers directly handle raw shell eggs and broken shells may come in contact with the egg content during food preparation and processing, which could allow the pathogen to reach the egg contents and increase the prevalence.

In current study ciprofloxacin (85.2%) showed maximum susceptibility and followed by Tetracycline (77.7%) and gentamycin (66.6%). Clindamycin was the most resisted (51.8%), followed by ampicillin (44.4%), amoxicillin (40.7%) and erythromycin (33.3%). While penicillin (33.3%) and spectinomycin (33.3%) were intermediate resistant. In this study, most of the *Salmonella spp.* isolates are resistant to the different antimicrobial agents tested, especially for clindamycin, amoxicillin, ampicillin, and streptomycin. The high resistance pattern of *Salmonella spp.* isolates against the commonly used antimicrobials is probably due to the irrational use of these antibiotics in veterinary medicine practices which creates problems in the treatment of salmonellosis [33]. In a study carried out in Eastern Ethiopia by [33], the susceptibility of the *Salmonella spp.* isolates were 0.0%, 0.0%, 14.2%, 28.6%, and 92.8% for ampicillin, amoxicillin, tetracycline, chloramphenicol, and gentamicin, respectively.

In [34] study, *S. newport* was showed the highest level of streptomycin resistance (66.7%). *S. heidelberg* and *S. haifa* serovars were resistant to streptomycin. The ampicillin, streptomycin and tetracycline antibiotics were resistant for the two isolates of *S. haifa*.

According to [35] study, 98 isolates of *Salmonella* serovars was recovered from food in Addis Ababa, Ethiopia and assessed their antimicrobial resistance. The results showed that 32 *Salmonella spp.* isolates were resistant to one or more of the 24 tested antimicrobials. Streptomycin (75%), ampicillin (59.4%), tetracycline (46.9%) and spectinomycin (40.6%) were the most common resistance antibiotics compared to the present study (Table 5). In study conducted in Haramaya area [22], all isolates of *Salmonella spp.* were resistant to clindamycin (100%), followed by erythromycin (62.5%), ampicillin (37.5%), amoxicillin (37.5%), and tetracycline (25%) antibiotics. From the tested antibiotics, gentamicin (37.5%), nitrofurantoin (50%), trimethoprim (50%), chloramphenicol (87.5%), and ciprofloxacin (100%) were susceptibility, respectively.

Improving biosecurity measures, vaccination, use of competitive exclusion products, introduction of novel immuno-potentiators, and limited use of antimicrobials are some of the approaches to prevent and control the disease in food animal industry [36]. The frequent use of antimicrobials in food animals causes in the development of antimicrobial resistance [37], through mutation and acquiring of resistance encoding genes [38].

In questioner survey the consumers were subjected to enquire for the source of egg that they used to consumption. It was noted that 26% of consumers preferred to purchase from market, 51.5% were preferred to consume from homemade and 22.5 % from both market and homemade. Questionnaire were also forwarded to know customers preference on eating raw eggs, 32.5% egg consumers were prefer for eating raw egg, while 67.5% of the consumer not like to eat raw eggs. This difference might be to their feeding habit. The reasons given by the respondents eating raw eggs were due to the nutritious purpose (29.3%) and medicine purpose (70.7%), respectively. Eating of raw egg could have negative effects on health. From eating raw egg respondents, 29(44.6%) them were faced a problem like nausea and vomiting, abdominal discomfort and diarrhoea, while thirty six (55.4%) were not facing problems. This problems were may be arises from contamination of egg by the pathogen in the study area.

The types of materials where the eggs were stored before preparation showed that 65.5% of the respondents used open containers such as carton and box, while 28.2% and 5.9% were used together with crops and refrigerators respectively. The majority of the respondents (90%) were used this storage practice to protect from breakage and the rest 10 % were to prevent egg from spoilage. The survey on storage of egg before preparation showed that 72.2% stored between 1-7 days, 24.4% stored between 7-15 days and only 3.2% stored between 15- 30 days (Table 3).

Conclusion

In conclusion, in the present study respective of 7.1% and 7.6% prevalence of *Salmonella spp.* from farm and market in Mizan Teferi suggest that egg products might be the source of human salmonellosis. Study on antimicrobial resistance results point to poultry as a potential reservoir of multi resistant *Salmonella* isolates, which are a serious public health concern now a days. Antibiotics should be used following assessment of antibiogram profiles and unlimited access to antimicrobial agents must be avoided to prevent the spread of multi resistant isolates. On the questionnaire survey result, most of the consumers have practiced eating raw eggs for medicinal value and this has considerable negative effect on their health. However, people consume raw and cracked eggs in the area, indicating a lack of awareness of zoonosis. Therefore, the public should be made aware of risks associated with consumption of raw chicken eggs and raw eggs cracked during storage and transportation. The prevalence of *Salmonella spp.* in egg contents was low. However, mixing of several eggs during food preparation particularly in undercooked or raw foods could result in high risk of *Salmonella* food poisoning.

Abbreviations

BPW: Buffered Peptone Water; CLSI: Clinical and Laboratory Standards Institute; ESR: Environmental Science and Research Limited; FDA: Food and Drug Administration; FRI: Food Research International; MAR: Multiple Antimicrobial Resistance; MAP: Modified Atmosphere Packaging

Declarations

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Data Availability

The data used to support the findings can be obtained from the corresponding author upon reasonable request.

Statement of author

The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations and the study was carried out in compliance with the ARRIVE guidelines. The authors also would like to confirm the research was approved by the board of Haramaya University.

Conflict of Interests

The authors declare that there is no conflict of interests.

Author contribution statements

Authors list

A. Shimelis Mengistu Hailu, Design the work, wrote the paper, performed the analysis and interpretation of data

B. Melkamu Melese, collected the data

C. Berhanu Sibhat, collected the data

D. Pawlos Wasihun, performed the analysis and interpretation of data

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Ethical Statement

The study was approved by the Institutional Review Boards of the Haramaya University. Informed consent was obtained from study participants.

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Tables

Table 1: Prevalence of *Salmonella* spp. by type and source of sample examined

Source of sample	No. examined	Egg shell	Egg content	Total	Prevalence (95% CI)
Farm	183	12	1	13	7.1 (3.8-11)
Market	183	11	3	14	7.6 (4.0-12)
Total	366	23	4	27	7.4 (4.9-10)

Table 2: The prevalence of *Salmonella spp.* between farm and market on egg shell and content

Sample type	Farm (n=183)	Market (183)	2	p-value
Egg shell	12 (6.5%)	11 (6.01%)	0.04	0.842
Egg content	1 (0.5%)	3 (1.6%)	14.43	0.000
Total	13 (7.1%)	14 (7.6%)		

Table 3: The prevalence of *Salmonella spp.* in egg shell and egg contents

Source of sample	No. of positive	Prevalence (%)	2*	p-value
Egg shell (366)	23	6.3	13.88	0.000
Egg content (366)	4	1.1		

(The 2* used is Fisher's exact.)

Table 4: Handling and consumption practice of eggs among egg sellers and consumers

Question Items		Respondents response			
		yes	%	No	%
What is the source of egg used for consumption?	Home made	103	51.5	97	48.5
	Purchased from market	52	26	148	74
	Both from market and home made	45	22.5	155	77.5
Do you consume raw egg?		65	32.5	135	67.5
Does your family consume raw egg?		89	44.5	111	55.5
If yes, for what reason do they consume?	For medicinal	63	70.7	26	29.3
	For nutritious	26	29.3	63	70.7
Have you ever had any sickness FF raw egg consumption?		29	44.6	36	55.4
If yes, what was the symptom?	Nausea and vomiting	11	37.9	18	62.1
	Abdominal discomfort	12	41.3	17	58.7
	Diarrhea	6	20.6	23	79.4
Do you know that disease could be transmitted by raw egg?		55	27.5	145	72.5
Do you wash the egg before preparation to consumption		45	22.5	155	77.5
Do you keep the egg at home?		184	92	16	8
If yes, for how many days?	1-7days	133	72.2	51	27.8
	7-15days	45	24.4	139	75.6
	15-30days	6	3.2	178	96.8
In what way do you keep the egg at home?	Open container	121	65.8	63	34.2
	Together with crops	52	28.3	132	71.7
	Refrigerator	11	5.9	173	94.1
What do you do with cracked eggs?	Consumed	45	22.5	155	77.5
	Rejected	155	77.5	45	22.5
Which cooking method do you used?	Boiling with shell	85	42.5	115	57.5
	Scrambling	104	52	96	48
	Baking	11	5.5	189	94.5

Open container-carton and box, FF-following

Table 5: Antimicrobial susceptibility of *Salmonella spp.* isolates (n=27)

Antimicrobial tested	Resistance isolates		Intermediate isolates		susceptible isolates	
	No.	%	No.	%	No.	%
Gentamycin	4	14.8	5	18.5	18	66.6
Chloramphenicol	3	11.1	7	26	17	62.9
Penicillin	7	25.9	9	33.3	11	40.7
Ampicillin	12	44.4	8	29.6	7	25.9
Erythromycin	9	33.3	6	22.3	12	44.4
Kanamycin	8	29.6	7	25.9	12	44.4
Amoxicillin	11	40.7	5	18.6	11	40.7
Streptomycin	10	37	-	-	17	63
Tetracycline	6	22.3	-	-	21	77.7
Ciprofloxacin	3	11.1	1	3.7	23	85.2
Clindamycin	14	51.8	3	11.1	10	37.1
Spectinomycin	7	25.9	9	33.3	11	40.7

Table 6: Resistance patterns exhibited by *Salmonella spp.* isolates against 12 antimicrobial agents.

<i>Salmonella spp.</i> isolates	No. Isolates with same pattern	Antimicrobial resistance pattern	No. of antimicrobials developed resistance
M21S,M36S,M57C,M80S, M99S	5	CLN,AMP,AMX	3
M10S,M126S,M245S	3	CLN,AMP,AMX,STR,GEN	5
M29S,M209S,M47C, M303S,M309S,M322S	6	CLN,AMP,AMX,STR,ERY,KAN	6
M43S, M66S, M109C	3	CLN,AMP,STR,PEN,TET,KAN,CIP	7
M328S,M346S, M227S, M317S	4	CLN,ERY,CHL,SPS,AMP,PEN,GEN,KAN	8
M89S,M338C,M342S,M355S,M360S, M363S	6	CLN,AMP,AMX,STR,KAN,PEN,CHL,TET,CIP,ERY	10

CLN: clindamycin; ERY: erythromycin; KAN: kanamycin; TET: tetracycline; AMP: ampicillin; AMX: amoxicillin; GEN: gentamycin; SPC: spectinomycin; PEN: penicillin; STR: streptomycin; CIP: ciprofloxacin; CHL: chloramphenicol.

Figures

Figure 1

Map of study area

Supplementary Files

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- [Questionnaire.docx](#)