

Received on (16-03-2016) Accepted on (06-08-2016)

## Bacteriological Occurrence of Salmonella spp. in Hens Eggs and their Environment in Selected Farms in Gaza Strip

Abboud Y. ElKichaoui<sup>1,\*</sup>  
Abdelraouf A. Elmanama  
Ashraf K Msallam

<sup>1</sup>Department of Biology Biotechnology  
Faculty of Science Islamic University of  
Gaza, Gaza Strip, Palestine.

\* Corresponding author

e-mail: [akichoi@iugaza.edu.ps](mailto:akichoi@iugaza.edu.ps)

### Abstract

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Poultry and eggs are considered as major sources for these pathogenic microorganisms.

To investigate the occurrence of Salmonella in Gaza Strip, 596 samples (100 egg pools, 88 feed samples, 320 chicken excreta and cloacal swabs and 88 water samples) were collected from 12 poultry farms in Gaza strip. Sampling program was between January 2007 and December 2007. Samples were tested at the Public Health Laboratory of MOH in Gaza strip. Data were collected through direct interview and structured questionnaire. The questionnaire was applied on 12 poultry farms to evaluate the level of cleaning in certain farms, which contain a number of questions that were answered by farmer owners.

The study showed that egg pools, feed samples and water samples were negative for Salmonella spp., whereas one Salmonella spp. was isolated from chicken excreta pools from Khan-Younis poultry farms. The questionnaire results supported the findings of the study in terms of quasi-absence of infection with Salmonella.

To the best of our knowledge, this is the first study in Gaza investigating the occurrence of Salmonella spp. in eggs and environment in selected local egg production farms.

### Keywords:

Salmonella,  
egg quality,  
poultry,  
Gaza strip.

### Introduction:

Salmonellosis is one of the most common and widely distributed food-borne diseases (Herikstad et al., 2002). They cause an estimated 1.4 million cases of food-borne disease each year in the United States alone (Mead et al., 1999). In addition, salmonellosis results in annual economic costs of approximately \$ 2.3 billion (Frenzen et al., 1999).

The genus Salmonella is a typical member of the family Enterobacteriaceae and consists of Gram-negative, non-spore forming bacilli (Samuel, 1996). The genus Salmonella is divided into two species, Salmonella enterica, which consists of six subspecies, and Salmonella bongori, currently the genus includes 2,500 serotypes (Popoff, 2003).

*Salmonella enterica* subspecies *enterica* (subspecies I) is responsible for 99.5 % of infection in man and animal (Pignato et al., 1998). The predominant serotypes responsible for human salmonellosis are *Salmonella enteritidis* and *Typhimurium* (Herikstad et al., 2002).

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 (World Health Organisation, 2002) Human illness by *Salmonella enteritidis* has increased worldwide in the last two decades, due to ingestion of contaminated eggs, and until now, it is considered the primary cause of salmonellosis in the world (Guard-Petter, 2001). In addition, it is estimated that, in the U.S., *Salmonella* transmission through contaminated shell eggs or egg products results in 700,000 cases of salmonellosis and costs \$1.1 billion annually (Rodriguez-Rom and Yousef, 2005).

Several governmental agencies including the FDA (Food and Drug Administration) have implemented an egg safety action plan to eliminate *S. enteritidis* illnesses due to eggs. In Gaza strip, however, there are no directives to control the process of egg production (Food and Drug Administration, 1997; Hope et al., 2002). In addition to the absence of control steps, there are no published data on eggs quality in Gaza strip. This investigative work is proposed to address this issue and will focus on egg produced for commercial purposes in local farms.

## 1. Material and Methods:

### 1.1 Sample collection

During the period from January to December 2007, Five hundred ninety six samples (egg pools, feed, chicken excreta pools, cloacal swabs and water samples) were collected from twenty poultry farms in Gaza strip and were distributed geographically according to governorates, as shown in table 1 below:

Table (1): Geographical distribution of poultry farms by governorates

Governorates	Number of farms	%
North Gaza	7	35
Gaza	7	35
Mid- zone	3	15
Khan-Younis & Rafah	3	15
Total	20	100

Batch samples (6 eggs per batch), fresh chicken feces, animal feed and water were collected from egg-laying chicken. Samples were transported to the analyzing laboratory with 2 hours of collection in an icebox.

### 1.2 Questionnaire

A questionnaire was used to evaluate the level of cleaning procedures in egg-production farms, which included a number of questions that were answered by farms owners.

### 1.3 Data analysis

All result and data collected from the production farm (questionnaire) were tabulated using Microsoft Excel.

### 1.4 Microbiological analysis

#### 1.4.1 Egg samples

*Salmonella* were isolated from egg pools according to (Bacteriological Analytical Manual methods (BAM). Egg were aseptically cracked , whites were discarded, 25 g of egg yolk was homogenized with 225 ml of pre-enrichment medium, Tryptic soy broth (TSB) and incubated for 24 h at 37°C. The pre-enriched culture (0.1 and 1 ml respectively) was transferred to Rappoport-Vassiliadis broth and Selenite broth and were incubated for 24h at 42°C and 37°C respectively. Following incubation, a loopful from each broth was streaked on SS agar, XLD agar and HE agar, and plates were incubated for 24 h at 37°C. The suspected *Salmonella* colonies were transferred into Triple sugar iron agar, Lysine iron agar and Urea. Following another overnight incubation at 37°C the *Salmonella* cultures were further identified biochemically, using API 20E system and by agglutination specific O and H antisera (Andrews et al., 1998).

#### 1.4.2 Chicken excreta and feed

*Salmonella* were isolated from chicken excreta and feed according to standard methods (International Organization for Standardization 6579, 1993). A 25-g of each sample was homogenized with 225 ml of pre-enrichment medium, Buffered Peptone Water and incubated for 18 h at 37°C. The pre-enriched culture

(0.1 and 1 ml respectively) was transferred to Rappoport-Vassiliadis broth and Selenite broth and incubated for 24h at 42°C and 37°C. respectively. The procedure was completed as egg samples **(International Organization for Standardization, 1993)**.

### 1.4.3 Water sample

*Salmonella* were isolated from water sample according to standard methods **(Health Protection Agency, 2006)**

Five hundred ml of water samples were filtered through membrane filter; the membrane filters were transferred to a container containing, typically, 90 ml of buffered peptone water and were incubated for 24 h at 37 °C.

The pre-enriched culture (0.1 and 1 ml respectively) was transferred to Rappoport-Vassiliadis broth and Selenite broth and incubated for 24h at 42°C and 37°C. respectively. The procedure was completed as egg samples **(Health Protection Agency, 2006)**.

## 2. Result

### 2.1 Description of sample types

Samples collected from twenty poultry farms were divided into three groups (A, B, C) according to period of collection (Group A, from January to March, Group B, from June to September and Group C from October to December).

The percentages of sample type's in-group A, B, C are presented in table 2

**Table (2): Sample type's percentages in-group A, B, C**

Group	Types of samples				
	Egg pools	Feed	Chicken excreta	Water	%
Group A	24	24	60	24	132 (22.1)
Group B	40	40	200	40	320 (53.7)
Group C	36	24	60	24	144 (24.2)

<b>Grand total</b>	100	88	320	88	<b>596</b>
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### 2.2 Questionnaire result

All samples were collected from Hyline chicken, the egg-laying chicken numbers ranged between 3,000-10,000 and egg production numbers ranged between 1,520-7,500, all poultry farms were of the open system type and manually feed. Eggs were collected manually and cracked eggs were separated from the intact eggs. According to farms owners, cracked eggs do not go to factories.

The water sources, which supply the farms, are private wells in some farms and municipal in others. Some farms add drugs to water while others did not. All farm owners claimed that all egg-laying chickens receive update of vaccinations. The dead chickens were disposed through burial.

**Table (3): Questionnaire results**

Questions	Percentage %	
	Close	Open
1. What is the system of the farm	0%	100%
	No	Yes
2. Are the chickens vaccinated	0%	100%
3. Do the chicken excreta mix with fodder	100%	0%
4. Are the cracked eggs distinguished from the untouched eggs	0%	100%
5. Is the farmer clean Periodically	0%	100%
6. Are the stained eggs clean	41.6%	58.3%
7. Do other people enter the farm other than the farmer	58.3%	41.6%
8. Do the chicken excreta mix with the eggs	75%	25%
9. Are there drugs used in the farm water	25%	75%
10. Does the farmer wear special wear when he enters the farm	58.3%	41.6%

The results of our questionnaire (table 3) showed that farmers are taking full caution in dealing with their farms of laying hens and that the work system in the farms is subject to strict procedures.

These results are clearly supports our research results in terms of the lack of infection with *Salmonella*.

There is no doubt that the questions from No. 2 to No. 5 supports these results, particularly in terms of chickens vaccinating, separation between chicken excreta and eggs, periodic cleaning of the farm and speed elimination of broken eggs where the percentages in all these cases was 100% positive.

### 2.3 *Salmonella* isolation and identification

The study showed that 100 egg pools (600 eggs) samples, 88 feed samples, and 88 water samples were negative for *Salmonella spp.*, whereas one *Salmonella spp.* was isolated from chicken excreta pools from Khan-Younis poultry farms.

## 3. Discussion

Eggs and egg products are nutritious foods and they form an important part of the human diet. However, they are perishable just like raw meat, poultry, and fish. Eggs and egg products that are improperly handled can be a source of food-borne diseases, such as salmonellosis.

The aim of this study was to determine the occurrence of *Salmonella spp.* in eggs and environment in selected local egg production farms in Gaza strip, Palestine. To the best of our knowledge, this is the first study in Gaza strip to tackle this issue.

The results obtained after the bacteriological investigation of the 100 egg pools, 88 feed samples and 88 water samples were negative for *Salmonella spp.*, whereas the results obtained from 320 Chicken excreta pools and cloacal swabs showed only one *Salmonella spp.*, despite increasing the number of samples and the period from four months to one year.

### 3.1 *Salmonella* and eggs

There are a large number of publications referring to studies of *Salmonella* contamination of eggs. In these studies, eggs have usually been batched and shell and contents contamination not differentiated. The findings of the present work are to some degree consistent with the results obtained by others. It is assumed that in the USA one in 10,000 eggs is infected with *Salmonella spp.*, in Great Britain one in 15,000 eggs (**Radkowski, 2001**).

The prevalence of *Salmonella spp.* recorded by this study is similar or in general agreement with the literature that reported by other investigators in many countries but the differences were in the numbers of pools egg as shown in the following studies:

In a survey done in New Zealand by Environmental Science and Research Limited (ESR) in 1994 found that no *Salmonella* were detected on the shells of 341 samples of 6 eggs (2,046 eggs in total) or in the contents of 339 samples of 6 eggs (2,037 eggs in total) (**Environmental Science and Research Limited, 2004**). In a study done in Poland between June 1997 and December 1998 *Salmonella* was not found on the shell or inside of a total of 1,200 eggs (**Radkowski, 2001**).

In a study done in Ireland from 2000-2002 on table eggs which consist of 208, 229 and 230 eggs respectively, no *Salmonella spp.* was detected from these eggs. In another study done in Ireland in 2003 on eggs produced under the Bord Bia Egg Quality Assurance Scheme (EQAS), *Salmonella spp.* was not detected in any samples of a total of 1,169 egg samples (each sample comprised of 6 eggs) (**Results of 3rd Quarter National Survey, 2003**).

Studies of eggs appear to indicate that those originating from some countries outside the UK have a higher rate of *Salmonella* contamination compared to UK-produced eggs. In 1996/97, a survey of non-UK eggs intended for retail sale found that 2% of samples contained *Salmonella spp.*, 1.3% contained *S. enteritidis* (**Little et al., 2007**). The Health Protection Agency (HPA) outbreak-associated examination of eggs during 2002

to 2004 showed a higher rate of *Salmonella* contamination in or on eggs from outside the UK and used in catering premises. Most *Salmonella* isolates were *S. enteritidis* (5.5% in Spanish eggs; 6.3% in eggs of country of origin not known) (Little *et al.*, 2007).

In another study done on table eggs in 8 countries of European Union from 2000-2002, *Salmonella* spp. was detected in 0 - 8.1% of eggs. For 2002, a *Salmonella* prevalence above 1% in table eggs was reported in four (Austria 1.1%, Greece 3.8%, Italy 3.1%, Spain 8.1%) out of eight reporting countries (European Commission, 2004).

In another survey done by Food Standards Agency's (FSA's) of *Salmonella* contamination in eggs produced outside the UK and on retail sale in England, which was carried out between March 2005 and July 2006. *Salmonella* spp. was detected from 13.3% and 0.6% of eggs samples that were produced in Spain and France, respectively. *Salmonella* were not recovered from eggs produced in Belgium, Germany, Portugal, Republic of Ireland or The Netherlands (Food Standards Agency, 2006).

In contrast, rates of *Salmonella* contamination in UK-produced eggs appear to have decreased significantly, clearly demonstrating an improved situation which is most likely to be due to the control measures introduced towards the end of the 1990s (1995/6; 1.0%, 2003; 0.3%) (Food Standards Agency, 2004; Little *et al.*, 2007).

A survey in Northern Ireland found an overall contamination rate of 0.43% of the shells and contents of 2,090 of six raw eggs batches of six eggs (Wilson *et al.*, 1998). In a study done in Hawaii on one hundred and six dozen eggs, *Salmonella* were detected in 10 cartons (9.4 percent) of the 106 dozen eggs sample, positive samples were from shells only and no *Salmonella* were detected from the content of eggs sample (Ching-leeM *et al.*, 1991).

In a study done in United States, the prevalence of *Salmonella* in eggs samples range from 0 to 62.5/10,000 eggs (mean, 0.0264%) (Henzler *et al.*, 1998). In 2005 a study done in Mexico city on four hundred (400) eggs, one *S. enteritidis* contaminated egg

was obtained, representing (0.25%) (Martinez *et al.*, 2005).

In a study done in Albania seventy-nine shell egg lots, representing a 22,945,520 eggs imported into Albania from many countries during the 2-years period 1996–1997. *Salmonella* spp. was detected in 1 out of 79 (1.26%) analyzed pooled samples; the lot consist of 275,000 eggs, originating from Bulgaria. *Salmonella* strain was isolated only from the eggshell, but not from the liquid part (Telo *et al.*, 1999).

In Brazil a study done on 614 boxes corresponding to 12 flocks (A-M) of white laying hens., found that the percentage of contaminated eggs was 0.2 and 2.0% in Flocks A and L respectively out of 2,500 eggs were examined (Gama *et al.*, 2003).

Eggs from known infected flocks could be expected to have higher levels of *Salmonella* contamination. In a study done in United Kingdom on eggs from known infected flocks suggested an overall minimum contamination rate of shells and contents combined of 0.24% (Poppe *et al.*, 1992; Whiting & Buchanan, 1997) found less than 1% contamination (range 0 to 19%) of individual eggs from known infected birds (World Health Organization, 2002). Whereas (Morris, 1990) found only 0.1%, overall from infected flocks and 0.5% from known infected individual birds (World Health Organization, 2002). In Canada, a study done by (Poppe *et al.*, 1992) found a prevalence of less than 0.06% of contaminated eggs from two infected flocks (Poppe *et al.*, 1992). Generally, the majority of estimates of egg infection rates in infected flocks were less than 1% (Rural Industries Research and Development Corporation, 2003).

Environmental sampling has been shown to be an accurate indicator of the presence of *Salmonella* in poultry flocks and there is a good agreement between the level of environmental contamination and the prevalence of caecal infection, the level of internal egg contamination and associated human disease (Poppe *et al.*, 1992; Henzler *et al.*, 1998; Gama *et al.*, 2003; Davies & Breslin, 2004; European Food Safety Authorit, 2006).

Despite the high frequency of *S. Enteritidis* in egg-laying flocks, the frequency of *S. Enteritidis* contamination of individual eggs is low. (1 in 20,000 eggs) as reported in

United States (Ebel & Schlosser, 2000), and the majority of egg infection rates in infected flocks in other previous studies are less than 1% as (Telo *et al.*, 1999; Results of 3rd Quarter National Survey 2003; Gama *et al.*, 2003; Rural Industries Research and Development Corporation, 2003; Davies & Breslin, 2004; European Commission, 2004; Little *et al.*, 2007). Whereas the high frequency of *S. enteritidis* in egg-laying flocks was reported from 1.12 - 64% (Ebel & Schlosser, 2000; World Health Organisation, 2002; Rural Industries Research and Development Corporation, 2003;).

The results obtained in this current study after the bacteriological investigation of the 320 Chicken excreta pools and cloacal swabs was one isolate of *Salmonella* spp., which was similar to a study done in "Israel" (Ministry Services and Animal Health, 2005), and was similar to a study done in Finland and Norway in 2002 where the prevalence of *Salmonella* spp. was 0% (European Commission, 2004), and in general agreement with a study done in 1996 in Finland, Norway and Sweden which was less than 1% (European Commission, 2004). Whereas there are a high differences with other previous studies in United Kingdom, Canada and Brazil (Poppe *et al.*, 1992; European Commission, 2004; Davies & Breslin, 2004), and to other studies in many countries which range from 1.12 - 64% (Ebel & Schlosser, 2000; World Health Organisation, 2002; Rural Industries Research and Development Corporation, 2003).

These high differences in this current study and other studies may be due to differences in number of flocks and samples. Geographical variations are thought to play important role in the prevalence and distribution of microorganisms including *Salmonella*.

### 3.2 *Salmonella* in feed samples

The prevalence of *Salmonella* spp. as shown in this current study is similar to that reported by other investigators in many countries but the differences was in the numbers of feed samples. In a study done in 2001 in the European Union, the prevalence of *Salmonella* spp. in Belgium, Greece, Italy and Norway was 0% and in 2002 was 0% in Greece, Italy and Norway, whereas there are differences in the prevalence of *Salmonella* spp. in this current study and in Netherlands, Spain and

Austria which range from 0.45% to 4.8% (European Commission, 2004). In addition, in a study done in Japan 4,418 of commercial layer feeds, *S. enteritidis* were isolated from 143 feed samples (3.3%). This result conformed that the commercial layer feeds are contaminated with *Salmonella* and the layer feed is one of the most important sources of chicken farm contamination to *S. enteritidis* (Shirota *et al.*, 2000).

### 3.3 *Salmonella* in water samples

The results obtained in this current study after the bacteriological investigation of the 88 water samples for the presence of *Salmonella* were in general in agreement with the result of water samples reported in Gaza strip by Public Health Laboratory received water samples from different part of Gaza strip (Ministry of Health, 2007).

In a study done in Iran, all of the wells were contaminated, with a less severity, with coliforms. Fecal coliform was detected in the water of all farms. From the drinking water of 5 farms, *Salmonella* serotypes were isolated. This problem could be due to the drainage from the contaminated streams adjacent to the farms, since the wells were shallow and didn't placed at a proper distance from the streams (Jafari *et al.*, 2006).

The use of open drinkers in the majority of the farms was favorable to contamination, and the presence of *Salmonella* in the litter was considered an important contamination route of the water provided to the birds as in a study done in Canada, the status of floor litter and drinking water; 223 of 226 (98.8 %) were culture positive in the litter and 63 of 226 (27.9 %) were positive in water (Renwich *et al.*, 1992). Furthermore, *S. serotypes Typhimurium* and *Meleagridis* were isolated from the water of troughs used by weaned dairy calves in California (Kirk *et al.*, 2002).

In addition, contamination of a tank water supply system led to an outbreak of *S. Saintpaul* with 28 cases of gastroenteritis amongst over 200 workers at a large construction site. Frogs and/or mice may have been the original source of the contamination **(Taylor et al., 2000)**.

This negative result may be due to a good quality of water sources which supply the farms whereas in studies done in Iran, Canada, USA and in Australia the use of open drinking water supply to farms, litter, troughs of water by weaned dairy calves and stored water without further chlorination lead to contamination of water with *Salmonella* as in previous studies **(Taylor et al., 2000; Jafari et al., 2006;)**.

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### مدي انتشار السالمونيلا في بيض الدجاج وبيئته في بعض مزارع قطاع غزة

#### كلمات مفتاحية:

سالمونيلا،  
بيض جودة،  
دجاج، غزة

تعتبر السالمونيلا من أهم الأمراض المنقولة للإنسان عن طريق الغذاء حيث أنها تشكل عبئاً كبيراً على الصحة العامة وتمثل تكلفة كبيرة في كثير من البلدان. ويعتبر الدجاج والبيض من أهم مصادر نقل هذه الميكروبات الضارة. ولمعرفة مدى انتشار السالمونيلا في قطاع غزة، تم جمع 596 عينة من مزارع الدجاج وهي تمثل الآتي: (100 عينة بيض، 88 عينة أعلاف، 320 عينة من براز ومسحات الدجاج و88 عينة ماء) في الفترة ما بين يناير 2007 إلى ديسمبر 2007، وتم فحصها في مختبر الصحة العامة الخاص في وزارة الصحة في قطاع غزة. تم جمع المعلومات من خلال مقابلة شخصية وباستخدام استبيان منظم أعد من قبل الباحث. وطبق هذا الاستبيان على 12 مزرعة في قطاع غزة لتقييم مستوى النظافة في مزارع قطاع غزة، حيث أنه كان يحتوي على بعض الأسئلة تمت الإجابة عليها من قبل المزارعين ودعمت نتائج الاستبيان ما خلصت إليه الدراسة من حيث شبه انعدام الإصابة بالسالمونيلا. أظهرت نتائج الدراسة بأن كل من عينات البيض والعلف والمياه خالية من السالمونيلا، بينما بينت الدراسة إلى وجود عينة واحدة موجبة للسالمونيلا في براز الدجاج في إحدى مزارع خانينونس. يشار إلى أن هذه أول دراسة تجرى في قطاع غزة للكشف عن مدى انتشار السالمونيلا في مزارع الدجاج والبيض وبيئته.