

Base study for the establishment of national *Salmonella* control program in hatching farms and table eggs in Turkey

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Abstract: Foodborne infections due to *Salmonella* are still a major concern worldwide. Particularly contaminated egg and egg related products are the primary sources for human salmonellosis. It is necessary to determine the risk factors associated with *Salmonella* contamination of eggs within the scope of farm to table and environment. The objective of this study was to develop the “National *Salmonella* Control Program in Laying Hens” and report the prevalence and serotype distribution findings of *Salmonella* in laying hens and eggs in Turkey. A total of 2122 samples were collected and analysed according to ISO 6579:2002 after the isolation and identification procedures. All *Salmonella* isolates were serotyped including 726 eggs and 1396 farm specimens from 241 epidemiological units (EpUs) that were located in 9 different provinces between 2015 and 2017. *Salmonella* contamination was detected in 14.9% of 241 EpUs. The results indicated that almost half of the flocks have multiple contamination sources. The highest contamination rate was obtained from environmental (11%) followed by faeces (7.5%) and the lowest was from water samples (1.6%). The overall contamination rate was detected as 7.46% for farms and 3.3% for eggs. As *S. Enteritidis* and *S. Typhimurium* are the most frequently seen serotypes all over the world, in Turkey *S. Typhimurium* was not detected and *S. Enteritidis* was the 5th most common isolated serotype. According to our results it can be concluded that differences in various countries, particularly geographical and egg hatching systems, may affect the contamination rate and serotype distribution of *Salmonella*.

Key words: Chicken farms, laying hens, prevalence, *Salmonella*

1. Introduction

The poultry industry is one of the most important food sectors in Turkey despite facing many problems such as microbial pathogens, high feed cost and the global financial crisis. In 2017, Turkey's poultry meat and hen egg production reached to 2.1 million tonnes and 1.25 billion tonnes, respectively [1], ranking 8th in the world for hen egg production [2].

In poultry and especially in egg industry, *Salmonella* is one of the major causes of foodborne infections. In the United States, European Union (EU), and Japan, *Salmonella* infections attributed to the food sources were more commonly linked to eggs compared to other food

sources [3]. This may affect the global trade of foods produced with eggs or contain eggs. [4].

Foodborne gastrointestinal *Salmonella enterica* infections are still a major concern worldwide. Particularly contaminated egg and egg related products are the primary sources for human Salmonellosis. Since *S. Enteritidis* is the dominant serotype isolated from commercial poultry and eggs worldwide, it is often found responsible for egg related food poisoning in humans [5]. *S. Enteritidis* was reported as the most common serotype (19%) followed by *S. Typhimurium* (14%), and *S. Newport* (10%) isolated in foodborne Salmonellosis in the United States [6]. *S. Enteritidis* was isolated from most of the foodborne

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Salmonellosis cases due to consumption of shell eggs [7,8], also other *Salmonella* serotypes have been reported from egg related *Salmonella* infections [8]. During poultry production *Salmonella* contamination to the external and internal egg is very complicated, depending on many factors. Therefore, it is very hard to implement appropriate control measures [9]. Egg can be contaminated with *Salmonella* both horizontally and/or vertically. Horizontal contamination generally occurs due to the faecal contamination of egg shell whereas vertical transmission is caused by the colonization of bacterium to the ovary and oviduct before the formation of egg shell [10].

Contamination with multiple serotypes of *Salmonella* on commercial layer farms is a common issue [11,12]. In a recent epidemiological study, *S. Mbandaka* (54.40%, 68/125) was reported as the most prevalent serotype on layer farms followed by *S. Typhimurium* (11.54%, 15/130) [11,13]. *S. Mbandaka* has also been reported previously in some other studies from animals, feed, egg shell, and sporadic *Salmonella* infections of humans [12,14,15]. Therefore, in order to reduce number of Salmonellosis cases due to the consumption of egg, effective methods are needed to control *Salmonella* in layers. For this purpose “Egg Quality Assurance Programs” (EQAPs) or specific guidelines to reduce *Salmonella* contamination of shell eggs have been developed in United States and EU [16]. Despite these programs, Salmonellosis caused by shell egg still remains as a public health problem highlighting the significance of revisiting the present EQAPs and specific guidelines [6, 17].

It is necessary to determine the risk factors associated with *Salmonella* contamination of poultry eggs within the scope of farm to table food hygiene in the laying hens’ environment. Monitoring, as the European Food Safety Authority (EFSA) has stated, is an effective way to learn about the prevalence of *Salmonella* in laying hens [18]. In this context, the EU has set a control program for *Salmonella* in layers for rearing and laying flocks with the regulation of EC 2160/2003 [18]. The main objective of this study was to develop the “National *Salmonella* Control Program in Laying Hens” and report the first 2 years’ prevalence and serotype distribution findings of *Salmonella* in laying hens and eggs in Turkey.

2. Materials and methods

2.1. Sampling

In this study, a sampling method including all variables such as poultry, feed, and environment in egg production was performed to obtain the isolates that will constitute the basis for the detection and control program of *Salmonella* prevalence. The units from which the materials are collected have been defined as the basic epidemiological units (EpUs) in the form of farms with specific borders,

common feed, and water use. The number of materials collected were not less than 10% of the total number of EpUs in Turkey. Sampling in eggs was performed in accordance with 517/2011/EC, considering the principles of the EU [19]. The EU Regulation of 152/2009/EC for feed specimens in poultry specimens and Regulation 28155 dated 27.12.2011 were taken as basis for the sampling and analysis of the feeds [20].

In the selection of the EpUs to be sampled, geographical settlement at the regional, provincial, district level, operational size, and the integrated facilities to which it belongs were taken into consideration. According to the origin of the material, the estimated prevalence in samples was 30% in laying hens, 5% in feed, 10% in egg storage and rodents. In all of these processes, random sampling method was used in stratified geographical sampling method and random sampling model was chosen from provinces selected with probability proportional to width (Sample width $\alpha = 0.01$, $P = 0.50$, d tolerance ± 0.04 ; population ratio calculated by simple random sampling), the probability of the difference between the ratio estimation being equal to or greater than a fixed number, such as predetermined d , must be equal to α [21]

In the study, faeces, litter, dust, environmental, rodent trap, feed, and water samples taken from EpUs were collected under special rules (all samples were taken according to the prestudy training program in order to make the same sampling). Litter samples were taken with at least 100 steps by boot swab (3M, Maplewood, Minnesota, USA) method to cover different regions of the poultry house. Samples were collected from the manure channels when the laying hens did not have litter. Feed samples were collected from feeders in different areas of the pen. Rodent samples were collected from stations as swabs and/or stool samples. Dust samples were collected from different places in the farms (beams, columns, ventilation pads etc.) with a moist sponge swab (3M, USA). Environmental samples were collected from the pens as a maximum of 5 steps away, such as mud, water deposits, wastes, and soil.

A total of 2122 samples were collected and analysed, including 726 eggs and 1396 farm specimens from 241 EpUs (each unit’s capacity was between 10,000 and 20,000 birds) between 2015 and 2017 that were located in 9 different cities (Afyon, Amasya, Bursa, Çorum, Denizli, İzmir, Konya, Manisa, and Samsun) in Turkey. The numbers of the collected samples and epidemiological units are shown in Table 1.

During the collection of samples (poultry house environmental and table eggs), the samples were barcoded by entering the information for each sample to *Salmonella* Control (SALKON) software program developed for this control program. Barcoded samples were transported in cold chain to the official and university laboratories. The

samples submitted to the laboratories were scanned with a barcode reader so their entries into the SALKON software program were made.

2.2. Isolation and identification

The isolation and identification of *Salmonella* was performed according to the ISO 6579:2002 protocol [22]. In terms of applying the method in the same way between laboratories, visual and practical ISO 6579:2002 protocol coordination training was given for 1-week to laboratory staff.

Samples taken from litter, dust, environment, rodent, feed, and water were preenriched in buffered peptone water (Oxoid CM0509) at 37 °C for 18–24 h. Egg samples were analysed by pooling 6 eggs. In the enrichment step, 0.1 mL of the preenrichment was transferred to semisolid Rappaport-Vassiliadis Medium (Oxoid CM0669) and incubated at 42 °C for 18-24 h. After incubation period, a loopful of medium was subjected to XLD agar (Xylose Lysine Deoxycholate agar, Oxoid CM0469) and incubated at 37 °C for 24 h. *Salmonella* suspect colonies were selected and plated on Nutrient agar (Oxoid CM0003) (incubation for 24 h at 37 °C in aerobic conditions) to obtain the pure colonies. Typical *Salmonella* colonies were confirmed by Microgen™ GNA+B-ID System (Microgen, UK) test kits including glucose (+), sucrose (-) and lactose (-) fermentation, gas (+) and hydrogen sulphide (H₂S) production (+), urea hydrolysis (-), indole formation (-), Voges Proskauer-VP (-), the lack of β-galactosidase (ONPG), and lysine decarboxylation (+). After the biochemical tests, *Salmonella* suspected colonies were identified by agglutination test with polyvalent *Salmonella* antiserum.

2.3. Serotyping

Serotyping of the *Salmonella* isolates was performed with the scheme of White–Kaufmann–LeMinor using lam agglutination and serum neutralization tests (Statens Serum Institute, Copenhagen, Denmark and Denka Seiken, Tokyo, Japan) [23].

3. Results and discussion

In this study, *Salmonella* contamination was detected in 36 (14.9%) of 241 EpUs investigated, without considering the type of sample. When pooled faeces were taken as the basis of EU criteria, 18 (7.5%) of 241 EpUs were reported as *Salmonella* positive. This indicated that almost half of the flocks have multiple contamination sources. The isolation frequency of *Salmonella* from various types of 1203 samples is shown in Table 2. Highest contamination rate was obtained from environmental materials 24 (11%) followed by pooled 18 faeces (7.5%) and the lowest was from 3 water samples (1.6%).

Considering the prevalence of *Salmonella* in 9 densely populated provinces, *Salmonella* positive EpU was not detected in 4 of these provinces which include 37 EpUs.

Table 1. Total number of samples examined for the poultry *Salmonella* control program in laying hens.

Samples	Number of samples	
Egg	726	
Epidemiological units	241	
Samples from epidemiological units	Litter*	262
	Dust	237
	Environment	239
	Rodent	162
	Feed	219
	Water	209
Total	1328	
Total samples	2054	

*Samples were collected from the manure channels when the laying hens did not have litter.

Table 2. The isolation frequency of *Salmonella* from various types of materials in laying hen's production.

	Number of studied	Number of positive	Positive %
Faeces	241	18	7.5
Dust	216	8	3.7
Environment	218	24	11
Rodent	142	3	2.1
Feed	198	5	2.5
Water	188	3	1.6
Total	1203	61	5.1

Four of 10 EpUs were (40%) found to be contaminated with *Salmonella* in Manisa within the positive EpUs. In other locations the prevalence was 20% in Çorum, 16.4% in Konya, and 12.7% in Afyon, respectively (Table 3).

Totally 14 different serotypes were identified in laying hens (Table 4). Examining the frequency of occurrence of any material in the EpU, the most commonly detected serotype is *S. Kentucky*. The prevalence of this serotype in pooled faeces was determined as 3.7%. *S. Infantis*, *S. Mbandaka*, *S. Agona*, and *S. Enteritidis* were the other most frequent serotypes, respectively. However, *S. Typhimurium* was not observed in laying hens in Turkey. The diversity of serotypes was highest in environmental samples with 10 different serotypes, followed by dust and pooled faeces with each 6 serotypes. *S. Mbandaka* was found in all types of sample materials. On the other hand, *S. Enteritidis* was

Table 3. Prevalence of *Salmonella* in EpUs and collected samples from different provinces of Turkey.

Province	Epidemiological unit			Material	
	Number	Any Positive* %	Faeces Positive %	Number	Positive %
Afyon	71	12.7	5.6	332	5.1
Amasya	5	0	0	27	0
Bursa	5	0	0	28	0
Çorum	55	20.0	12.7	297	6.4
Denizli	1	100.0	100.0	6	16.6
İzmir	19	0	0	108	0
Konya	67	16.4	5.9	309	5.5
Manisa	10	40.0	20.0	55	12.7
Samsun	8	0	0	41	0

*Accepted from the faeces samples

Table 4. Frequency of *Salmonella* serotypes in epidemiological units and total isolates of laying hens (%).

Serotype	% of Epidemiologic units		% within all serotypes
	In any samples	In pooled faeces	
Infantis	3.7	1.2	14.7
Kentucky	10.8	3.7	42.6
Enteritidis	1.2	0.4	4.9
Senftenberg	0.8	0	3.3
Mbandaka	3.3	0.8	13.1
Hadar	0.4	0	1.6
Virchow	0.4	0	1.6
II	0.4	0	1.6
Corvallis	0.4	0	1.6
Anatum	0.4	0	1.6
Agona	1.6	0.8	6.5
Paratyphi	0.4	0	1.6
Paris	0.4	0.4	3.3
Montevideo	0.8	0	1.6

found in 1.2% of laying hens' materials and in 0.4% of pooled faeces. *S. Enteritidis* could be detected in pooled faeces, dust, and environmental samples, but not in other type of materials (Table 5).

Evaluating the frequency of the *Salmonella* serotypes in provinces, *S. Kentucky* was isolated as the most dominant serotype in all *Salmonella* positive provinces (Table 6). *S. Infantis* and *S. Enteritidis* were isolated in 4 and 2 different

provinces, respectively. On the other hand, 8 different serotypes were detected in Afyon province, 5 in Çorum, and 4 in Manisa and Konya.

Salmonella contamination was detected in 24 (3.3%) of 726 table egg samples purchased from different regions of Turkey. According to the serotyping results, 75% and 25% of contaminated eggs carried only *S. Enteritidis* or *S. II* (*S. enterica* subsp. *salamae*), respectively.

Table 5. Presence of *Salmonella* serotypes in 1203 laying hens' material (%).

Serotype	Pool faeces	Dust	Environment	Rodent	Feed	Water
Infantis	1.2	0.4	1.8	0	0	0.5
Kentucky	3.7	1.4	5.5	0.7	0	0.5
Enteritidis	0.4	0.4	0.4	0	0	0
Senftenberg	0	0	0.4	0.7	0	0
Mbandaka	0.8	0.4	0.4	0.7	1.0	0.5
Hadar	0	0	0.4	0	0	0
Virchow	0	0	0.4	0	0	0
II	0	0	0.4	0	0	0
Corvallis	0	0.4	0	0	0	0
Anatum	0	0	0	0	0.5	0
Agona	0.8	0	0	0	1.0	0
Paratyphi	0	0	0.4	0	0	0
Montevideo	0	0.4	0.4	0	0	0
Paris	0.4	0	0	0	0	0

Table 6. Number of epidemiological units with *Salmonella* serotypes in each province (Total number of EpUs are shown in parenthesis).

Serotype	Afyon (71)	Çorum (55)	Denizli (1)	Konya (67)	Manisa (10)
Infantis	1	2	0	3	1
Kentucky	1	7	1	6	1
Enteritidis	1	1	0	0	0
Senftenberg	1	0	0	0	0
Mbandaka	0	0	0	4	1
Hadar	0	0	0	1	0
Virchow	0	0	1	0	0
II	1	0	0	0	0
Corvallis	0	0	0	0	1
Anatum	1	0	0	0	0
Agona	2	0	0	0	0
Paratyphi	1	0	0	0	0
Montevideo	0	2	0	0	0
Paris	0	1	0	0	0

This is the first comprehensive study on the prevalence and distribution of *Salmonella* in chicken layer farms and table eggs from all over Turkey. In Turkey, laying hen farms are located in 9 different provinces. Therefore these 241 EpUs represent almost all EpUs in the country. The overall contamination rate was detected as 18 (7.5%) for farms and 24 (3.3%) for eggs. The prevalence of the EpUs was ranged from 0% to 100% but the rate of *Salmonella*

contamination in provinces where EpUs are intensive (more than 20 EpUs) were varied from 12.7% to 20%. This obtained prevalence in laying hens in this study is higher than the EU's 2016 average contamination level which was 3.17% [24].

Our results showed that environmental samples and pooled faeces are the most important steps in collecting samples within the national programs. It is mentioned that

Salmonella contamination from the environment in to the flock can be the major sources of highest prevalence and same with the environmental transmission, faeces is one of the most important risk factor for *Salmonella* contamination in pens. It is also accepted that elimination of *Salmonella* from the environmental samples would reduce the contamination rate of table eggs [25,26].

As a predominant serotype *S. Kentucky* was detected in all of the sample matrices collected from laying hens except for feed samples. *S. Kentucky* has been rapidly spreading across different geographical regions of the world in recent years and has become an increasingly important serotype in terms of public health. In the United States, *S. Kentucky* is the most frequently isolated serovar from chickens [27]. Approximately 1% of the *Salmonella* cases seen in humans in the EU, originated from serovar *Kentucky*, which ranks 7th in total human cases [24]. Despite available information about *S. Kentucky* throughout the world, it is not one of the major serotypes seen in humans, but according to the results of previous studies this serotype may likely cause significant future prospective health problems with a high resistance of antibiotics [5].

Interestingly, among the serotypes most frequently seen in humans all over the world, *S. Typhimurium* was not detected and *S. Enteritidis* was the 5th most common isolated serotype in this study. There is limited data relating with laying hen *Salmonella* contamination in Turkey but according to the previous studies *S. Typhimurium* contamination was rare in poultry meat and products [28, 29]. In another study performed in Turkey, overall *Salmonella* infection rate was 18.2% in 14 chicken layer breeder flocks analysed by PCR and conventional culture methods [30].

This study showed that 3.3% of 726 purchased table egg were contaminated with *Salmonella*. The results

obtained from this study is very important due to the lack of further work on this issue. In a small-scale study conducted in Turkey no *Salmonella* was detected from 50 table egg samples [31]. In another study performed to assess the microbiological quality of chicken eggs in terms of the presence of *Salmonella* spp., purchased for the need of 7 military units in Ankara Garrison, the results showed that *Salmonella* was not detected in 882 egg samples [32]. Although the findings of the laying hens' results are different, the predominant serotype in the egg samples is detected as *S. Enteritidis* (75% of the positive samples). This result was found as consistent with the findings of the studies conducted around the world [33]. This may be explained by the fact that *S. Enteritidis* can contaminate and colonize the reproductive tract of chicken during the egg production [34].

In conclusion, this study shows that differences in various countries, particularly geographical and egg hatching systems, may affect the contamination rate and serotype distribution of *Salmonella*. As this study is the first nationwide survey conducted in Turkey, the results are of great importance to understand the current status of *Salmonella* in hatching farms and table eggs. It is thought that a reduction in environmental and faecal contamination with future control programs and biosafety practices will not only protect public health but also provide economic benefits. It is essential that the national monitoring program should be sustainable in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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