Two Consecutive Outbreaks of Foodborne Gastroenteritis Caused by Salmonella Enteritidis in Turkey

Türkiye'de *Salmonella Enteritidis*'in Neden Olduğu İki Ardışık Besin Kökenli Gastroenterit Salgını

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ABSTRACT Objective: We report two foodborne outbreaks caused by *Salmonella enterica* serovar Enteritidis occurred in four youth hostels and two private schools in Diyarbakir, Turkey to identify the involving pathogen(s) in the possible source of infection, to control and to prevent further of the outbreaks. **Material and Methods:** An outbreak survey was conducted and food items, water supply, work surface samples and stool cultures were analyzed. **Results:** Out of 346 persons, a questionnaire was administered to 208 persons, 117 affected ill and 91 control healthy persons, to describe the illness and to identify its likely source. Salmonella enterica serovar Enteritidis was isolated from 51 stool cultures and also samples of chicken and potatoes meals in the first outbreak and samples of green salad in the second outbreak. **Conclusion:** In this study, we described how we identified the sources of both outbreaks and how we managed it with a case control study.

Key Words: Disease outbreaks; gastroenteritis; Salmonella enteritidis

ÖZET Amaç: Bu yazımızda, Diyarbakır ilinde dört yurt ve iki özel okulda Salmonella enterica serovar Enteritidis'in neden olduğu iki besin kökenli salgını sunuyoruz. Olası enfeksiyon kaynağına neden olan patojeni belirlemek ve ileride olabilecek salgınları önlemek amaçlanmıştır. Gereç ve Yöntemler: Bir salgın anketi kullanıldı ve besin türleri, su kaynakları, çalışılan alanlardan örnekler alındı ve gaita kültürleri analiz edildi. Bulgular: 346 kişiden 117'si hasta 91'i kontrol olgusu olmak üzere 208'ine hastalığı tanımlamak ve olası kaynağı belirlemek için anket uygulandı. Elli bir gaita kültüründen ve aynı zamanda birinci salgında patates ve tavuktan hazırlanmış yiyecekten alınan örneklerden ikinci de ise yeşil salatalardan alınan örneklerden Salmonella enterica serovar Enteritidis izole edildi. Sonuç: Çalışmamızda her iki salgının nedenlerini nasıl ortaya çıkardığımızı ve bir vaka kontrol çalışma dizaynıyla nasıl çözümle sonuçlandırdığımızı tanımlıyoruz.

Anahtar Kelimeler: Hastalık salgınları; gastroenterit; Salmonella enteritidis

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In many countries the incidence of Salmonella infection has increased markedly, although national or hospital based surveillance data was well-done in most of the cases. In 1994, *S. enteritidis* and *S. ty-phimurium* were the most common serotypes isolated from human sources. Nontyphoidal Salmonella infections in human are almost always associated with food products that were prepared in suboptimal conditions. Non-typhoidal Salmonellae are an important cause of bacterial foodborne infections worldwide. There are an estimated 1.4 million ca-

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ses of Salmonella infections annually in the United States.^{2,3} Salmonella has been found to be responsible for annual incidence of 6.8 % in healthy people in 2010.4 In Singapore, a total of 380 laboratory-confirmed cases of non-typhoidal salmonellosis were reported in 2006, an increase of 28.3% from 296 cases reported in 2005. Two hundred fifteen of these cases were caused by S.enteridis.⁵ Animal-originated foods such as meat, poultry, egg or dairy products can become contaminated with Salmonella. Eating uncooked or inadequately cooked food-or food cross contaminated with these products-may cause human infections. Changes in food consumption and the rapid growth of international trade of agriculture could incline the outbreaks. Recent foodborne outbreaks of salmonellosis have been found associated with fresh products including cantaloupe, freshly squeezed orange juice, sliced tomatoes, and alfalfa sprouts. ¹In this study, we reported two outbreaks occurred with an interval of five days in the largest city of southeastern Turkey, Diyarbakir (Figure 1, 2).

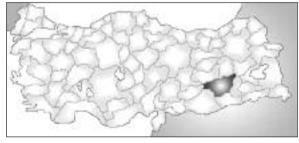


FIGURE 1: Location of Diyarbakir within Turkey.



FIGURE 2: Diyarbakir and its districts.

MATERIAL AND METHODS

BACKGROUND

An outbreak of non-typhoidal Salmonella gastrointestinal infection occurred in four youth hostels on February 18, 2009 and the second outbreak occurred in two private schools on February 23, 2009 in Diyarbakir, Turkey. Both outbreaks had a common feature. Foods were ensured from the same company. In both outbreaks, a total of 346 people suffered.

Informed consents were obtained from all patients. This study was approved by the Local Health Directorate and Administrative Committee of the Diyarbakir State Hospital. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki.

EPIDEMIOLOGIC INVESTIGATION

After the outbreaks were broken out with gastrointestinal symptoms of many affected people, analysis was first performed to determine the source meal. With initial findings, a subsequent survey was conducted on students, teachers and their staffs who had eaten the affected meal to determine the served and consumed various meals. Then, a case control study was performed to determine the likely foods in youth hostels on February 18, 2009 in the first outbreak and in two private schools on February 23, 2009 in the second outbreak. A questionnaire was applied to 208 individuals including 91 healthy people and 117 affected ill people. They were asked about diarrhea, that was defined loosing stool three times or more during 24-hour period. If diarrhea was reported, the respondent was asked whether medical care was sought, and what treatment, if any, was initiated. All respondents were asked if they had participated in the February lunch and were asked to recall consumed foods. An epidemiologic curve was drawn based on the onset of symptoms. An initial survey conducted within five days of outbreak in private schools and youth hostels. The survey involved the presence of clinical symptoms and the probable source of the outbreak that were described by students, teachers and their staffs of the youth hostels and private schools.

LABORATORY INVESTIGATION

Stool samples were collected from 346 symptomatic students, teachers and their staffs and 23 food handlers to examine for commonly seen enteric pathogens. Salmonella isolates were subtyped using standard procedures. Blood cultures were taken during diarrheal period.

ENVIRONMENTAL INVESTIGATION

Diyarbakir Health Department was informed of the outbreak and conducted environmental and sanitary inspections. Laboratory tests were performed on the water supply of youth hostels and private schools to exclude it as a possible source of infection. In relation to the results of the survey and laboratory tests, further investigations into the food preparation process, inspection of the areas and interview with food handlers were conducted to determine the source of the outbreak.

STATISTICAL METHODS

For the descriptive analysis, attack rates were calculated to determine the food item that most likely caused the outbreak. Chi-square test was used to compare the categorical outcomes for statistical significance. A probability value of less than 5% was defined as significant. Odds ratio (OR) and corresponding 95% confidence intervals (CI) were calculated. Logistic regression analysis was performed to determine the probable causative food or foods. The input data was evaluated by using percentages for intermittent variable, arithmetic mean in continuous variable and standard deviations. All statistical analyses were performed using SPSS 10.0 soft—ware (Statistical Package for the Social Sciences).

RESULTS

A total of 346 people including students, teachers and their staffs were resident in four youth hostels and two private schools during two outbreaks. Fifty nine of 94 people answered the questionnaire (response rate: 63%) in the first outbreak and 117 of 252 people answered the questionnaire (response rate: 46%) in the second outbreak. The non-responders could not be promptly contacted for the epidemiological investigation. The median age of the patients in the first outbreak was 19.3 years

(range: 15-28 years), thirty eight (65%) of them were males. During the epidemiologic studies in the both outbreaks, the affected patients were grouped according to their ages (Table 1, Table 2).

A case was defined as presence of fever and diarrhea (three or more times stool loosing in 24- hour period), abdominal cramps, nausea and/or vomiting within three days after eating in youth hostels or private schools. All patients had symptoms of gastroenteritis. Myalgia was reported in 51 patients (86.4%), headache in 46 (78%), fever in 40 (68%) and diarrhea in 33 (56%) The symptoms during the outbreaks were shown in Table 3. The symptoms appeared between six and 36 hours after eating suspected meal. The median duration of

TABLE 1: The age distribution of affected patients in the first outbreak.

Number of affected patients %100									
Age interval	(n=59)	(percentage)							
15-19 age	35	59.3							
20 years and older	24	40.7							

TABLE 2: The age distribution of affected patients the second outbreak.

Age interval	Number affected (n= 117)	%100 (percentage)
Under 9 years	3	2.6
10-14 years	18	15.4
15-19 years	82	70.1
20 years and olde	r 14	11.9

TABLE 3: The distribution of symptoms among the patients of first outbreak (n= 94).

Nur	nber of affected patients	%100
Symptoms	(n=59)	(percentage)
Myalgia	51	86.4
Headache	46	78.0
Fever	40	67.8
Nausea	38	64.4
Abdominal pain/colic	35	59.3
Diarrhea	33	55.9
Vomiting	27	45.8
Muscle and joint pain	24	40.7
Night sweating	11	18.6
Blood in stool	1	1.7

diarrhea was four days (range: 1-8). Hospitalization was reported for 53 of 59 patients (90%). Acute kidney injury (1.6%) was diagnosed only in one patient. There was no exitus.

In the second outbreak, the median age of the patients was 16.9 years (range: 8-33 years), 85 (73%) were males. All patients had symptoms of gastroenteritis. Myalgia was reported in 112 (96%), diarrhea in 93 (89.5%), headache in 98 (84%) and fever in 98 (83%) (Table 4). The symptoms appeared between six and 36 hours after eating suspected meal. The median duration of diarrhea was five days (1-9 days). One hundred and seven people were (91.4%) admitted to the hospital. Hospitalization was reported in 53 (45.2%) patients. No mortality was observed.

Initial symptoms of 176 people were revealed in Table 3 and 4. All patients have suffered from gastrointestinal symptoms. Hospitalized 54 patients were treated with symptomatic intravenous fluid and antimicrobial therapy in the Diyarbakir Education and Research Hospital. The attack rate was 34.6% in the first outbreak and 28.4% in the second outbreak. No mortality was observed. The survey was conducted in relation to hospital data including the admission date to hospitals after the onset of the outbreaks (Figure 3).

The questionnaire was applied to 208 people that were containing 91 healthy people and 117 affected ill people. The relationship between eating a

TABLE 4: The distribution of symptoms in the second outbreak (n= 252).

	oatbroak (11 202)1	
Numbe	er of affected patients	%100
Symptoms	(n=117)	(percentage)
Myalgia	112	95.7
Diarrhea	93	89.5
Headache	98	83.8
Fever	97	82.9
Abdominal pain/colic	96	82.1
Muscle and joint pain	87	74.4
Nausea	92	78.6
Vomiting	78	66.7
Night sweating	59	50.4
Blood in stool	1	0.8

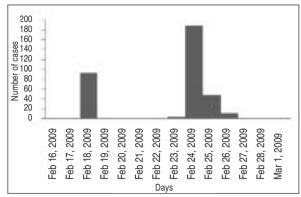


FIGURE 3: Distribution of patients with symptoms of food poisoning according to admission date to hospitals between February 18-23, 2009.

meal and developing illness was assessed during the last two days of second outbreak. The relationship between eating the meal and the number of cases were shown in Table 5, 6, 7 and 8.

From analysis of Table 5, people who had lunch and dinner at 23 February were in risk of illness between 6.11 and 6.59 times more than those who had at 24 February (P<0.001).

As shown in Table 6 those who ate meat braised in its own fat had a 17.28 times relative risk for infection more than those who did not ate (P<0.001) (95% CI, 8.23-36.25).

As shown in Table 7, consumed meals at dinner including fried egg, pilaf and green salad were more likely contaminated (Odd ratios for fried egg and pilaf were 5.905 and 6.597, respectively).

The distribution of patients that consumed foods on February 24 lunch was shown in Table 8. There was a significant difference in patients that consumed wheat pilaf at lunch menu on February 24 (OR=0.477).

Logistic regression analysis was shown in Table 9 about foods that were thought to be the source of salmonellosis. Braised meat was found 9 times higher contamination rate whereas green salad had 4.6 times contamination rate on February 23.

Stool samples were collected from 346 symptomatic students, teachers and their staffs for bacteriological tests. Stools were examined for Salmonella, Shigella, Campylobacter and *Escheric*-

TABLE 5: The distribution of patients according to consumed meals in private school.

		Ca	se	Con	trol	To	tal				95% cor interv	nfidence als(CI)
Date	Eating	Number	%	Number	%	Number	%	X 2	Р	OR	Min	Max
At February 23,	Yes	107	93.3	65	71.4	172	83.9					
lunch	No	7	6.1	26	28.6	33	16.1	18.85	<0.001	6.114	2.512	14.884
At February 23,	Yes	25	25.8	4	5	29	16.4					
dinner	No	72	74.2	76	95	148	83.6	13.81	<0.001	6.597	2.188	19.891
At February 24,	Yes	16	31.4	60	65.9	76	53.5					
lunch	No	35	68.6	31	34.1	66	46.5	15.69	0<0.001	0.236	0.113	0.492

TABLE 6: The distribution of patients according to the sort of meals in lunch menu on February 23, in private school.

		Ca	se	Con	trol	То	tal					nfidence als (CI)
		Number	%	Number	%	Number	%	X 2	Р	OR	Min	Max
Breakfast	Yes	7	6.2	1	12.5	8	3.9	3.47	0.620	5.943	0.718	49.221
Dieakiasi	No	106	93.8	90	45.9	196	96.1	3.47	0.620	3.943	0.710	45.221
Meat braised in	Yes	102	89.5	30	33	132	64.4	70.45	<0.001	17.283	8.239	36.254
its own fat	No	12	10.5	61	67	73	35.6	70.43	V0.001			30.234
Pilaf	Yes	104	91.2	59	64.8	163	79.5	01.60	-0.001	.001 5.641	2.589	12.288
Filai	No	10	8.8	32	35.2	42	20.5	21.63	<0.001			
Green	Green Yes	74	64.9	15	17.2	89	44.3	45.44	-0.001	8.88	4.515	17.464
salad	No	40	35.1	72	82.8	112	55.7	45.44	5.44 <0.001	0.00	4.515	17.464

TABLE 7: The distribution of patients according to the sort of meals in dinner menu on February 23 in private school.

		Case		Control		Total					%95 cor Interva	
		Number	%	Number	%	Number	%	X 2	Р	OR	Min	Max
Eriod ogg	Yes	23	23.7	4	5	27	15.3	11.87	<0.001	5.905	1.948	17.901
Fried egg	No	74	76.3	76	95	150	84.7	11.87	<0.001			17.901
Pilaf	Yes	25	25.8	4	5	29	16.4	40.04	-0.001	6 507	0.100	10.001
Pilai	No	72	74.2	76	95	148	83.6	13.81	31 <0.001	6.597	2.188	19.891
Green	Yes	17	17.7	4	5 21 11.9	6.71	-0.001	4.000	1.016	10.705		
salad	No	79	82.3	76	95	76	88.1	6.71	<0.001	4.089	1.316	12.705

hia coli O157:H7 in the Department of Public Health Laboratories. All strains isolated from stool samples that were taken in first outbreak were identified as *S.enteridis*. In the second outbreak, 30

(77%) of 39 stool samples yielded *S. enteritidis. Salmonella* isolates were serotyped in the Department of Public Health Laboratories.

		Case		Control		Total					%95 cor Interva	
		Number	%	Number	%	Number	%	x 2	Р	OR	Min	Max
Meat with	Yes	13	25.4	38	41.7	51	35.9	3.76	0.053	0.477	0.224	1.015
pea	No	38	74.6	53	58.3	91	64.1					1.015
Pilaf with	Yes	15	20.8	57	62.6	72	50.7	14.40	14.43 <0.001	0.249	0.119	0.510
wheat	No	36	79.2	34	37.4	70	49.3	14.43				0.519
Diakla		10.3	16	11.6	0.26	0.550	1.070	0.40	2.050			
Pickle	No	44	56.3	78	89.7	122	88.4	0.36	0.36 0.550	1.379	0.48	3.958

TABLE 8: The distribution of patients according to the sort of meal in lunch menu on February 24, in private school.

Water supplies in youth hostels and private school were examined for possible source of outbreak. In addition, stool and other microbiological samples of 23 food-handlers were cultivated. Any pathogenic bacteria were not cultivated.

Food and environmental samples were cultivated at the Department of Agriculture Laboratories to measure the concentrations⁶ of isolated microorganisms from samples. *E.coli, S.aureus* and *Salmonella enteritidis* were isolated from chicken and potatoes samples. *B.cereus* and *S.aureus* were isolated from pilaf, *E.coli* and *S. enteritidis* was isolated from green salad.

DISCUSSION

Gastroenteritis is the most common infection of non-typhoidal Salmonellosis.1 The severity of the illness and onset duration of symptoms are related to the ingested amount of bacteria (infectious dose). Diarrhea is not usually severe and it proceeds to 3– 7 days. Diarrhea lasting more than 10 days or fever lasting more than 72 hours should suggest other diseases. In our study, all patients had mild symptoms in relation to both outbreaks. The onset time of symptoms was between six and 36 hours after eating contaminated meals in both outbreaks. It is consistent with incubation period of Salmonella gastroenteritis as reported from 6 to 48 hours. 7 Myalgia, headache, fever and diarrhea were the most common symptoms in both outbreaks.8-10 In our study, the median duration of diarrhea was four days (range: 1-8 days). Among these 59 pati-

TABLE 9: Logistic regression analysis of the probable causative food.

				95.0% C.	I.for OR
	Beta	р	OR	Lower	Upper
Pilaf (lunch)	-0.425	0.417	0.654	0.234	1.826
Green salad	1.539	<0.001	4.661	2.072	10.487
Meat braised in					
its own fat	2.203	<0.001	9.055	3.722	22.030
Fried egg	0.292	0.809	1.339	0.125	14.292
Pilaf (dinner)	20.560	0.999	1.540	0.129	12.123

ents, six (10%) remained hospitalized, acute renal failure was diagnosed in one patient and there was no exitus in the first outbreak. In second outbreak, the median duration of diarrhea was five days (range: 1-9 days). Hospitalization was reported for 48 (41%) of 117 patients and there was no exitus.

Salmonella enteridis is the most common food-borne species of Salmonellosis in humans and comprises approximately 80% of salmonellosis cases in Europe. In the first outbreak, all stool samples were positive for *S. enteritidis*. In the second outbreak, 30 (77%) of 39 stool samples were positive for *S. enteritidis*. No microorganisms were isolated from blood cultures in both outbreaks.

In this study, during the first outbreak *E.coli*, *S.aureus* and *Salmonella* enteritidis were isolated from chicken and potatoes samples. In the second outbreak, *B.cereus* and *S.aureus* were isolated from pilaf; *E.coli* and *S. enteritidis* were isolated from

green salad. Salmonella infections are associated with contaminated meals including animal foods and their products. ^{12, 13} In France, foodborne disease outbreaks recorded according to the frequencies of the agents between 1999 and 2000 and *Salmonella sp, S. aureus* and *B. cereus* was found the most responsible agents. ¹⁴ Kumar et al emphasized the importance of *B. cereus* and *S. aureus* as foodborne pathogens for food safety in terms of frequency and severity of the disease. ¹⁵

Certain groups including acutely ill or an asymptomatic food handlers can transmit *Salmonella* infection during preparation of foods. Thus, periodic education programs about foodborne infections and their transmission routes should be made and routine control procedures should be implemented where food products are made. In our study, in both outbreaks no pathogenic bacteria were isolated from 23 food handlers.

Health regulations in Turkey routinely prompt to examine food handlers before employment and while working for being vector of foodborne infections. In Turkey, The Ministry of Health prohibited patients to return to school/work until cultures were negative and symptoms were resolved because of the probability of contamination or transmission.

In our study, in the second outbreak, The Division of Public Health Laboratories and The Department of Agriculture Laboratory coordinated abruptly and worked together. This coordination made easy to identify the pathogens and help to control the outbreak quickly. This cooperation is important to prevent further outbreaks and develop food safety policy.

In the prevention of outbreaks, rapidly implement control measures must be taken rapidly and strictly and also public and media interest should

be sustained for early intervention. Improvements in the commercial catering trade are needed to realize further public health benefits. A specific surveillance system should be set for outbreaks. Once an outbreak has been recognized, it is necessary to determine the extent of the outbreak in terms of person, place and time. Identifying the source usually depends on study of large numbers of sporadic cases, for instance in an epidemiologic case-control study, or on identifying a group of infections among the relevant sporadic cases and investigating that cluster intensively. ⁴

This study emphasizes not only the potential effect of Salmonellosis but also the importance of cooperation of relevant states in the occurrence of outbreak. Control and prevention measures were not taken during the first outbreak by responsible authorities. This was the main cause of the occurrence of the second outbreak. Two meals which caused two outbreaks were made by the same catering company. During the first outbreak, health department did not warn catering company to take required measures so there was a short interval between the two outbreaks.

In conclusion, in order to prevent secondary cases, when a foodborne outbreak is suspected, control measures should be implemented as soon as possible. In each level of food process, all staffs must be alert and when outbreak is broken out; all staffs should be quickly coordinated to control the outbreak. This study also emphasized that neglectance and applications can cause outbreaks affecting a large population and can cause a big financial loss.

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