Yonsei Medical Journal
Vol. 44, No. 2, pp. 198 - 202, 2003

A Food-Borne Outbreak Caused by Salmonella Enteritidis

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This study was designed to define the epidemiology of a food-borne outbreak caused by Salmonella enteritidis that affected only one squadron of a military battalion located in the vicinity of the city of Edirne in Turkey.

The outbreak was analyzed by a standard surveillance form of the Centers for Disease Control and Prevention. The relationship between the eaten foods and cases was analyzed by Fisher's exact chi-square test, and odds ratios were calculated by a case-control study.

The outbreak affected 60 of 168 soldiers in the squadron, 16 of whom were hospitalized. S.enteritidis was cultured in stools from 13 of the hospitalized soldiers and from 3 soldiers who had prepared the food. All strains were completely susceptible to antibiotics; their plasmid profiles were also identical.

The highest attack rate detected was 55.7% in an omelet eaten 24 hours before ($p<0.001$). Furthermore, it was the riskiest food according to the case-control study (OR=7.88; 95% CI=3.68-16.89). The food samples were unobtainable because they had been discarded. All of the hospitalized cases recovered, and none of the control cultures of stools yielded the pathogen after three weeks.

In conclusion, although our results didn't indicate the exact source of the outbreak microbiologically, the omelet was considered to be the source based on the epidemiological proofs.

Key Words: Food-borne outbreak, salmonella enteritidis, outbreak surveillance, surveillance, outbreak

INTRODUCTION

Food-borne outbreaks are an important health problem and are prevalent in different parts of the world. In particular, mess halls and dining halls at schools, dormitories and military battalions are risky places. S. enteritidis is a common source of food-borne outbreaks. The eating of undercooked eggs or meals prepared with uncooked eggs increases the risk for food-borne diseases. The diagnosis of food-borne disease should be considered when an acute illness with gastrointestinal or neurologic manifestations affects two or more persons who have shared a meal during the previous 72 hours.[1] A lack of sufficient reporting is a key cause of new cases of food-borne disease because this insufficiency hinders prevention. Therefore, surveillance of food-borne diseases exposes the size and complication of the problem and is essential for identifying the required prevention strategies.

The aim of the study was to find the source of a food-borne outbreak at a military battalion. The source of the outbreak was investigated with both microbiological laboratory techniques and epidemiological research techniques.

MATERIALS AND METHODS

Definition of the outbreak: The outbreak occurred at a squadron of a military battalion located in the vicinity of the city of Edirne in Turkey. The military battalion contained 9 squadrons and 1500 soldiers. Because of the restoration of the kitchen, the battalion meals were cooked in temporary tents built for a certain period by a civilian cook and 37 soldiers, and were delivered in smaller pots to each squadron by the same soldiers. Each of the squadrons, consisting of 150 soldiers approximately, had their meals in differ-

Received June 20, 2002
Accepted October 19, 2002

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ent places with separate dinner services. Also each squadron had its own dormitory, toilet, bathroom and cafeteria. The outbreak was seen in only one squadron. A case was defined as a soldier with diarrhea accompanied by at least one of the following symptoms: fever, vomiting, nausea, or abdominal pain during the outbreak period.[2] The first cases applied to the doctors on Friday June 30, 2000, and the applications continued the next day. The data of the research were obtained by using the 'research form for food-borne outbreaks' designed by the Centers for Disease Control and Prevention (CDC).[3] For the epidemiological research of the outbreak, the meals that were eaten in the 72 hours prior to the first application were questioned in accordance with the incubation period of S.enteritidis. A total of 168 soldiers, who ate their meals in the same dining room, were interviewed according to the questions in the research form.

Microbiology

Stool samples were collected from the soldiers who cooked the meal and/or who were hospitalized, and cultured in Selenit F broth (Oxoid, UK), Salmonella-Shigella (SS) agar (Acumedia, USA) and Eosin Methylene Blue agar (Pronadisa, Spain). Isolates that gave appropriate, preliminary biochemical reactions were serogrouped with commercially available polyvalent antisera (Denka Seiken, Japan).[4]

Water samples taken from the squadron's toilet and cafeteria and egg samples taken from the firm that supplied the eggs for the battalion were also cultured. As a typing method the following antibiotics were tested: ampicillin, amoxicillin-clavulanate, cefazolin, cefuroxime, cefotaxime, cefoperazone, ceftriaxone, ceftazidime, aztreonam, imipenem, ofloxacin, gentamicin, amikacin, netilmicin, tobramycin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline. The antimicrobial susceptibilities of the strains were determined by using the disk diffusion technique according to the National Committee for Clinical Laboratory Standards (NCCLS),[5] and disks were obtained from Oxoid, UK. The alkaline-lysis method was applied for plasmid DNA isolation. Samples were analyzed by electrophoresis on agarose gel. The gel was stained with ethidium bromide and photographed under UV illumination.[6]

Statistical analyses

Statistical analyses of the collected data were done by using statistical software SPSS for Windows Ver. 8.0. The relationship between the kind of meal and disease was evaluated by chi-square and Fisher's exact tests. The odds ratios and 95% confidence intervals were calculated after constructing cross tables.

RESULTS

Sixteen of the outbreak cases who applied to the doctors were hospitalized. An additional 60 cases, who completed the research form in the squadron, were found by the survey on the fourth day of the outbreak. Table 1 presents the clinical complaints found in the cases. Abdominal pain seemed to be the most common symptom, followed by diarrhea and fever. Abdominal pain was the first symptom for an important ratio of the patients (Table 1).

Table 1. Symptoms Found During the Survey of Soldiers (N=168)

Symptoms	N	%
Fever	51	31.4
Nausea	48	28.6
Vomiting	39	23.2
Abdominal pain	67	39.9
Diarrhea	60	35.7

The meals eaten within 72 hours before the first case's application were determined from the menu and five that might have accounted for the outbreak were investigated: turkey roast, boiled turkey, turkey with potatoes, omelet and fried chicken. The attack rates were determined for each meal (Table 2).

When the meals suspected to be a potential source of the outbreak were investigated with a case-control study, the omelet was observed to have the highest risk (55.7%) (Table 2). According

Table 2. Special Attack Rates of Suspected Foods

Foods	Soldiers who ate the food			Soldiers who did not eat the food			p
	Cases	Others	Attack rate (%)	Cases	Others	Attack rate (%)	
Turkey Roast	35	47	42.6	25	61	29	0.077*
Boiled Turkey	39	45	46.4	26	58	30.9	0.260*
Turkey with potatoes	38	55	40.8	22	53	29.3	0.146*
Omelet	49	39	55.7	11	69	13.7	<0.001†
Fried Chicken	42	65	39.3	18	43	29.5	0.243†

*chi-square test.
†Fisher exact test.

Table 3. Odds Ratios and 95% Confidence Intervals of Suspected Foods

Food	Odds Ratios	95% Confidence Intervals
Turkey Roast	1.82	0.96 - 3.44
Boiled Turkey	1.52	0.80 - 2.86
Turkey with potatoes	1.66	0.87 - 3.18
Omelet	7.88	3.68 - 16.89
Fried Chicken	1.54	0.79 - 3.03

to the analysis of attack rates for the five meals, a significant relationship was found between the food poisoning and eating only omelet ($p<0.001$). The odds ratios found in the case control study are presented in Table 3. When the odds ratios and 95% confidence intervals were taken into consideration, the highest risk was found in the group who ate omelet (odds ratio=7.88; 95% CI= 3.68 -16.89). Other meals were found to be risk free and not responsible for the outbreak (Table 3). As the epidemiological studies were done on the fourth day of the outbreak, the meal samples couldn't be taken for culture. Stool cultures were done in 14 of the hospitalized cases and S. enteritidis was isolated in 13 of them. Their blood cultures were negative. In the stool cultures of the dining room staff, S. enteritidis was isolated in three staff responsible for meal delivery in the same squadron. One of the three had no complaints, while the other two had only abdominal pain. All of the hospitalized cases recovered and no pathogenic microorganisms were found in their control cultures three weeks later. All of the isolates were fully susceptible to all antibiotics studied, and they had three plasmids at weights of 80, 34 and 2.4 MDa. The firm that supplied the eggs for the squadron was also the principal egg-supplier to Edirne city with a weekly production of 2.5 million eggs. Salmonella spp. couldn't be isolated in cultures from 250 eggs chosen randomly. Furthermore, the cultures of the water samples were negative.

DISCUSSION

To investigate food-borne outbreaks, a description of the food causing the outbreak is required. The most important result of this study is that the possible source of an outbreak, which cannot be detected by microbiological methods, can be found by epidemiological methods. We used the attack rates for the meals eaten within 72 hours before the onset of symptoms in the first case, and the odds ratios and 95% confidence intervals of the meals were determined from the case-control study. As in this study, epidemiological and statistical techniques can be use alternatively, if it is impossible to find the source of the outbreak with laboratory techniques. It is recommended that determining attack rates is the first technique to be attempted for finding the source. Attack rates for each food can indicate the responsible source of the outbreak. However, for sufficient evidence, the responsible food should have a

significantly higher attack rate.[1] Outbreaks of S. enteritidis infection have been associated with the ingestion of uncooked or soft-boiled eggs, egg-containing food products, and inadequately cooked poultry.[7] In this study, two kinds of food were found by the questionnaire to have higher attack rates and were therefore thought to be responsible for the outbreak: the omelet eaten in the breakfast one day before the outbreak, and the boiled turkey eaten in the same day. To determine the relative roles of the two meals in the outbreak, a case-control study was designed and the omelet was found to be the only food responsible for the outbreak. The analysis showed that the omelet was the only meal to have an odds ratio with a confidence interval of greater than 1. To definitely prove the source of the outbreak, the omelet was cultured. In order to define the responsible food for such outbreaks, it was a rule in the battalions to store a certain amount from each meal in a refrigerator for 72 hours. However, the outbreak occurred on a Friday, we obtained permission from the army on the following Monday, and when the epidemiological study began on the Tuesday we were informed that the stored meals had been discarded by a uniformed soldier.

The contamination ratio of eggs with S. enteritidis may vary from 0.01% to more than 0.1%.[7] We could take only 250 eggs (0.01%) for culture, and as the egg production in the firm supplying eggs to the squadron was so high this rate might not have been sufficient. It might be concluded that the soldiers preparing the food were the source of the outbreak. S. enteritidis was isolated in stool cultures from three soldiers. Long-term carriage develops in 0.2 to 0.6% of patients with non-typhoidal salmonellosis.[7] Although these soldiers could be considered non-symptomatic carriers, their control cultures were negative three weeks later. Hence, it is more logical to conclude that they were themselves infected during the outbreak, rather than being carriers.

Traditional epidemiological methods, including antimicrobial susceptibility testing, serotyping and phage typing of isolates, and molecular methods, including plasmid analysis, pulsed-field gel electrophoresis and ribotyping, have been used to investigate S. enteritidis outbreaks.[8] As the same plasmid profile was obtained in the isolates in this study, we thought that this was a common-source outbreak. Their complete susceptibility to the antibiotics which were studied by disc diffusion tests also supported the common-source outbreak hypothesis. On the other hand, this result decreased the value of the antibiotic typing method.[8] S. enteritidis isolates have been found to be sensitive to all antibiotics in the same region before.[9] Therefore, resistance tests for the purpose of typing and following-up are not suitable.

Although our results didn't indicate the exact source of the outbreak microbiologically, the omelet was considered to be the source based on the epidemiological proofs. The incubation period of this outbreak varied from 24 to 63 hours, which is in accordance with the incubation period known for salmonella infections.[1] Furthermore, the outbreak occurred in summer months when salmonellosis is more prevalent.[1]

In the last 20 years, the number of infections related with S. enteritidis has increased in many countries.[7,8,10,11] S. enteritidis latently/secretly infects the ovaries of healthy-appearing hens and contaminates the eggs before the shells are formed. To prevent the recurrence of similar outbreaks, the control of poultry, the keeping of eggs in refrigerators and the consumption of only well cooked eggs are recommended.

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