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A Mexican restaurant-associated outbreak of *Salmonella* Enteritidis type 34 infections traced to a contaminated egg farm

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SUMMARY

In May 1996, the Georgia Division of Public Health was notified about a cluster of persons with *Salmonella* Enteritidis (SE) infections in Waycross, Georgia. A matched pair case-control study to determine risk factors for illness found a statistically significant association of SE infection with a history of having eaten at Restaurant A during the 5 days before onset of illness (relative risk = 13 [95% confidence interval (CI) = 3–62, $P < 0{\cdot}01$]). In a second case-control study, to determine specific food exposures, consumption of a deep-fried Mexican dish (chile relleno) (4 of 21 cases *vs.* 0 of 26 controls, odds ratio undefined, 95% CI $> 1{\cdot}46$, $P = 0{\cdot}034$) was found to be significantly associated with SE infection. An environmental investigation found evidence of suboptimal food storage and cooking temperatures at Restaurant A; cross contamination of foods may have contributed to the low attributable risk identified for chile rellenos. Five of 37 Restaurant A food and environment specimens yielded SE strains. All five positive specimens were from chiles rellenos. Of the seven outbreak-associated strains (six patient isolates and one food isolate from Restaurant A) for which phage typing was conducted, all were phage type 34. A FDA traceback investigation through Restaurant A's single-egg supplier identified the potential source as three interrelated farms in South Carolina. Environmental culture from one of these farms yielded SE phage type 34. As a result of this outbreak, FDA helped institute a statewide egg quality-assurance programme in South Carolina to minimize SE contamination of eggs.

INTRODUCTION

During 1980–90, *Salmonella* Enteritidis (SE) infections were recognized as an increasingly important public health problem in the United States and Europe [1]. In the United States, the proportion of all salmonella isolates that were SE increased from 5% in 1976 to 26% in 1994; consequently, SE was the most common serotype reported in the United States [2]. Consumption of raw or undercooked eggs was a major cause for this increased incidence [1–13]. In the United States, the phage types most commonly associated with human outbreaks of SE have been phage types 8, 13a, and 13 [6, 14]. However, SE phage type 4, the predominant phage type in Europe, caused infections among patrons of a Texas restaurant attributed to eating egg rolls prepared using pooled

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shell eggs [3] and has now been reported to be rapidly emerging in the western United States, particularly Southern California [4].

On 30 May 1996, the State Public Health Laboratory notified the Epidemiology and Prevention Branch of the Georgia Division of Public Health of the identification of 15 strains of SE from Waycross, Ware County. These specimens had been collected during 7–23 May. This was a dramatic increase compared to 1994–5, when Ware County reported only 42 salmonella strains of any serotype and no SE strains. Initial discussions with district health officials showed that they had begun to interview cases, but no specific single source was suspected.

The objectives of the investigation were to: (1) determine whether the SE cases in Ware County were epidemiologically related; (2) identify a common food source responsible for these SE infections; and (3) identify and institute public health preventive measures to control these SE infections.

METHODS

Epidemiologic methods

Case definition

For the purpose of our investigation, a case of SE infection was defined as a patient with onset of gastrointestinal illness after 5 May 1995, and isolation of SE from stool.

Case ascertainment

The infection control department of the major area hospital and local area physicians were contacted to search for unreported SE cases.

Initially, hypothesis-generating interviews were conducted, with in-depth exploration of the foods patients had ingested during the 5 days before illness onset. These interviews led to development of hypotheses about possible sources of infection, which were then tested through two case-control studies. In both case-control studies, questionnaires asked for demographic data, clinical features of the disease, and source of drinking water. All interviews were conducted by telephone.

Case-control study I

To test the hypothesis that Restaurant A was the source of infection, control subjects were selected by asking cases for a well co-worker or neighbourhood friend of the same sex and within 10 years of the same age. Each case and his/her matched control was administered a questionnaire asking if he/she had eaten food from Restaurant A in the 5 days before the case's onset of illness.

Case-control study II

To determine which food or food components in Restaurant A were associated with SE infection, an attempt was made to interview all identified case-patients and a convenience population of well meal companions identified by cases who served as the control group. Cases and controls were administered a questionnaire that asked in detail about the foods they ate at Restaurant A.

Microbiologic methods

Stool specimens from food workers, foods from multiple restaurants, and environmental specimens from Restaurant A were submitted to the Georgia State Public Health Laboratory for culture and testing for antimicrobial susceptibilities [15–17]. This laboratory also received all patient isolates of Salmonella for serotyping [18]. Environmental samples from the farm investigation were cultured by the FDA South East Regional Laboratory, Atlanta, Georgia. Phage typing was performed by the Foodborne and Diarrheal Diseases Laboratory, Centers for Disease Control and Prevention (CDC) [19].

Environmental investigation

Once Restaurant A was suspected to be a possible source of the infection, the investigators worked with restaurant management to determine food suppliers to Restaurant A, components of all menu items, and food storage and cooking practices. Several food samples from the suspect restaurant (and three other Waycross restaurants) and stools from all Restaurant A employees were obtained for culture at the Georgia State Public Health Laboratory.

Statistical methods

McNemar's χ^2 test was used in the matched analysis, and univariate odds ratios and exact 95% confidence intervals were calculated using Epi-Info, version 6 (CDC, Atlanta). The two-tailed Fisher's exact test was used to compare differences in categorical variables.

RESULTS

Descriptive epidemiology

Forty-four persons had culture-confirmed SE or untyped *Salmonella* sp. infections; all were white, 27 (61%) were female, and the mean age was 27 years (range: 1 month–81 years). Eight (36%) patients were hospitalized, and one patient underwent appendectomy for presumed appendicitis.

Analytic epidemiology

The cases for the first case-control study were the 29 persons with culture-confirmed SE infection known to investigators by 12 June. After completion of interviews of 16 case-control pairs, local health authorities asked that the telephone interviews cease at least temporarily until a rapid analysis was done. Because the results were conclusive, no additional interviews were subsequently completed. The study found a striking and highly statistically significant association between SE infection and a history of having eaten at Restaurant A during the 5 days before illness onset (relative risk = 13 [95% confidence interval (CI) = 3–62, $P < 0{\cdot}01$]) (Table 1). Limited information on restaurant exposures was secured through brief telephone interviews with additional SE-infected persons identified after 12 June. The dates of onset of illness for all 37 SE-infected persons who reported eating or not eating at Restaurant A during the 5 days before illness onset are shown in Figure 1; those who reported they were unsure were excluded.

For the second case-control study, 8 of the 29 persons with culture-confirmed SE infection known to investigators by 12 June were excluded because four did not recall eating at Restaurant A and four did not complete the questionnaire. Twenty-six well meal companions identified by 15 of the 21 cases served as the control group. To determine whether specific foods served at Restaurant A could be associated with SE infection in patrons, we examined consumption histories for 54 foods and found that eating chile relleno (4 of 21 cases *vs.* 0 of 26 controls, odds ratio (OR) = undefined, 95% CI $> 1{\cdot}46$, $P = 0{\cdot}034$) was significantly associated with SE infection. The four chile relleno-associated cases all had onset during a 3-day period, 31 May–2 June (Fig. 2). Because eating chile relleno accounted for only 4 (19%) of 21 cases, we looked further at specific food ingredients and found that consumption of any egg dish (5 of 21 cases *vs.* 0 of 26 controls, OR = undefined, 95% CI $> 1{\cdot}93$, $P = 0{\cdot}013$) and fried corn tortilla (11 of 21 cases *vs.* 6 of 26 controls, OR = 3·67, 95% CI = 1·07–2·49, $P = 0{\cdot}038$) were significantly associated with SE infection. The numbers were too small for meaningful stratified analyses.

Table 1. *Association between Restaurant A with* Salmonella *Enteritidis infection, matched-pair case-control study 1 (cases and co-worker/friend controls), Waycross, Georgia, 5 May–3 June, 1996*

	Control*		
	Ate at Restaurant A	Did not eat at Restaurant A	Total
Case			
Ate at Restaurant A	2	13	15
Did not eat at Restaurant A	1	0	1
Total	3	13	16

* Controls are co-workers or friends of cases asked if ate food from Restaurant A (dine in or take out) in 5 days preceding the case's date of onset.
Matched Relative Risk = 13 (95% CI, 3–62), $\chi^2 = 10{\cdot}3$, $P < 0{\cdot}01$.

Laboratory results

Five of 45 specimens from restaurant foods and environments (37 from Restaurant A) yielded SE strains. All 5 strains were from 3 samples of a single type of food, chile relleno (3 strains) or from a tray that had contained chiles rellenos (2 strains), and all 5 were from Restaurant A. In addition, SE was detected in stool from one asymptomatic food worker from Restaurant A. All SE strains from patients showed a similar antimicrobial susceptibility pattern (susceptible to all antimicrobials tested). Of the 7 outbreak-associated strains (6 patient isolates and 1 food isolate from Restaurant A) that underwent phage typing at CDC, all were an unusual phage type (Type 34).

Environmental investigation

Investigation of food-handling practices at Restaurant A revealed several problems. In particular, the restaurant's cool room was too warm, and large amounts of precooked meat and other foods were stored in deep plastic containers without labels identifying the dates of preparation or storage. A survey on 7 June found the temperatures of food stored in these containers to be too warm; on the

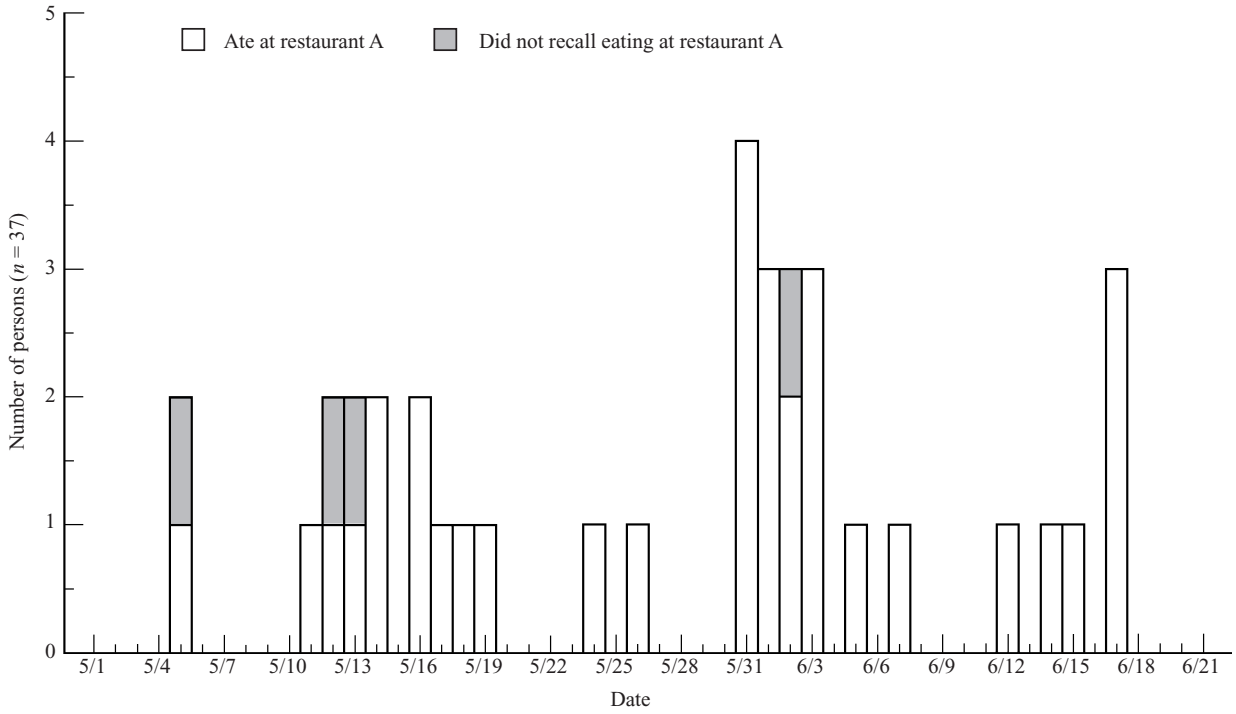

Fig. 1. *Salmonella* Enteritidis infections in persons whose history of eating or not eating at Restaurant A is known, by date of onset of illness, Waycross, Georgia, 1 May–21 June, 1996.

advice of county environmentalists, the contents of these containers and any foods in the restaurant prepared from them were discarded. Our interviews with restaurant employees found that they lacked knowledge of safe food-handling practices and, in particular, lack of awareness of the need to adequately chill precooked food and to rotate stock. In addition, employees were observed eating Restaurant A food in the restaurant's food-preparation areas.

Chiles rellenos were reportedly prepared in Restaurant A's kitchen twice a week. Their preparation was not observed, but the process was described by the manager through an interpreter. Approximately 44 shell eggs were broken into an industrial blender and mixed with flour. The egg-flour mixture was then poured over a tray of green peppers pre-stuffed with cooked ground beef. These were then removed from the tray with bare fingers and briefly deep fried in multiples of 10–15. After cooking, they were placed on a plastic tray, wrapped with clear plastic wrap, and placed in the freezer compartment of a refrigerator in the restaurant kitchen. As needed, a tray of frozen chiles rellenos was thawed above the range in the kitchen and then stored in the cool room. Approximately six thawed chiles rellenos were kept on a separate tray near the food-preparation area in the kitchen for ready access. When ordered, a thawed chile was removed from this tray and cooked in a gas oven for approximately 4 min or in a microwave for approximately 1 min. The three positive food cultures were obtained from a frozen chile relleno, a thawed chile relleno, and a ready-to-serve cooked chile relleno (internal temperature 129 °F). Interviews with employees revealed that on occasion, fully thawed chiles rellenos remaining at closing time would be refrozen.

Restaurant A's manager reported that shell eggs were used in only three menu food items – chiles rellenos, huevos rancheros, and flan (egg custard); in addition, chopped boiled eggs were available at the self-serve buffet. All the restaurant's eggs came from a single supplier with headquarters in South Carolina. A review of the restaurant's invoices found that it had received monthly shipments of 30 dozen eggs on 22 April and 20 May 1996.

The corn tortillas were purchased ready-made by the restaurant.

Egg traceback investigation

The 22 April egg shipment to Restaurant A was traced back to three interrelated South Carolina farms. Culture surveys were performed on all three farms. A gooey mass, accumulated at the end of an egg

conveyor belt in one of the two chicken houses on one farm, yielded SE of the same rare phage type (Type 34) as that found in the patients. Study of egg packaging and distribution records showed that eggs from the positive chicken house could have been delivered to Restaurant A on 22 April. On the basis of this investigation, all eggs from the implicated chicken house were then diverted for pasteurization until the current flock ceased producing eggs.

DISCUSSION

During the 1980s, SE infections became increasingly common in the northeastern United States, and as a result, this became the most common salmonella serotype in that area [5, 6]. Subsequently, the SE epidemic spread into the Middle Atlantic states and then moved West [11]. However, SE infection had been rare in the southeastern states. The high rates of SE were initially a mystery, but were ultimately found to be caused by entry of SE from infected chickens into eggs while the eggs were being formed, with subsequent dissemination to humans in intact shell eggs [5, 20]. During 1985–9, shell eggs accounted for 78% of the SE outbreaks in which a food vehicle for transmission was identified [14].

The proportion of eggs infected with SE, even eggs from implicated flocks of chickens, has always been small (an estimated 0·01%) [11]; therefore, the risk for infection from eating a single undercooked or raw egg has always been small. A major source of outbreaks has been the practice of pooling shell eggs, especially with subsequent temperature abuse promoting bacterial multiplication, which allows a single contaminated egg to contaminate many servings of food. Many states now require that pasteurized liquid eggs (available in cartons, like milk) be used instead of pooled shell eggs for scramble eggs, French toast, and similar foods.

One limitation of our investigation was the use of controls identified by cases in the first case-control study. However, the main problem with such controls is that they are likely to have the same exposures as cases, and we found salmonellosis to be associated with eating at the restaurant despite this potential bias. The second case-control study, which, like Boyce and colleagues [3], used well meal companions identified by patients as controls, had the same potential bias, but despite that we found a significant relationship between salmonellosis and eating chiles rellenos.

Our investigation was consistent with introduction of SE into Restaurant A in one or more shell eggs. Restaurant A used the same lot of shell eggs for at least 1 month and pooled shell eggs. The restaurant had numerous problems with failure to refrigerate foods properly and at adequate temperatures. This time and temperature abuse could have allowed multiplication of salmonellae. The one food implicated both statistically and by all five positive food and environmental cultures was chiles rellenos, which was prepared from pooled shell eggs. Although we found a significant association between disease and eating chiles rellenos, the chiles accounted for only four of the cases, and all four occurred in a tight cluster as part of the second peak in the epidemic curve (Fig. 2). This may reflect extensive cross contamination of multiple foods by raw egg batter during preparation of chiles rellenos in early May, followed by a second wave of cases almost a month later when contaminated chiles were thawed and served (Fig. 1). The foods eaten by persons with onset after 3 June were not determined because of the termination of the second case-control study, so we do not know if the 4–17 June cases ate chiles rellenos or foods that may have been cross-contaminated.

Although Restaurant A management reported that all the chiles rellenos were used within a few days after preparation, a photograph of the freezer showed heavy build-up of frost on the bottom trays of chiles, implying that they had been frozen for weeks. With erratic rotation of the prepared chiles, the contaminated batch could have been stored for a month, or served sporadically over a period of $\geqslant 1$ month. Further opportunity was available for multiplication of SE because trays of chiles were thawed near the range, held at ambient temperature ready for reheating, or kept in the insufficiently cool refrigerated room. In addition, opportunity existed for mixing contaminated with uncontaminated chiles because unused chiles were refrozen at the end of the day.

Further dissemination of SE within the restaurant may have resulted from food workers becoming infected by eating SE-contaminated Restaurant A food and then failing to wash their hands adequately after defecation: restaurant workers were observed eating Restaurant A food, and one of the food workers had SE isolated from his stool. Another factor contributing to persistence of SE in the restaurant may have been inadequate cleaning of the mixer, which could contaminate subsequent batches of egg batter and any other foods (e.g. cheese dip)

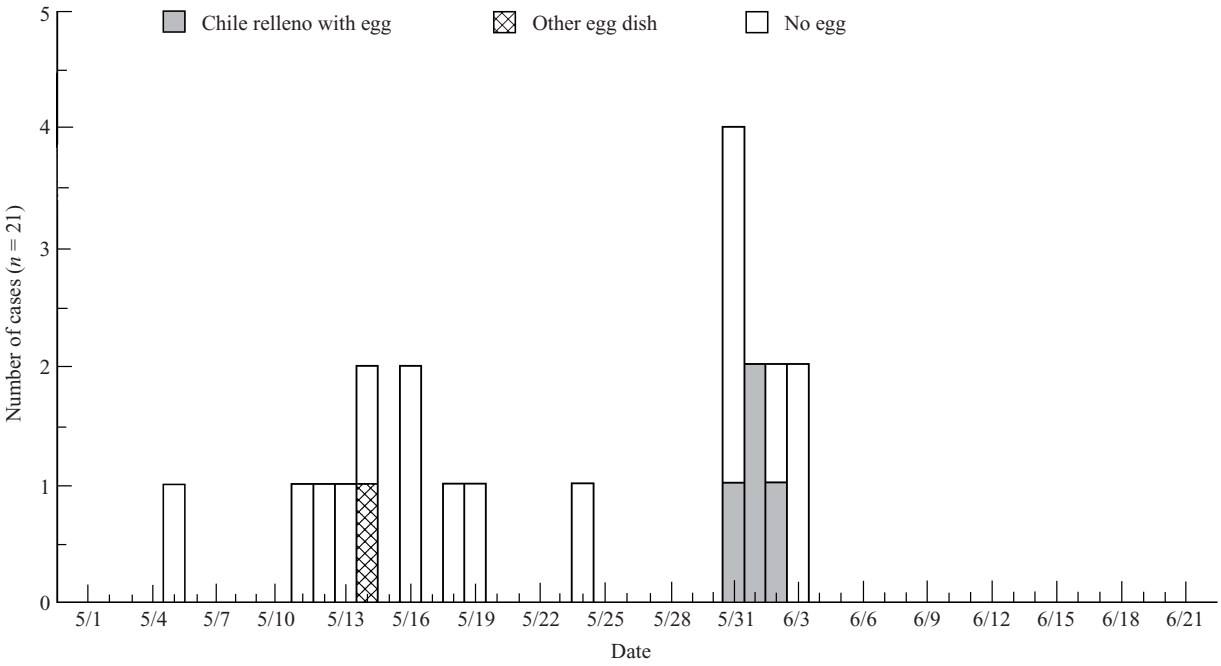

Fig. 2. *Salmonella* Enteritidis cases included in study of specific foods eaten at Restaurant A, by date of onset of illness, Waycross, Georgia, 1 May–21 June 1996.

prepared in the mixer. The staff were not aware that the plug in the mixer's lid should be removed for proper cleaning.

The deficiencies found were discussed with restaurant management, which was highly cooperative throughout the investigation. Vigorous efforts were made to improve sanitation while the restaurant continued to operate. However, when cases of SE infection continued to occur in early June in persons who had eaten at Restaurant A, the restaurant was voluntarily closed to address the deficiencies and interrupt the chain of transmission. The planned corrective measures included: (1) repairing and/or improving the air handling system in the cool room to enable maintaining an appropriate refrigeration air temperature; (2) using shallow food storage pans to allow rapid chilling of precooked foods; and (3) requiring all Restaurant A employees to attend a course on proper food-handling practices (e.g. labelling containers of prepared food with the date of preparation and rotating stock) taught by county environmentalists. No additional cases have been reported.

SE of phage type 34 is unusual and has been previously linked to shell eggs. In their review of 42 outbreaks in the United States, studied during 1988–9, traced to various sources, Hickman-Brenner and colleagues, identified only one due to phage type 34, and this outbreak was egg related [19]. In a small, multiple phage type (phage types 8 and 34), egg-associated SE outbreak in Eatonton, New Jersey, in September 1990, the authors hypothesized that phage type 34 may have been unrelated to the outbreak or may have reflected polyclonal infection in the suspect flock [14]. In addition, phage type 34 has recently emerged as a dominant phage type in Japan; however, no link has been established between Japan and the two earlier phage type 34 outbreaks in the United States [21]. To our knowledge, the Waycross, Georgia, outbreak is the first phage type 34 SE outbreak in the United States traced to an infected flock.

The evidence that egg-associated SE outbreaks have resulted from eggs contaminated on the farm is compelling. For example, in a study of 18 SE outbreaks during 1990–1 that were epidemiologically associated with shell eggs from identified egg farms, the predominant phage type associated with the human outbreak was recovered from the environment of all the farms and from the internal organs of chickens in 88% of implicated flocks tested [14]. In 1990, the US Department of Agriculture began an SE control programme based on tracing eggs implicated in human outbreaks to source egg farms, testing the farm environment and the internal organs of chickens on those farms, and diverting the eggs to pasteurization if cultures yielded SE [22]. As a result

of the Waycross, Georgia, outbreak, FDA helped institute a statewide egg quality-assurance programme in South Carolina to minimize SE contamination of eggs. Such programmes include routine bacteriologic sampling, disinfection of houses between flocks, rodent control, and fly control. Although egg-associated outbreaks can theoretically be prevented by safe food-preparation practices [23, 24] alone, universal safe practices cannot be guaranteed. Thus, reduction of SE in eggs is important to protect the consumer.

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