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The pandemic of *Salmonella* Enteritidis phage type 4 reaches Utah: a complex investigation confirms the need for continuing rigorous control measures

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SUMMARY

In 1995, *Salmonella* Enteritidis (SE) cases in the state of Utah increased fivefold. Isolates were identified as phage type 4 (PT4). Risk factors and sources of infection were investigated in two case-control studies, a traceback of implicated foods, and environmental testing. Forty-three patients with sporadic infections and 86 controls were included in a case-control study of risk factors for infection. A follow-up case-control study of 25 case and 19 control restaurants patronized by case and control patients examined risks associated with restaurant practices. In the first case-control study, restaurant dining was associated with illness ($P = 0{\cdot}002$). In the follow-up case-control study, case restaurants were likelier to use > 2000 eggs per week ($P < 0{\cdot}02$), to pool eggs ($P < 0{\cdot}05$), and to use eggs from cooperative 'A' ($P < 0{\cdot}009$). Eggs implicated in separately investigated SE PT4 outbreaks were traced to cooperative 'A', and SE PT4 was cultured from one of the cooperative's five local farms. We conclude that SE PT4 transmitted by infected eggs from a single farm caused a fivefold increase in human infections in Utah.

INTRODUCTION

Salmonella species cause an estimated 2 million infections annually in the United States. Between 1976 and 1996 the rate of infection with one serotype, *Salmonella* Enteritidis (SE), increased from 0·6/100000 to 3·6/100000 [1, 2] and during this time the proportion of all salmonella isolates that were SE increased from 9·9% in 1985 to 26·1% in 1994, making SE the most common serotype in this country [3, 4]. Most SE outbreaks are due to the consumption of raw or under-cooked shell eggs [5, 6]. Between 1985 and 1998, 82% of outbreaks with a known vehicle of transmission were associated with eating eggs [1]. Implementation of control measures in the 1990s may have led to a drop in SE incidence between 1996 and 1998, although outbreaks in the western United States have not decreased [1].

Worldwide, the incidence of SE also increased dramatically [8], driven largely by the emergence of a single phage type (PT), SE PT4. During the 1980s, this PT replaced previously dominant SE PTs in Europe, Russia, and Mexico; its appearance has been followed

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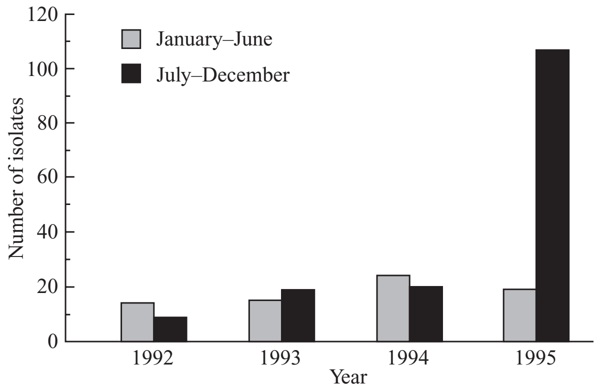

Fig. 1. *Salmonella* Enteritidis cases in Utah, 1992–5.

by increases of five- to tenfold in the incidence of human SE infection [9–11]. Before 1993 this PT was rare in the United States, except for cases associated with travel [6, 7]. The first recognized outbreak of SE PT4 associated with a domestic source in the United States occurred in a restaurant in El Paso, Texas, in 1993. Egg rolls made with pooled egg batter were implicated [12]. In 1994 SE PT4 emerged in Los Angeles County, California, became the dominant SE subtype and caused a fivefold increase in SE incidence within 4 months. Consumption of undercooked eggs was a risk factor for illness [13]. These events raised concerns that SE PT4 could spread to other regions of the United States.

Routine surveillance data showed that between July and December 1995, 102 SE cases occurred in Utah, a fivefold increase over the average for these months during the preceding 5 years (Fig. 1). No changes in surveillance or culture practices were noted by the state health department, and no changes in the incidence of other salmonella serotypes occurred. We subtyped SE isolates to determine if PT4 had reached Utah, and investigated risks for infection.

METHODS

Case finding and case definitions

We attempted to contact by telephone every person in Utah with a stool culture that yielded SE between 1 February and 19 March 1996. We identified these persons from reports from the state public health laboratory and by contacting daily all clinical laboratories culturing stools for salmonella in Utah. A case was defined as a diarrhoeal illness in a resident of Utah, reported between 2 November 1995 and 19 February 1996 with a stool culture that yielded SE.

Case-control study 1: Risk factor for human SE infection in Utah

To determine risk factors for infection with SE PT4 in Utah, we conducted a patient case-control study, using all available cases of SE identified in Utah during this period. Only the first case in each household or the first case in a known common-source outbreak was included in the study. Two controls per patient were recruited. A control was defined as a person matched by age and sex to a case-patient and identified by random-digit dialling within the patient's telephone exchange. Between 12 and 26 February we used a standardized questionnaire to ask case-patients and controls about food intake and food handling during the 3 days preceding the date of the patient's illness. For comparison, we asked the same questions of case-patients and controls regarding the most recent equivalent 3 weekdays preceding the interview.

Case-control 2: Risk factors for SE infection in restaurants

After the patient case-control study implicated eating at restaurants as a risk factor for SE infection, we conducted a follow-up case-control study of restaurants in Salt Lake County. We sought to define characteristics, practices, and food sources of establishments where patients had eaten, and we chose Salt Lake County because most restaurants patronized by patients in the case-control study were there, and because of the availability of a detailed computerized database of inspections from the county's Bureau of Food Protection.

A case restaurant was defined as a food establishment in Salt Lake County patronized by an SE case patient from the case-control study during the 3 days before illness, and a control restaurant was defined as a Salt Lake County food establishment patronized by a matched control from the patient case-control study during the 3 days preceding the matched patient's illness. One restaurant was patronized by both a case and a control, and it was excluded from analysis. Only restaurants patronized by patients or controls who had eaten at two or fewer restaurants during the patient case-control study were included.

Case and control restaurants were compared according to (1) type of restaurant, size, employee/seat ratio, number of meals served, and any history of a

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foodborne outbreak during the period the restaurant has been in existence, variables all of which were available in the city-county health department's database; (2) the three most recent inspection scores given by city-county inspectors, typically occurring in the previous 3 years; and (3) foods served and suppliers of foods such as poultry, beef, and eggs, as determined by a telephone questionnaire administered to restaurant employees.

Traceback of eggs

A traceback of shell eggs used by case and control restaurants during the 3 days before the case-patient's illness was undertaken to determine if the farms supplying case restaurants differed from those supplying control restaurants. Invoices from the suppliers named by restaurant personnel as their source of eggs used during this period, as well as invoices from intermediate distributors and egg cooperatives, were used to determine as nearly as possible the farm of origin of each restaurant's eggs. If exact dates of shipments were uncertain, we traced all egg shipments to the restaurant during the 2 weeks preceding the date of the case-patient's meal.

Human isolate identification

Salmonella isolates from hospital laboratories in Utah were forwarded as isolated colonies to the Utah public health department laboratory for serotyping and confirmation. Isolates were grown on triple sugar iron agar (TSI), lysine iron agar (LIA), citrate, and urea motility agars. Those identified as salmonella were grouped; group D isolates were serotyped with Difco reagents (Detroit, MI) as H antigen g and m.

Phage typing

Randomly selected SE isolates from Utah patients from November–December 1995, and representative human isolates from Utah from each month between March 1995 and February 1996 were phage-typed by the CDC Foodborne Diseases Laboratory Section, using the system described by Ward and colleagues [14] and the methods described by Hickman-Brenner and colleagues [15]. These methods were used to phage type isolates from poultry farms and eggs, as described below.

Farm inspection and testing

Egg farms belonging to Cooperative A were inspected twice. First, an inspection of the five Cooperative A egg farms was conducted by the Utah State Department of Agriculture and Foods in March 1996, in which 1–2 swabs were used to culture each poultry house. In May 1997, an outbreak of SE from shell eggs in a restaurant in another state resulted in a traceback implicating Cooperative A, which led to a second inspection of the five Cooperative A farms. The US Food and Drug Administration (FDA), used the US Department of Agriculture (USDA) protocol for SE investigations to test the implicated farms. In each poultry house of the implicated farms, 10 moistened gauze swabs were dragged through chicken manure pits the length of the house and back again. Ten swabs were taken of dried egg whites and yolk accumulated on egg conveyer belts. Specimens were placed in tetrathionate-brilliant green selective enrichment broth and cultured on XLT4 agar plates and brilliant green novobiocin agar plates for salmonella [16]; group D isolates were serotyped at the FDA laboratory in Minneapolis, MN. On farms where SE was recovered, 1000 randomly selected eggs from each hen house were cultured for salmonella and group D isolates were serotyped by Silliker Laboratories in Chicago, IL. All SE isolates from farms and eggs were phage-typed at CDC as described above.

Statistical analysis

Odds ratios with exact 95% confidence intervals are reported as measures of association for categorical data. For undefined odds ratios, the Fisher exact 2-tail test was used. For continuous variables, the Kruskal–Wallis test was used as the measure of association.

RESULTS

Patient case-control study

Eighty-nine cases of SE were identified in Utah between 2 November 1995 and 19 February 1996. Of these, 43 (48%) were enrolled in the patient case-control study. Forty-six individuals with culture-confirmed SE infection were not included in the study; 26 (57%) were non-index cases in point-source outbreaks or another infection in the same household, 5 (11%) refused to participate, 4 (9%) could not

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Table 1. *Risk factors for SE infection in case-control study. Proportion of cases and controls reporting exposures during the 3 days preceding illness in case-patients*

Risk factor	Case no. exposed/total	(%)	Control no. exposed/total	(%)	Matched OR	95% CI
Eating at restaurant	38/42	(91)	48/77	(62)	5·7	1·7–21·4
Dinner at restaurant	19/43	(44)	17/86	(20)	3·2	1·3–7·8
Eating cookie dough containing raw egg	0/43	(0)	11/81	(14)	0	0·0–0·83
Cracking an egg	7/39	(18)	36/78	(46)	0·3	0·09–0·7
Nibble while cooking	4/41	(10)	26/81	(32)	0·2	0·06–0·8
Eat green salad	1/38	(55)	60/78	(77)	0·4	0·2–0·9
Eat any dish with egg	24/37	(65)	49/74	(66)	0·9	0·4–2·4
Eat any dish with runny or raw eggs	11/35	(31)	19/68	(28)	1·2	0·4–3·1

Table 2. *Comparison of restaurants based on inspection data gathered by Salt Lake City-County Health Department*

Characteristic	Case ($n = 25$)	Control ($n = 19$)	OR	95% CI	P
Number (%) with history of foodborne outbreak	4 (16)	4 (21)	0·71	0·12–4·2	—
Number (%) sit-down restaurants	15 (60)	12 (63)	0·88	0·21–3·6	—
Number (%) fast food restaurants	6 (24)	5 (26)	0·88	0·18–4·3	—
Mean (S.D.) of last three inspection scores	87·8 (4·9)	85·1 (7·7)	—	—	0·49
Mean (S.D.) last inspection score	85·2 (6·4)	84·1 (6·2)	—	—	0·7
Mean (S.D.) number of employees	18·3 (13)	35 (36)	—	—	0·084
Mean (S.D.) number of seats	103·5 (83)	162 (130)	—	—	0·19
Mean (S.D.) employees/seat	0·18 (0·19)	0·22 (0·21)	—	—	0·77

identify the date of disease onset, 4 (9%) had been interviewed in depth before the study, and 2 (4%) each were under 2 years of age, out of state during the 3 days preceding illness, or could not be reached. Controls could not be found for one patient.

The mean age of case-patients was 35·6 years, 21 (54%) were male, and 38 (97%) were white. Controls were of similar age, sex, and race distribution. Thirty-seven (86%) had fever, 35 (81%) cramps, and 34 (79%) had nausea. Thirty-three (79%) were treated with antimicrobials, and 5 (12%) were hospitalized. The median duration of diarrhoea was 7 days (range, 2–45 days), and the median maximum number of bowel movements in a 24-h period was 20 (range, 4–90). Recall was considerably aided by requesting interviewees to consult daily journals, which are kept as a practice among adherents of the Church of the Latter Day Saints, to which most case-patients and controls belonged.

Thirty-eight (91%) of 42 patients, compared with 48 (62%) of 77 controls ate at a restaurant during the incubation period [matched odds ratio (mOR) = 5·7, 95% CI, 1·7–21·4] (Table 1). The number of restaurants visited during the incubation period was not significantly higher for patients ($2{\cdot}2 \pm 1{\cdot}8$) compared with controls ($2{\cdot}0 \pm 1{\cdot}8$, $P > 0{\cdot}4$). Dinner was the only meal significantly associated with illness (19 of 43 case-patients *vs.* 17 of 86 controls, mOR = 3·2, 95% CI, 1·3–7·8) (Table 1). No significant positive association was found between a specific food item or class of items and illness, including any egg item and any undercooked or raw egg item. Food preparation activities at home, such as nibbling while eating, cracking an egg, and eating cookie dough were protective. Neither eating at a restaurant nor food-preparation activities during the comparison period of the 3 weekdays preceding the interview were significantly associated with illness.

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Table 3. *Risk factors associated with case restaurants in restaurant risk factor study (categorical variables)*

(A) Continuous variables Risk factor	Case no. exposed/total	(%)	Control no. exposed/total	(%)	OR	95% CI
Use any shell eggs	20/25	(80)	13/19	(68)	1·9	0·38–9·2
Use > 2000 eggs/week	7/25	(28)	0/19	(0)	Undef., $P = 0{\cdot}014$	
Pool > 3 eggs	14/25	(56)	4/19	(21)	4·8	1·0–23·8
Serve raw eggs	2/23	(9)	1/19	(5)	1·7	0·10–53·2
Serve hamburger	19/25	(76)	13/19	(68)	1·5	0·31–6·9
Use any ground beef	23/25	(92)	15/19	(79)	3·1	0·39–28·5
Add eggs to ground beef items	6/24	(24)	2/19	(11)	2·8	0·41–24·1
Use any non-ground beef	23/25	(92)	14/19	(74)	4·1	0·57–36·5

(B) Categorical variables Risk factor	Mean (S.D.) ($n = 25$)	Median (range) ($n = 19$)	P
Eggs used per week			
Case	1631·6 (2216·6)	360·0 (0–8100)	
Control	493·9 (675·9)	99·0 (0–1980)	0·11
Maximum number of eggs pooled per week			
Case	28·0 (71·0)	10·0 (0–360)	
Control	19·8 (55·6)	1·0 (0–240)	0·13
Meals served per week			
Case	574·8 (534·7)	321 (100–2000)	
Control	438·7 (375·6)	275·0 (35–1286)	0·45

Restaurant case-control study

Because eating at a restaurant was associated with illness in the initial case-control study, we compared food-handling practices and sources of foods in restaurants patronized by case-patients and their matched controls. Of 118 restaurants patronized by patients and controls from the first case-control study during the 3-day incubation period, 69 were located in Salt Lake County. Of these, 20 (29%) were excluded because they were patronized by an individual who ate at ⩾ 3 restaurants during the incubation period; 3 (4%) were excluded because the restaurant could not be contacted; 1 restaurant (2%) was excluded because it was patronized by both a patient and a control; and 1 (2%) restaurant owner refused to participate. Managers or owners of 25 case restaurants and 19 control restaurants studied were interviewed by telephone on 13 and 14 March 1996. Of these, 10 case and 12 control restaurants were patronized by patients or controls who had patronized only 1 restaurant in the 3 days of interest; the remainder were patronized by patients or controls who ate at 2 restaurants during the incubation period.

Review of the city-county database of licensed restaurants showed that case and control restaurants did not differ with respect to a history of outbreaks, type of restaurant, inspection scores, number of employees, number of seats, or employee-to-seat ratio (Table 2). Excluded restaurants did not differ significantly from those included.

Case restaurants were significantly more likely to use large numbers of eggs per week. Seven (28%) of 25 case restaurants used ⩾ 2000 eggs per week, compared with none of 19 control restaurants (OR undefinable, lower 95% CI, 1·5, $P < 0{\cdot}014$). Case restaurants were significantly more likely to pool eggs (i.e. mix raw eggs together, enhancing the risk of infection from a single contaminated egg). Fourteen (56%) of 25 case restaurants, but only 4 (21%) of 19 control restaurants pooled ⩾ 3 eggs (OR = 4·8, 95% CI, 1·01–23·8, $P = 0{\cdot}043$). No other practices, such as serving hamburgers or eggs *per se*, were associated with being a case restaurant (Table 3), and case and control restaurants did not differ by suppliers of poultry and beef.

Phage typing of isolates

Nine randomly selected Utah isolates of SE from November and December 1995 were all PT4. Two SE isolates were then randomly selected from each month between March 1995 and February 1996 and phage-typed. In March and April 1995, 1/2 isolates each

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month were PT4. In May, June and July 1995 0/2 isolates each month were PT4. During August 1995–February 1996, 2/2 isolates each month were PT4.

Traceback of eggs used by case and control restaurants

Because practices associated with the use of eggs differed between case and control restaurants, we traced the sources of eggs used by the restaurants. Of 44 restaurants enrolled in the case-control study, 33 (75%) used shell eggs. Eight (6 case and 2 control) restaurants used eggs from several sources during the incubation period of the patient who patronized the restaurant, while 25 (14 case and 11 control) restaurants used eggs exclusively from one source. For all restaurants, including those using eggs from multiple sources, case restaurants were significantly more likely to use eggs from Egg Cooperative A during the incubation period. Nineteen (95%) of 20 case restaurants used eggs from Cooperative A, compared with 8 (62%) of 13 control restaurants (OR = 11·8, 95% CI, 1·01–590, $P < 0{\cdot}025$). When analysis was limited to the 25 restaurants that obtained eggs from only 1 source, all 14 case restaurants used exclusively Cooperative A eggs, compared with 6 (55%) of 11 control restaurants (OR = undef., $P < 0{\cdot}009$).

Traceback of eggs implicated in an eggnog-related cluster of SE cases

On 17 December 1995, three members of a family in Utah became ill with a febrile diarrhoeal illness; a stool culture from one patient yielded SE PT4. Interviews revealed that the only food exposure shared by the three was home-made eggnog consumed on 16 December 1995. The eggnog was made of cream and 6–8 raw eggs purchased during the preceding week at a local supermarket. Supermarket invoices showed that all shell eggs sold during the period 1–15 December 1995 were supplied by Egg Cooperative A.

Cooperative A: source of implicated eggs

Cooperative A consists of five egg farms located in Utah (farms 1, 2, 3, 4, 5): Additionally, the co-operative receives weekly egg shipments from a farm in Colorado and intermittent shipments from suppliers in California. Between 2 November 1995 and 19 February 1996, the period examined in the two case-control studies described, Egg Cooperative A received four shipments from Farm Y in California, in January, and three shipments from Farm Z in California, in February. Because many cases occurred in Utah during November and December 1995, when Cooperative A obtained no eggs from California, egg shipments from California do not explain the increase in SE. Because the cooperative received egg shipments almost daily from the five Utah farms and weekly from the Colorado farm, traceback alone could not implicate a specific farm or farms.

Investigation of Cooperative A farms

All cultures from the environmental inspection of the five Cooperative A farms carried out by the Utah State Department of Agriculture and Foods in March 1996 were negative. The extensive inspection of Cooperative A egg farms by the FDA in June 1997 yielded the following results: All environmental cultures from farms 1, 2 and 3 were negative. Twenty-five environmental cultures from farm 4 and 1 environmental culture from farm 5 grew SE. Of the 25 environmental isolates from farm 4, 3 isolates from 2 of 3 hen houses were SE PT4, 12 isolates from 3 hen houses were SE PT7, 2 isolates from 2 hen houses were rough and could not be phage typed, and 1 was reactive but did not conform to a known PT. The isolate from farm 5 was PT8. One thousand eggs from each hen house on farm 4 were pooled in 50 batches of 20 eggs each and cultured for salmonella. One of 50 pooled batches of eggs collected at each of 2 hen houses yielded SE PT4, and 3 of 50 pooled batches of eggs from the third hen house yielded SE. All were PT4. Farm 4 had numerous hygienic infractions. Old and new layer flocks were housed together, allowing transfer of infection to incoming flocks from aged ones. Farm 4 eggs were diverted to an egg pasteurization plant on 1 July 1997, and the positive farm 4 flock was depopulated. Subsequently, farm 4 discontinued egg production.

DISCUSSION

In Europe, Russia and Mexico, the introduction of SE PT4 was followed by dramatic increases in human infections. Our data suggest that SE PT4, transmitted in part through restaurants through shell eggs from a single farm, caused a fivefold increase in human SE

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infections in Utah within 6 months. The epidemic of SE infections in Utah resembled the one in southern California in 1994, where SE PT4 also caused a sustained fivefold increase in SE infections within a few months [13]. Despite recent declines in the overall incidence of SE in the United States, including the western United States, the number of SE outbreaks in the western United States has not decreased, and therefore the strain's potential to produce widespread illness continues to be an important public health concern [1].

The study of sporadic SE infections showed that illness was associated with eating in restaurants. The study of restaurants in Salt Lake County showed that restaurants patronized by case-patients with sporadic SE were significantly more likely than those patronized by controls to use more than 2000 eggs per week and to pool more eggs. The use of $\geqslant 2000$ eggs per week by a restaurant was associated with being a case restaurant, probably because of the increased likelihood of encountering an infected egg. The risk of egg pooling has been documented in previous investigations [4–6, 17, 18]. This evidence of amplification suggests that restaurants are an important control point for SE control. Food codes and foodhandler training should prohibit the pooling of eggs and emphasize the use of pasteurized egg products [20]. Hospitals and nursing homes should use exclusively pasteurized egg products. Consumers need to be educated to cook eggs completely at home, and to insist they be cooked well in restaurants.

Results of the epidemiological studies and microbiological testing of implicated farms suggest that the increase in SE PT4 in Utah was due to the appearance of this strain in flocks of one farm, and their widespread distribution through the distributor, Cooperative A. Investigations, such as ours, confirm the need for such farm safety measures as isolating hens from the environment, maintaining a clean food and water supply, controlling rodents, as well as for regular microbiological monitoring to assess the effectiveness of these practices. A quality assurance programme incorporating these practices, 'the Pennsylvania quality assurance program', may account in part for a reduction in SE cases in the northeastern United States [19], and similar programmes in 12 other states may underlie the reported national decrease in SE incidence in 1996–8 [1]. The President's Council on Food Safety's Egg Safety Action Plan aims to further reduce egg-associated SE infections by 50% by 2005. The plan includes expanding surveillance for human and poultry SE infection, accelerated outbreak investigation and tracebacks and public education [21].

In conclusion, we describe a fivefold increase in the incidence of SE in Utah, caused by SE PT4. The infection may have been spread by eggs from farm 4 in Utah. While control measures appear to have decreased the overall SE incidence in the United States, the success of these measures in controlling SE PT4 has not yet been documented. Farm level controls must be expanded, and restaurants and consumers must safely handle eggs. Failure to undertake these steps may allow the spread of SE PT4 to other regions in the United States.

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