Large outbreak of *Salmonella* Enteritidis PT8 in Portsmouth, UK, associated with a restaurant

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(Accepted 17 November 2011; first published online 14 December 2011)

SUMMARY

Seventy-five individuals with *Salmonella* infection were identified in the Portsmouth area during August and September 2009, predominantly *Salmonella* Enteritidis phage type 8. Five patients were admitted to hospital. A case-case comparison study showed that a local restaurant was the most likely source of the infection with a risk of illness among its customers 25-fold higher than that of those who did not attend the restaurant. A case-control study conducted to investigate specific risk factors for infection at the restaurant showed that eating salad was associated with a threefold increase in probability of illness. Changing from using ready washed lettuces to lettuces requiring washing and not adhering strictly to the 48 hours exclusion policy for food handlers with diarrhoea were likely to have contributed to the initiation and propagation of this outbreak. Possibilities for cross-contamination and environmental contamination were identified in the restaurant.

Key words: Epidemiology, Salmonella, Salmonella enterica, Salmonella typing, outbreaks.

INTRODUCTION

Salmonella Enteritidis is one of the Salmonella serotypes frequently associated with morbidity and mortality in humans [1]. S. Enteritidis phage-type 4 was the most common phage type isolated during the 1980s and the 1990s in England and Wales [2]. However, the incidence of S. Enteritidis phage-type 8 (SE PT8) and other more unusual phage types of

S. Enteritidis has increased in the last 10 years [3]. Studies have reported SE PT8 as the predominant phage type in Poland, Slovak Republic, Czech Republic, Denmark and the USA in the 1990s and at the beginning of this century [4–6]. SE PT8 has also been implicated in large outbreaks in the USA in 2002 and Italy in 2006 affecting over 650 and 150 patients, respectively [7, 8].

The number of reported cases of SE PT8 in England and Wales in recent years was: 599 (in 2005), 1119 (in 2006), 1384 (in 2007) and 386 (in 2008). In 2007 the Health Protection Agency (HPA) conducted a national case-control study to explore the 2006/2007

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increase. This study showed eating egg dishes away from home was strongly associated with acquiring SE PT8 infection [multivariate analysis: odds ratio (OR) 8.52, 95% confidence interval (CI) 1.42-51.1, P=0.009]. Overall, SE PT8 accounted for 7% of all salmonellae in the period 2005-2008 (Centre for Infection, personal communication).

In August 2009, Portsmouth Hospitals NHS Trust (PHT) microbiology laboratory informed the Hampshire and Isle of Wight Health Protection Unit (HIOW HPU) of the HPA of a sudden increase in Salmonella infections. The HIOW HPU, which serves an area with a population of 1.8 million, routinely monitors reports of infectious diseases from local laboratories. Between 2003 and 2008, the PHT laboratory reported a mean number of five S. Enteritidispositive isolates per month, of which about 10% were PT8. Specimens received in late August 2009 were retested to exclude laboratory cross-contamination and the outbreak was subsequently confirmed. All individuals with S. Enteritidis lived in Portsmouth and adjoining areas of southeast Hampshire. The majority of the Salmonella isolates were identified as SE PT8. At the time there were no other identified outbreaks caused by this strain elsewhere in the UK.

An outbreak investigation was conducted in order to confirm and quantify the size of the outbreak, determine risk factors for its occurrence and advise on control measures.

METHODS

HIOW HPU routinely receives reports of *Salmonella* infections from local laboratories. On 28 August 2009, PHT microbiology laboratory informed HIOW HPU of a suspected increase in *Salmonella* infections. Following retesting of specimens to exclude a laboratory artefact, results were reported to HIOW HPU on 1 September 2009.

On the same day HIOW HPU sent an alert to local authorities and neighbouring HPUs informing them of the increase in *Salmonella* infections and issued a standardized general *Salmonella* questionnaire to be administered to all individuals with a stool sample positive for *S.* Enteritidis identified by the PHT laboratory. The questionnaire collected data on demography, symptoms and risk factors within 7 days of the onset of symptoms, including travel history, contact with animals, food, water, environmental, recreational exposures and shopping habits. The

questionnaire was administered by the local authority or HPU staff and a flexible approach was permitted (telephone or postal response) to expedite data collection and increase the response rate.

Questionnaires were double-entered using EpiData software (EpiData Association, Denmark) with correction of discordant records. Data from the questionnaires were analysed to generate a hypothesis for the source of infection and vehicle of transmission.

Microbiological investigations

At the PHT laboratory stool samples were plated onto xylose-lactose-deoxycholate (XLD) agar and inoculated into selenite broth. The broth was subcultured the next day onto *Salmonella* ABC chromogenic medium (Lab M Ltd, UK). Culture plates were incubated for 16–24 h and suspicious colonies were further characterized by agglutination tests and biochemical tests.

Speciation and phage-typing were performed at the *Salmonella* reference laboratory at the Centre for Infection, HPA.

Descriptive analysis

Cases were defined as persons with laboratory-confirmed *Salmonella*, where the sample was taken after 14 August 2009 and the result was provided by 30 September 2009. They were described in terms of demography, illness (date of onset, hospitalization, severity), and exposures within the incubation period.

Analytical epidemiology

A case-case comparison study was subsequently conducted to test a hypothesis generated from the descriptive analysis implicating a particular exposure setting, hereafter referred to as restaurant A. The study population included the same individuals as in the descriptive analysis. Cases were defined as any individual with SE PT8 infection. The comparison group was defined as individuals ill with *Salmonella* infection which proved to be due to a strain other than SE PT8 (classified as 'Other salmonellae'). The standardized questionnaire was administered to cases and controls. Unconditional logistic regression was used to examine associations between having the phage type of interest and exposure variables. EpiData

analysis and Stata v. 11 (StataCorp., USA) were used for data analysis.

In addition, an unmatched case-control study was performed to identify specific risk factors at restaurant A. Cases were defined as patients with confirmed SE PT8 from stool samples, who had an acute gastrointestinal illness (diarrhoea with ≥ 3 stools per day and/or abdominal pain) with an onset within 7 days of visiting restaurant A and a laboratory result provided by Friday 25 September with a stool specimen date after 14 August. Further cases were identified following local and national alerts. Controls were defined as individuals without gastrointestinal symptoms who had accompanied a confirmed SE PT8 case on their visit to restaurant A within 7 days of the onset of illness in the confirmed case. A second questionnaire, including the menu at restaurant A, was posted by HPU staff to cases and controls. Owing to the complexity of the menu, the different menu items were analysed individually and in addition, common ingredients were grouped together and analysed as a group, e.g. any dish containing bacon, any dish containing whole egg (fried or scrambled), chicken, tuna, sausage, ham, and cheddar cheese, etc. Statistical analysis was performed using R [9, 10].

In both of the analytical studies we explored the association between study variables and SE PT8 infection using univariate analysis. Those variables found to be associated in the univariate analysis at P < 0.1 were included in the multivariate analysis. For each analysis we estimated the OR and 95% CI.

Environmental investigations

Restaurant A was inspected by environmental health officers from the Portsmouth City Council (PCC) Environment and Public Protection Service. Methods of preparation, working environment and food safety practices were reviewed in detail.

Twenty-three environmental swabs from the kitchen preparation area, basins, containers, dishes and food samples were collected and sent for microbiological testing on 7 September.

Staff were interviewed on several occasions to determine procedures used in restaurant A. Twenty-five staff completed a staff questionnaire regarding the presence of symptoms and the consumption of food at work. Stool specimens were collected from those staff members who had reported symptoms. Screening was later extended to all current staff.

RESULTS

Microbiological investigations

Seventy-five individuals with a *Salmonella* infection were identified by the PHT laboratory from stool samples collected after 14 August 2009 and results provided by 30 September 2009. Of the 75 isolates, 54 were PT8. In addition, six further patients with *Salmonella* PT8 isolates, linked to restaurant A were identified through other laboratories. All isolates were subsequently confirmed at the Salmonella Reference Unit at the HPA Centre for Infection.

Descriptive analysis

Out of 75 cases, questionnaires were completed for 68 (a response rate of 90%). Of these 68, 53 (78%) were diagnosed with SE PT8 (see Fig. 1). The dates of onset of 41 (77%) of the 53 PT8 cases were concentrated around a 6-day period between 18 and 23 August with a single peak on the 19 August (Fig. 2). Seventy-four per cent of the PT8 cases were female with a median age of 43 years (range 1-73 years). Median age for males was 16 years (range 5-73 years). Of the 68 cases, five (all SE PT8 cases) were admitted to hospital. Hospital stays ranged from 1 to 28 days (median 10 days). For the 38 (56%) patients for whom duration of illness was available, 19 reported illness lasting \geq 10 days. Of the 68 cases, 50 (74%) had visited restaurant A within 7 days prior to onset of symptoms; one was a member of staff at this restaurant. The median duration between the visit and onset of illness was 3 days (range 0-7 days). Of the 18 individuals who did not report a visit to restaurant A, four (22%) had a SE PT8 infection. The hypothesis generated was that this outbreak of SE PT8 in the catchment area of the PHT laboratory was associated with a visit to restaurant A. This was further examined by analytical methods.

Analytical epidemiology

Case-case comparison study

The results of our univariate analysis (Table 1) showed an association between SE PT8 infection and having eaten food from any restaurant, café, tea shop, pub or hotel (OR 13·53, 95 % CI 2·98–72·24). Having eaten at restaurant A showed the strongest association with SE PT8 infection (OR 36·79, 95 % CI 4·81–1689·50). Being a SE PT8 case was also found to be associated with being aged >35 years, having

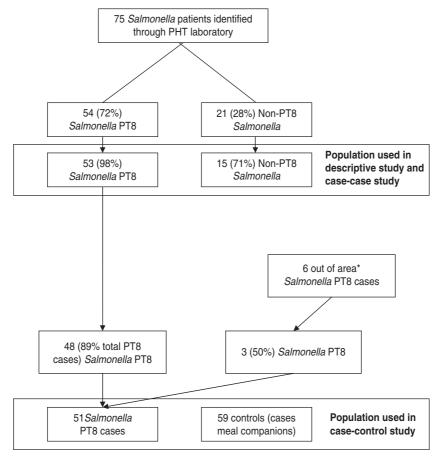


Fig. 1. Flow diagram describing descriptive, case-case comparison and case-control study populations. * *Salmonella* PT8 cases linked to restaurant A identified by other laboratories than Portsmouth Hospitals NHS Trust (PHT).

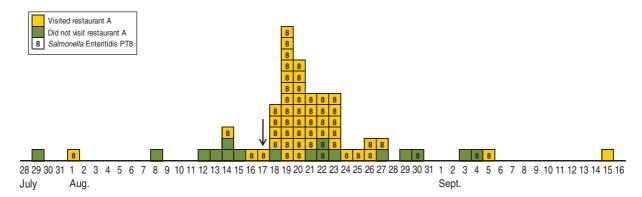


Fig. 2. Epidemic curves of cases of *Salmonella* in the catchment area of the Portsmouth Hospitals NHS Trust microbiology laboratory, showing date of onset, PT8 status (n = 53) and restaurant A visit (n = 50). n = 67 (one case with onset before 1 July 2009 is not shown). The vertical arrow (\downarrow) indicates the earliest illness onset of a food worker.

eaten food bought in a supermarket and having eaten salad, but no association was found with any particular supermarket or with any particular source or brand of salad. Travel outside the UK in the 3 days prior to onset of symptoms showed a negative association with SE PT8 infection (OR 0.04, 95% CI 0.01–0.23). No association was found between being

a SE PT8 case and having had close contact with anyone suffering from diarrhoea before the onset of symptoms, or with consumption of eggs, chicken, milk, or handling poultry.

The multivariate model included age, eating at restaurant A, travel outside of the UK and eating salad and food from a supermarket. The only variable

Table 1. Univariate and multivariate analysis of the risk factors associated with Salmonella Enteritidis PT8 infection (case-case study)

Exposures in last 3 days before illness onset	PT8 cases n (%)	Other salmonellae $n (\%)$	Crude OR (95 % CI)	P value	Adjusted OR (95 % CI)	P value (Wald test)
Ate in any restaurant	40 (00 4)	C (10.0)	12.52 (2.00 52.21)	0.0004.hth		
Yes	48 (90·1)	6 (40.0)	13.53 (2.98–72.24)	0.0001**	_	_
Ate in restaurant A						
Yes	39 (73.6)	1 (6.7)	36·79 (4·81–1689·5)	< 0.0001**	24.55 (1.62–371.63)	0.02
Age groups (yr)						
< 15	9 (17.0)	7 (46.7)	1	_	1	_
15–34	15 (28·3)	4 (26.7)	2.91 (0.66–12.82)	0.16***	_	_
35–65	16 (30·2)	2 (13·3)	6.22 (1.06–36.57)	0.04***	0.81 (0.34–18.98)	0.89
>65	13 (24.5)	2 (13·3)	5.06 (0.84–30.18)	0.07***	1.51 (0.05-46.72)	0.82
Shopped in supermarket						
Yes	43 (81·1)	6 (40.0)	13.30 (2.19–105.43)	0.001**	5.49 (0.25–121.74)	0.28
Ate salad						
Yes	34 (64·2)	5 (33·3)	3.86 (0.87–18.71)	0.04**	2.04 (0.14-30.69)	0.52
Travel abroad						
Yes	3 (5.7)	9 (60.0)	0.04 (0.01-0.23)	< 0.0001**	_	_

OR, Odds ratio; CI, confidence interval.

Table 2. Univariate and multivariate analysis of the food items associated with Salmonella Enteritidis PT8 infection (case-control study)

Food item eaten in restaurant A	Cases exposed (% total cases)	Crude OR (95 % CI)	P value (Wald test)	Adjusted OR (95 %CI)	P value (Wald test)
Ate salad Yes	27 (52·9)	3.84 (1.65–8.96)	0.006	2.93 (1.19–7.19)	0.02
Ate coleslaw Yes	20 (39·2)	6·31 (1·69–23·55)	0.01	2.81 (0.64–12.29)	0.17

OR, Odds ratio; CI, confidence interval.

associated with being a SE PT8 case was eating at restaurant A (adjusted OR 24.55, 95% CI 1.62–371.63).

After restricting the analysis to individuals ill with *Salmonella* not exposed to restaurant A, no association was found between being a SE PT8 case and any single exposure.

Case-control study

A total of 109 individuals were enrolled in the case-control study: 51 were cases and 58 controls (see Fig. 1). Out of the 51 cases responding the questionnaire, 48 originated from the same population of the case-case comparison study; three further cases were enrolled after they were identified by out of area

laboratories. Cases had a mean age of 37 years (range 3-71 years) and 76% were female. Controls had a mean age of 36 years (range 3-77 years) and 54% were female.

On univariate analysis, having eaten salad was associated with being a SE PT8 case (OR 3·84, 95% CI 1·65–8·96) (Table 2). There was also an association between being a SE PT8 case and having eaten coleslaw (OR 6·31, 95% CI 1·69–23·55).

Twenty-seven (53%) cases had been exposed to salad and 20 (39%) to coleslaw. In total, 28 (55%) cases had been exposed either to salad or coleslaw.

Fitting both exposures into a multivariate model, there was no longer an association between coleslaw consumption and being a SE PT8 case, while eating

^{**}P value from Fisher's exact test; *** P value from Wald test.

salad was still associated with a threefold increase in risk for illness with SE PT8 (adjusted OR 2.93, 95% CI 1.19–7.19).

Environmental investigations

SE PT8 was isolated from a cloth in the pot wash area. A sample of precooked pasta had a total viable count of $1.3 \times 10^8/g$ of SE PT8 suggestive of poor hygiene.

The supplier of lettuce had been changed prior to the outbreak resulting in the replacement of ready washed lettuce to lettuce which required washing. The new lettuce was washed in a sink also used for washing raw chicken.

Although some staff had defined working responsibilities, there was a complex system of rotas and responsibilities between full-time/part-time staff due to long opening hours (07:00–20:00 hours) with up to 250 customers per day.

During the investigation, it became clear that processes and procedures were complex as well. Staff would quickly change working responsibilities at short notice, depending on the demands at the time, increasing the risk of contamination unless satisfactory standards of hygiene were continually observed.

Staff were assigned to one of four roles on the work rota. These were front of house, chefs and kitchen, wash up and runners, with a large amount of interchange and multitasking. Positive *Salmonella* results were obtained from staff in each of these work areas.

Six staff tested positive for *Salmonella* of which five had SE PT8 and the sixth had S. Bredeney. Two SE PT8 results and the different strain were obtained from asymptomatic staff. An analysis of the onset dates of the confirmed SE PT8 in symptomatic staff members showed that the three developed symptoms on 17 and 18 August. Staff records showed that three (one of whom was symptomatic) of the five staff worked in the tea rooms on the 17 August.

Outbreak control

The Outbreak Control Team (OCT) first focused on ensuring that the premises were safe to operate, and second, to investigate the cause of the outbreak. Officers from PCC Environment and Public Protection Service worked to ensure that food safety practices/procedures in the restaurant were satisfactory. Changes in practice that were advised included increased changing of cloths, replacing terry hand

towels with paper towels and using bowls to give greater separation when washing different food items in the same sink. The use of sanitizers and gloves was reviewed to ensure that the sanitizer was always available and that gloves were changed appropriately, especially after handling potentially contaminated food items. Advice was also given on the Food Standards Agency criteria for the return to work of food handlers with gastrointestinal symptoms. The business owners cooperated fully with every aspect of the investigation and adopted advice, guidance and information where appropriate. The premises remained open throughout the outbreak investigation.

The incident was considered to have ended at the OCT meeting on 17 September 2009; however, heightened surveillance was continued until the end of October.

DISCUSSION AND CONCLUSION

We report a large outbreak of SE PT8 involving 60 confirmed cases. Most of the cases were resident in the Portsmouth area, South of England, and were ill between August and September 2009. The first case of SE PT8 was reported on 1 August 2009, the last on 5 September 2009. The true number of SE PT8 cases is likely to have been higher due to underreporting [11].

Salad consumed at restaurant A was statistically implicated as the vehicle; however, it is likely that it was not the only vehicle, as only 53% of cases reported eating salad. In this restaurant, salad was a common garnish on many dishes. The lettuce, which had recently changed from ready washed lettuce to lettuce requiring washing, was washed in the same sink used for washing raw chicken. Raw chicken has been described as a potential source of Salmonella [12–14]. It is unlikely that lettuce would have been contaminated from the supplier as there were no other identified outbreaks of SE PT8 at the time. When we restricted the analysis to individuals ill with Salmonella, who had not been exposed to restaurant A, no association between being a PT8 case and eating salad, chicken or any other specific food item was found.

Although SE PT8 has been found to be associated with eating chicken outside the home and keeping a pet lizard [15], and S. Enteritidis infections are commonly associated with eggs and poultry [16], in recent years reports of gastroenteritis originating from contaminated salad have significantly increased. Little & Gillespie, in a review of foodborne general outbreaks

of infectious intestinal disease between 1992 and 2006 in England and Wales, found that four percent of the outbreaks (n=82) were associated with salad [17]. Berger *et al.* [18] reported that *Salmonella* can efficiently and successfully adhere to salad leaves in any phase between plant growth and human consumption.

A dish cloth from the area where pots were washed was positive for SE PT8 on 7 September. A positive result could occur if a newly introduced contaminated item had just been washed. However, it is likely from the descriptive epidemiology that the outbreak strain persisted in the restaurant for around 3 weeks. The epidemic curve, indicating a propagated outbreak, suggests that the initial source of *Salmonella* infection persisted over a period of about 8 days in the restaurant or was propagated by infected staff members, resulting in the large number of people affected.

Out of the five members of staff found to be infected with SE PT8 during the outbreak investigation, three developed diarrhoea symptoms on 17 and 18 August. This preceded the peak of illness in customers but was after the date of the first case. Prior to the investigation, the restaurant management had not specifically advised symptomatic members of staff to stay away from work until 48 h after the first normal stool as recommended under Food Safety Agency guidance [19, 20]. Staff may have been infected in a similar way to customers or been unwitting sources of infection, or both. As staff stool specimens were collected from 7 September onwards, it is possible that Salmonella could have cleared before testing was undertaken leading to an underestimate of the number of staff infected.

Food handlers have previously been implicated in *Salmonella* outbreaks [21–23]. In 2007 Greig *et al.* estimated that the incidence of *Salmonella* outbreaks involving food handlers had increased in recent years [24]. Food handlers can become infected by consuming contaminated food, handling contaminated raw materials or through environmental contamination. Regardless of the initial source of the outbreak, food handlers often serve as a reservoir for the infection, contributing to its prolonged transmission [25].

Salmonella outbreaks involving implicated food handlers can be associated with a longer incubation period prior to illness. This can be explained by the low number of bacteria shed by food handlers [25]. This outbreak reflects a similar situation. Eighteen per cent of our PT8 cases (eight individuals) had an

incubation period between 4 and 7 days, considerably longer than the characteristic 12 h to 3 days window generally defined for *Salmonella* [26, 27].

The combination of the two different analytical study designs gave flexibility to the investigation, making a two-stage approach to identify a source of this large outbreak possible. Case-case studies using surveillance data have a reduced risk of being affected by selection bias than case-control studies. In casecase studies, cases and controls are subject to the same selective process of being reported into the surveillance system [28]. The major drawback of the case-case comparison study design is the possibility that aetiological exposures were systematically different between genotypes, which would affect the validity of the study if the number of different genotype cases included as comparators is small. Our comparison group included four other species of Salmonella and six other S. Enteritidis phage types, which would reduce the likelihood of this.

In the case-control study there may be an increase of recall bias in cases compared to controls. Because we used the same cases in both analytical studies, they may have had better recall as they had previously completed the standardized questionnaire and also due to their illness status. Since a standardized questionnaire was used for all cases of *Salmonella* as well as the cases involved in the outbreak, using the same source population for the case-control study, as for the initial case-case comparison study, was thought to be a reasonable approach.

Our study has some limitations. In the case-control study, controls were meal companions of cases. This could have been considered for a matched design, as controls were selected on the basis of their association with a particular case. As the information linking cases and controls was not recorded when data were collected, a conditional logistic regression to cross-check the finding of the unmatched case-control study was not done. However, the unmatched multivariate analysis appears robust and we believe that a matched estimate would have had a very similar odds ratio, with a more precise (less wide) 95% confidence interval.

We conclude that this outbreak was associated with restaurant A and that salad containing lettuce was implicated as the primary vehicle of transmission. The precise source of contamination was not detected but cross-contamination from raw chicken, infected food handlers and environmental contamination may have been contributory factors.

The outbreak investigation involved multiple organizations over a 2-month period including the HPA, several local authorities, the local acute trust, and two primary care trusts. This demonstrates the importance of good communication channels and the contribution of different components of the public health system.

To our knowledge this is the first outbreak of SE PT8 associated with a restaurant reported in the UK. With the increased incidence of this strain it is likely that new outbreaks of PT8 will occur in the future in the UK. Phage-typing of *Salmonella* isolates was extremely useful in identifying this outbreak.

DECLARATION OF INTEREST

None.

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