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An outbreak of *Salmonella enteritidis* phage type 34a infection in primary school children: the use of visual aids and food preferences to overcome recall bias in a case control study

E. LINNANE[1]*, R. J. ROBERTS[1] AND P. T. MANNION[2]

[1] *Department of Public Health Medicine, North Wales Health Authority, Preswylfa, Mold, Flintshire, CH7 1PZ, UK*
[2] *Chester Public Health Laboratory, Countess of Chester Health Park, Liverpool Road, Chester CH2 IUL*

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SUMMARY

Outbreaks of infectious intestinal disease are common in schools. Case control studies are useful in the investigation of infectious disease outbreaks but the time interval between illness and investigation can lead to recall bias, particularly in young children. We describe an outbreak of *Salmonella enteritidis* phage type 34a infection involving 54 clinical cases in two adjacent schools, and a novel approach to overcome recall bias. The likely dates of infection were identified from the epidemic curve. We created a visual display of the menu from those days and asked 9 cases and 18 matched controls to identify their food preferences from this display. Preference for chocolate mouse was significantly associated with illness ($P = 0{\cdot}006$). The results of the case control study agreed with the findings of the environmental investigation. We believe our approach could be used in other circumstances, where subjects are young children or recall bias is a concern.

INTRODUCTION

Outbreaks of infectious intestinal disease are common in schools and nurseries. The PHLS Communicable Disease Surveillance Centre received reports on 4603 outbreaks of infectious intestinal disease in England and Wales between 1992 and 1999. Of these, 291 arose in schools and nurseries. Between 1992 and 1999, 939 of the general outbreaks of infectious intestinal disease in England and Wales were associated with Salmonella [1].

Central to the control of these outbreaks is the identification and removal of the source of infection. Case control studies are useful in the investigation of infectious disease outbreaks but the time interval between illness and investigation of the outbreak can lead to recall bias. Accurate recall is a particular problem in young children who may not fully understand the nature of the investigation.

* Author for Correspondence: Bro Taf Health Authority, Temple of Peace and Health, Cathays Park, Cardiff CF1 3NW, UK.

We describe an outbreak of *Salmonella enteritidis* phage type 34a infection involving two adjacent schools; in which we used a novel approach to overcome recall bias in our young subjects.

The outbreak

On 30 September 1998 the Public Health Department was informed by a community paediatrician of a diarrhoeal illness in pupils at a local school for children with severe learning difficulties. Salmonella had been isolated from one of the pupils. This school accepts children age 2–19 years (School 1). The 45 pupils that attended that school all needed hands on personal care, and approximately one third were totally dependent on their carers for feeding, dressing and toileting. A laboratory report had been received by the public health department that morning for one of the pupils who had been admitted to a local hospital with confirmed *Salmonella enteritidis* infection.

METHODS

Lunchtime school meals were produced in and transported to School 1 from a neighbouring school (School 2). School 2 also supplied a third school, School 3 which was on a different site. School Two was a mainstream primary school with 602 pupils catering for ages 4–11 years. It shared the same site as School 1 and there was some mixing of pupils.

We obtained the school menus for 21–25 September 1998 for School 1 and School 2. Approximately 40% of 4–7 year olds, 60% of 7–11 year olds and 40% of the staff from School 2 received school dinners.

Faecal specimens were requested from all suspected cases identified in the two schools during the outbreak. The PHLS Laboratory of Enteric Pathogens typed all isolates.

Samples of foodstuffs were taken from the kitchen of School1 on 30 September.

Case control study

We undertook a case control study at School 2 to test whether infection among children was associated with a specific food item served with a school dinner.

For the study we defined a case as a pupil at School 2 who developed diarrhoea or vomiting with the onset of symptoms on 24, 25, or 26 September 1998.

We asked the head teacher to nominate two friends of each case to act as controls. The parents of cases and controls were interviewed over the phone using a structured questionnaire which ascertained illness, symptoms, duration of symptoms, attendance at school, consumption of school dinners and any foodstuff which the child in question never ate. Verbal consent was received from the parents and the head teacher before talking to the children.

An outbreak curve (Fig. 1) was drawn showing a peak of primary infection on September 26 and a peak of secondary infection on September 28. The first positive case of *Salmonella enteritidis* phage type 34a infection had a date of onset of 24 September. The incubation period for salmonella infection is 6–72 h, usually 12–36 h, but can be up to 80 h when there is a small innoculum, or if the innoculum is incorporated in high fat foodstuff [2, 3]. Thus the 22 or 23 of September were the most likely dates on which, based on the outbreak curve, exposure had occurred.

Interviews took place in School 2 on 9 October 1998. Each child was interviewed using a standard protocol and a structured questionnaire, which listed all the foods served for school dinners on 22 or 23 September. The interviews took place beside a display of the foods served on these days. The children were shown the display of foods, and were then asked what they would choose as first and second choice. Odds ratios and the Mantel–Haenzel χ^2 test were used to examine associations between illness and each potential food item. Fisher's Exact test was used where expected values in any cell were less than five. The analysis was based on the first choice of each child interviewed. Statistical analysis was performed using Epi-info (Version 6.0) [4].

RESULTS

Environmental

Lunch was prepared at School 2 and delivered by car to School 1 at approximately 11:50 hours each day. The food was put on plates by a designated catering assistant and served in each class. A catering supervisor helped with feeding in each class.

School 2 had an adequately equipped kitchen and designated catering staff. Cooking temperatures were recorded and all foods were adequately cooked. In School 2 food was distributed directly to the tables for infant classes while older children and staff were served a choice from the serving counter.

The case control study (see below) suggested that chocolate mousse was associated with illness. On the 22 September a sponge cake (served at School 3) was made before preparing a chocolate mousse dessert. A freestanding mixing bowl with a whisk attachment was used for making both deserts. The sponge cake was prepared using raw shell eggs. The chocolate mousse was prepared immediately afterwards by whisking desiccated mousse with water before transferring to serving bowls. The mixing bowl and whisk were reported to have been washed in a sink, between preparing the two desserts. The temperature of this wash was approximately 52 °C, based on the measured temperature of a similar wash observed during a subsequent kitchen visit.

Epidemiological

The epidemiological picture was complicated by a pre-existing outbreak of diarrhoea and vomiting at School 2 with clinical and epidemiological features compatible with viral gastroenteritis.

Fifty-four clinical cases with either diarrhoea or

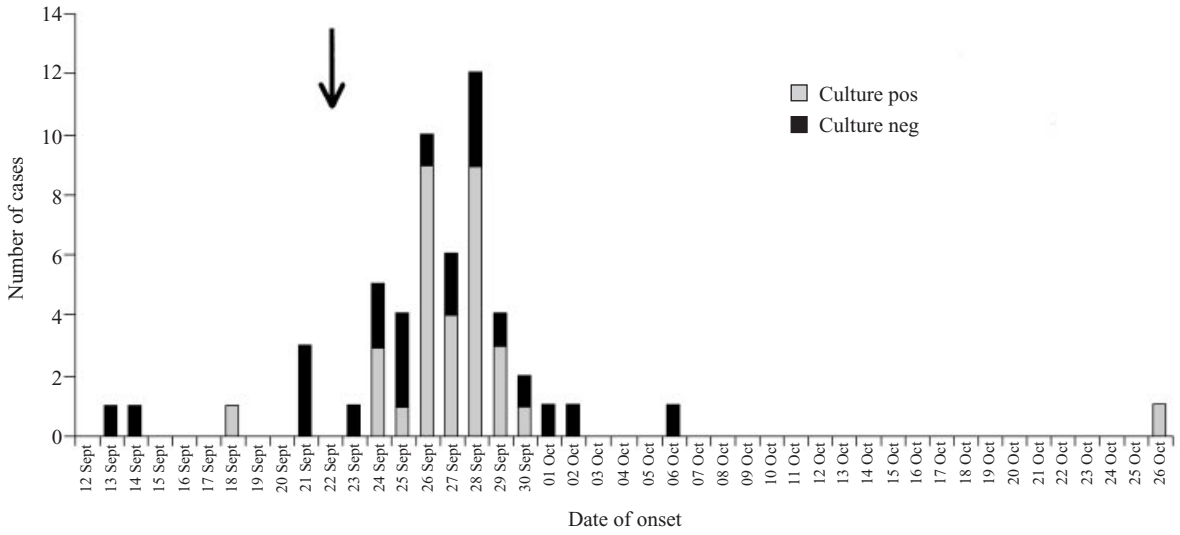

Fig. 1. Epidemic curve of all cases both clinical and microbiologically confirmed, according to date of onset. Number of cases on the *y*-axis. Date of onset of illness on the *x*-axis (day/month). Arrow indicates 22 September, the date the chocolate mousse was consumed.

Table 1. *Case control study of menu from 22 September*

Food: 22 September 1998	Cases		Controls		
	Preferred	Not preferred	Preferred	Not preferred	*P*-value
Spaghetti bolognese	3	6	5	13	NS
Hot dog	0	9	6	12	NS
Sausage roll	1	8	1	17	NS
Fish fingers	1	8	0	18	NS
Chicken drummers	3	6	1	17	NS
Vegetable lasagne	1	8	2	16	NS
Jacket potato	2	7	4	14	NS
Pasta twists	2	7	9	9	NS
Tinned spaghetti	3	6	3	15	NS
Tuna salad	0	9	1	17	NS
Cheese salad	2	7	2	16	NS
Bread rolls	3	6	9	9	NS
Sultana cookie	1	8	3	15	NS
Iced sponge cake	0	9	7	11	NS
Cheese and biscuits	2	7	5	13	NS
Chocolate mousse	6	3	2	16	$P = 0{\cdot}006$

Results of case control study carried out using a visual display of the food available on 22 September. Values indicate numbers of cases and controls that chose each food item from the display. *P* values refer to association between preference and illness.

vomiting were identified at the two schools (Fig. 1) of which 28 were males and 26 were females. Four were adults. The median age was 7·6 years (range 1·5–47·2 years). The median onset interval was 6 days (range 1–14 days). One case had developed diarrhoea and vomiting on 18 September and had a positive culture on 29 September. It was assumed that his initial symptoms were due to a viral infection that he subsequently became infected with *Salmonella enteritidis* PT 34a.

All those who were ill in School 1 and School 2 had received school dinners. School Three received school dinners prepared in School 2 on 22 September. The meals were identical except that School 3 received chocolate cake on that day instead of chocolate mousse. No illness was reported in School 3.

Microbiology

Samples were submitted from 41 of the 54 clinical cases and *Salmonella enteritidis* phage type 34a was isolated in 29 cases. Campylobacter was isolated in two cases, one of which was also infected with *Salmonella enteritidis* phage type 34a. No organism was identified in samples from 11 cases and no samples were available from 13. Of the 29 cases positive for *Salmonella enteritidis* phage type 34a infection, 13 were from School 1 and 16 were from School 2. Three of the children were admitted to hospital and had stool samples positive for *Salmonella enteritidis* phage type 34a.

None of the food samples tested positive for salmonella. The chocolate mousse was made from desiccated chocolate mousse, and water. No eggs or chocolate mousse were available from the batches used on the days in question but samples were taken from different batches as multiple batch analysis has been advocated in such cases [5].

Case control study

The case control study was carried out in School 2 as children in School 1 would have been unable to participate due to their learning difficulties. There were 24 clinical cases in School 2 of which 16 were microbiologically positive. Of the 16 microbiologically positive cases in School 2 we received parental consent to interview 10 cases and questionnaires were completed for 9 (one was absent from school) and 18 controls.

The results for each of the foodstuffs served on September 22 are given in Table 1. The risk of illness was significantly increased in those whose first choice was chocolate mousse (OR = 13·81, 95% CI = 1·58–207·26). No other food served on September 22 and some served on September 23 were significantly associated with illness. Those choosing sponge cake were significantly less likely to be ill ($P = 0{\cdot}05$).

DISCUSSION

We describe the investigation of an outbreak of *Salmonella enteritidis* phage type 34a infection in two schools, by a case control study using food preferences instead of recalled consumption of foods to indicate exposure.

Although there were 230277 cases of *Salmonella enteritidis* in England and Wales between 1981 and 1999, *Salmonella enteritidis* phage type 34a is uncommon. It was first described in 1996 when there were 45 cases in England and Wales. In 1997 there were 228 cases and 180 in 1998. The only food isolate of *Salmonella enteritidis* phage type 34a during this time was from a sample of pasteurized egg (personal communication, LR Ward, Laboratory of Enteric Pathogens, Central Public Health Laboratory). From an analysis of the epidemic curve we concluded that exposure had occurred on one of two days, 22 or 23 September. As eggs are commonly implicated in salmonella infections [6–10] we investigated the possibility of cross contamination or inadequate cooking. Following the finding in the case control study that chocolate mousse was associated with illness we re-interviewed the cook regarding the precise order in which items were prepared. This information supported the hypothesis that cross contamination occurred from the mixing bowl that contained raw egg mixture prior to mixing chocolate mousse. Specific enquiries about the order of preparation and washing of utensils confirmed the plausibility of this route of cross contamination. Further evidence was obtained from the fact that there were no cases in School 3, which received identical meals to School 2, except that they received chocolate cake instead of chocolate mousse on 22 September. Preferring iced sponge cake, which was the alternative on the menu that day in School 2, to chocolate mousse, was associated with a significantly reduced risk of illness.

We were concerned that children, aged 5–11 years, the age range of the children involved in the study, might not accurately recall food consumption of specific days more than 2 weeks earlier. Therefore, we hypothesized that those children affected would have a preference for the foods they actually ate in a visual 'line-up' of all the items. To avoid recall bias we asked the children to state their first and second choices from the menus under investigation rather than trying to remember what they ate. We found the visual display very useful in assisting the children identify favourite foods. In so far as we could, we presented the food as it would have been presented to them on the suspected date of exposure.

By using stated food preferences, rather than asking children to recall what they actually ate, we identified chocolate mousse as a food vehicle that was significantly associated with illness. This finding was consistent with other circumstantial evidence and led to the identification of a lapse in food hygiene and a biologically plausible route of contamination.

Observer bias may have occurred, as this was an unblinded study. We think that it was unlikely to have greatly affected our results as no attempt was made to coach or influence our subjects' preferences, and questions were uniformly phrased following a standard written protocol. It would be possible to have observers blinded in future applications of this method.

In accordance with The Food Safety (General Food Hygiene) Regulations, 1995 adequate cleaning and, where necessary, disinfection of utensils and equipment must be carried out in order to take all steps to avoid the risk of contamination of ingredients. Previous studies have documented outbreak sources originating from cross contamination of kitchen equipment [11,12]. Cross contamination may result in a low innoculum. However low levels of salmonella in food, and extremely low infectious doses, can cause disease in humans [13]. Longer incubation periods occur following ingestion of relatively few organisms, particularly in foods with high fat content such as chocolate mousse [2]. This is in keeping with our findings, where the median onset interval was 6 days, and even allowing for the fact that some or most of the later cases resulted from secondary transmission, suggests that some of our primary cases may have had an incubation period in excess of 80 h.

Three children from School1 required admission to hospital with severe gastroenteritis. This illustrates the importance of food hygiene in particular when dealing with a vulnerable population. Our findings highlight the importance of avoiding the use of shell eggs as a food ingredient where pasteurized egg is a reasonable alternative.

We suggest that the approach used here to overcome recall bias by using visual prompts and stated food preferences, could be used in other circumstances, where subjects are young children and recall is difficult.

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