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A national outbreak of infection with *Salmonella enteritidis* phage types 5c and 6a associated with Chinese food businesses in Scotland, summer 2000

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SUMMARY

An outbreak of salmonellosis, involving cases of infection with *Salmonella enteritidis* phage types (PT) 5c and 6a, occurred across Scotland between May and August 2000. In total, 70 outbreak cases were microbiologically confirmed. Preliminary investigation suggested that consumption of food, especially chicken dishes, from Chinese restaurants or take-aways (food businesses) was a risk factor for infection. A matched case-control study demonstrated a statistically significant association (OR 22·4, $P = 0{\cdot}0024$) between infection and consumption of food from Chinese food businesses. A cohort study of novel design suggested that chicken was an important vehicle of infection. However the result did not reach statistical significance (OR 1·7, $P = 0{\cdot}3$). Extensive environmental investigation was unable to identify the source of the suspected contaminated chicken.

INTRODUCTION

Surveillance of salmonella infection in humans in Scotland is based on the voluntary submission by diagnostic microbiology laboratories of salmonella isolates to the Scottish Salmonella Reference Laboratory (SSRL), which reports its findings to the Scottish Centre for Infection and Environmental Health (SCIEH). Local authority Environmental Health Officers (EHOs) or NHS board staff interview most cases using standard questionnaires (enteric surveillance forms). These forms differ in detail across Scotland but all elicit demographic, clinical, and exposure information from cases.

On 31 July 2000, SSRL reported to SCIEH an increase in the number of *Salmonella enteritidis* PT5c isolates from humans. From 1 January to 31 July 2000, SSRL had reported 13 isolates of *S. enteritidis* PT5c from people with no recent history of foreign travel, compared with three for the whole of 1999. It subsequently became apparent that a similar increase in reports of *Salmonella enteritidis* PT6a was occurring: SSRL had reported 37 isolates from 1 January to 31 July 2000 compared with 20 in the same period in 1999 (personal observation). Although reports of salmonellas in humans have declined in recent years, *Salmonella enteritidis* remains the commonest serotype identified in humans in Scotland accounting for 64% of all salmonellas reported from humans in 1999 [1]. Phage type 4 is the commonest phage type of *S. enteritidis*, accounting for 724 (60%) of the 1210 reports of all *S. enteritidis* infection in 1999 [2].

In contrast, *S. enteritidis* PT5c and PT6a are rare. In 1999 there were four reports of *S. enteritidis* PT5c and 82 of *S. enteritidis* PT6a, constituting 0·3% and 7% respectively of all *S. enteritidis* reported to SCIEH. Furthermore, in 1999, 1 of the 4 reports of *S. enteritidis* PT5c and 24 (29%) of the 82 reports of *S. enteritidis* PT6a infections were thought to have been acquired outside the UK, compared with only 77 (11%) of the 724 reports of *S. enteritidis* PT4 infection (personal observation).

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In 1999, SCIEH identified 16 general outbreaks of salmonella infection, of which 8 were of *S. enteritidis*: 5 were of *S. enteritidis* PT4, 1 of both *S. enteritidis* PT4 and PT5c, 1 of *S. enteritidis* PT6a, and 1 was not ascribed a phage type [3]. In only two outbreaks, both of infection with *S. enteritidis* PT4, was a food vehicle reported with evidence stronger than descriptive. In one the vehicles were tuna mayonnaise and coleslaw and the supporting evidence was statistical, in the second the vehicle was cooked chicken, and the supporting evidence was microbiological (personal observation).

In response to the increase in reports of UK-acquired *S. enteritidis* PT5c infections identified in July 2000 the Food Standards Agency (Scotland) (FSA(S)) convened an outbreak control team (OCT). This paper describes the epidemiological component of the investigation of the outbreaks of *S. enteritidis* PT5c and PT6a infection, referring to the environmental and microbiological components where appropriate. The OCT report describes more fully the environmental and microbiological components of the investigation [4].

METHODS

Epidemiological

Descriptive epidemiology

The investigators reviewed the routine laboratory reports to SCIEH of salmonellas, and contacted Consultants in Public Health Medicine (CPHMs) in whose NHS board area cases of infection with *S. enteritidis* PT5c or PT6a resided. CPHMs then forwarded to SCIEH completed enteric surveillance forms on cases in their area. Information from these forms was used to generate the descriptive epidemiology, and formulate hypotheses on the cause of the outbreak. Where additional information was required cases were re-interviewed using a specific pro-forma developed by SCIEH. The data were collated and analysed using SPSS [5] and EGRET [6]. The definition of an outbreak case for the descriptive epidemiology was: 'A person with *S. enteritidis* PT5c or PT6a infection reported to SCIEH between 3 June and 26 September 2000 and not reported to have acquired their infection outside the UK.'

Analytical epidemiology

Two studies were carried out to test two hypotheses generated from the descriptive epidemiology.

The first was a matched case-control study, which tested the hypothesis that a case was more likely than a control to have consumed food from a Chinese food business in the 7 days prior to the onset of the case's symptoms. For the case-control study a case was defined as 'a UK-acquired household primary or co-primary symptomatic case of infection with *S. enteritidis* PT5c or PT6a reported between 3 June and 26 September 2000'. Cases were asked to nominate up to five controls, matched for area of residence, age and sex. We did not approach cases identified from their enteric surveillance forms as being asymptomatic, or acquired outside the UK, or household secondaries (that is persons falling ill more than a day after another person in the household). We excluded from analysis cases who were identified at interview as falling into one of these categories, or who could not be contacted.

The second study included, as a single cohort, groups of people who had consumed a meal from a Chinese food business in the company of a case included in the case-control study during the 7 days prior to the onset of the case's symptoms. This cohort study tested the hypothesis that the consumption of chicken from a Chinese food business at which a case had eaten was associated with an increased risk of being a case. Names of cohort members were identified during interviews with cases who had eaten from Chinese food businesses. Cohort members who were outbreak cases were defined as confirmed cohort cases. Cohort members who had diarrhoea in the 7 days following their potential exposure but whose stools were either not cultured or were negative were defined as probable cohort cases. Cohort members who had no episodes of diarrhoea in the 7 days following their potential exposure were defined as non-cases. Analyses were performed both including and excluding probable cases.

The case-control and cohort studies were conducted concurrently by interviewers from SCIEH administering standard questionnaires by telephone. Questionnaires, interviewers' scripts, and matching criteria were written down for use by interviewers. Resulting data were entered manually onto an SPSS database. Data were analysed for each phage type separately, and then combined.

Environmental

EHOs from the appropriate local authority visited restaurants and takeaways from which cases had eaten in the 7 days before the onset of their symptoms (outbreak food businesses). EHOs described, and assessed by interview and observation, the practices, processes, equipment and premises utilized in food preparation and sale. EHOs informed the FSA(S) of suppliers to outbreak food businesses (primary suppliers). When primary suppliers who supplied more than one outbreak food business were identified, EHOs ascertained what cutting plants, importers or wholesalers had supplied them (secondary suppliers). Other outlets supplied by primary suppliers were also identified where possible, as were farms from which poultry served at outbreak food businesses had originated. Port Authorities throughout the UK were contacted for details of imports of chicken destined for Scotland, and attempts were made to identify any particular source of supply to Chinese food businesses in Scotland. The FSA(S) collated and reviewed all the available information to identify any possible common lines of supply [4].

Microbiological

EHOs took food and environmental samples for culture from outbreak food businesses. Priority was given to chicken, chicken products, eggs, and spices [4]. Isolates identified at local laboratories were sent to SSRL for typing. Routinely available surveillance data on the incidence of salmonella in animals and food in Scotland and the rest of the UK were reviewed.

RESULTS

Epidemiological

Descriptive

Between 3 June and 26 September 2000, SCIEH received 41 reports of cases of infection with *S. enteritidis* PT5c, of which 5 had a history of recent travel outside the UK. Of the 36 cases of UK-acquired infection (outbreak cases), 4 were household secondary cases and 1 person was asymptomatic.

Onset dates for the 35 symptomatic outbreak cases of *S. enteritidis* PT5c infection ranged from 28 May to 6 August 2000; two of these cases could not provide exact onset dates but first became symptomatic within this period (Fig. 1).

Between 3 June and 26 September 2000, SCIEH received 46 reports of cases of infection with *S. enteritidis* PT6a, of which 12 had a history of recent travel outside the UK. Of the 34 cases of UK-acquired infection (outbreak cases), 2 were household secondary and 1 person was asymptomatic.

The temporal distribution of cases of *S. enteritidis* PT6a infection was similar to that for cases of *S. enteritidis* PT5c infection (Fig. 1). Onset dates for the 33 symptomatic outbreak cases of *S. enteritidis* PT6a infection ranged from 6 June to 30 July 2000; one case could not provide exact onset dates but first became symptomatic within this period.

After excluding secondary and asymptomatic cases, most of the remaining 31 outbreak cases of *S. enteritidis* PT5c infection lived in the central belt of Scotland. Of these, 17 (55%) lived in the neighbouring NHS board areas of Greater Glasgow (10 cases) and Lanarkshire (7 cases). Two outbreak cases resident in Grampian had eaten from a Chinese food business in Lothian. Five outbreak cases resident in Shetland had all eaten from Chinese food businesses in Shetland.

After excluding secondary and asymptomatic cases, most of the remaining 31 outbreak cases of *S. enteritidis* PT6a infection lived in the west central belt of Scotland. Of these, 25 (81%) lived in either Lanarkshire (18 cases) or Greater Glasgow (7 cases): none lived in Shetland.

The ages of the 31 household primary outbreak cases of *S. enteritidis* PT5c infection ranged from 1–70 years, and for cases of *S. enteritidis* PT6a infection from 7–60 years. Sixteen (52%) of the 31 cases of *S. enteritidis* PT5c infection were young adults aged 18–40, as were 12 (39%) of the 31 cases of *S. enteritidis* PT6a infection.

One case of infection with *S. enteritidis* PT5c died, an elderly person whose date of onset was 1 June 2000. This was a household secondary case, whose spouse had been ill first, and was subsequently confirmed as having *S. enteritidis* PT5c infection. In total eight cases of *S. enteritidis* PT5c infection were reported to have been admitted to hospital. There were no deaths amongst cases of *S. enteritidis* PT6a infection, and only three known hospital admissions, but hospital details were not available for all cases.

By 11 August 2000, the hypothesis was formed that UK-acquired household primary infection with *S. enteritidis* PT5c and *S. enteritidis* PT6a was associated with consumption of food from Chinese food businesses, and that chicken dishes were an important vehicle of infection. No other hypothesis was identified that was worthy of testing. Of the 31 household primary outbreak cases of *S. enteritidis* PT5c infection, 25 (81%) had consumed food from at least 1 of

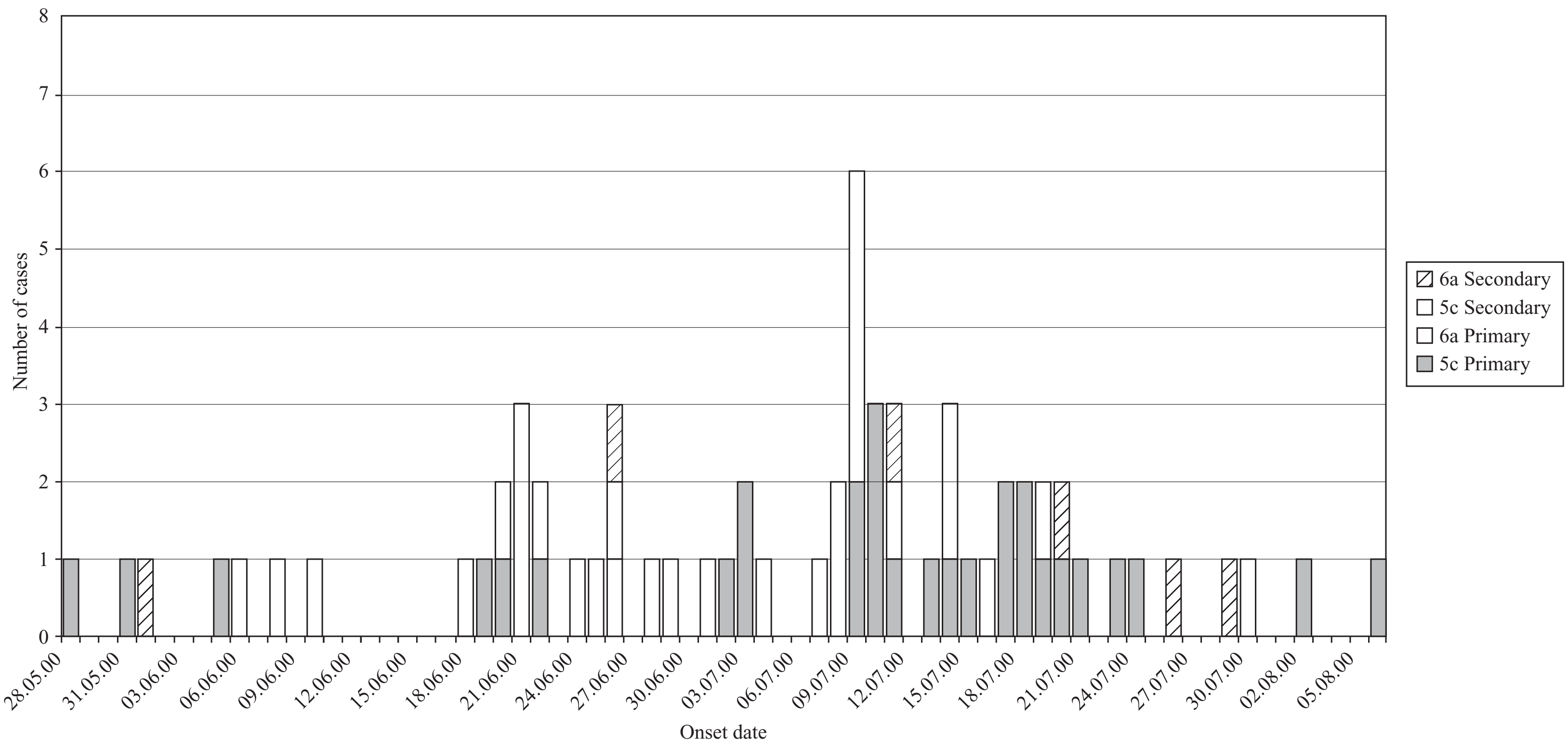

Fig. 1. Outbreak cases of *S. enteritidis* PT5c & PT6a infection: onset dates, where known ($n = 65$).

Table 1. *Results of matched case-control study*

Pathogen	Number of matched groups	Number of concordant matched groups	Number of discordant matched groups	Odds ratio	95% Confidence interval	*P*-value
S. enteritidis PT5c	19	8	11	> 12·7	1·83–552	< 0·001
S. enteritidis PT6a	17	6	11	9·5	1·2–74·6	0·032
S. enteritidis PT5c & PT6a	36	14	22	22·35	2·99–166·7	0·0024

13 Chinese food businesses. Nineteen (76%) of the 25 household primary outbreak cases of *S. enteritidis* PT5c infection who had eaten from Chinese food businesses, had eaten chicken.

Of the 31 household primary outbreak cases of *S. enteritidis* PT6a infection, 20 (65%) had eaten from at least 1 of 7 Chinese food businesses. Twelve (60%) of the 20 household primary outbreak cases of *S. enteritidis* PT6a infection who had eaten from Chinese food businesses, had eaten chicken.

Analytical

Matched case-control study. Of the 31 household primary outbreak cases of *S. enteritidis* PT5c infection, 28 had known onset dates and were available for interview. Matched controls were found for 19 of these cases. Of the 19 matched case-control groups, 8 were concordant, that is both the cases and their controls had consumed food from a Chinese food business during the relevant period. In all of the remaining 11 matched groups, the case had consumed food from a Chinese food business and the controls had not. Since all cases in the 11 discordant groups had been exposed to the risk factor and none of the controls had, the odds ratio is undefined. Introducing a hypothetical extra matched pair with the case not exposed and the control exposed produces an odds ratio lower than the true value. Employing this strategy, matched analysis was carried out using the conditional logistic regression programme available in EGRET [6]. This resulted in an odds ratio of 12·7 (95% CI: 1·83–552, $P < 0·001$). The true odds of exposure is therefore more than 12 times greater in the cases than in the controls. There was a highly statistically significant association between infection with *S. enteritidis* PT5c in household primary outbreak cases and consumption of food from Chinese food businesses (Table 1).

Of the 31 household primary outbreak cases of *S. enteritidis* PT6a infection, 27 had known onset dates and were available for interview. Matched controls were found for 17 of these cases. Of the 17 matched case-control groups, 6 were concordant. In the remaining 11 matched groups most, but not all, of the cases had consumed food from a Chinese food business during the relevant period and most, but not all, of the controls had not. Matched analysis using conditional logistic regression gave an odds ratio of 9·5 (95% CI: 1·2–74·6, $P = 0·032$). There was a highly statistically significant association between infection with *S. enteritidis* PT6a in household primary outbreak cases and consumption of food from Chinese food businesses (Table 1).

When all case-control study cases of *S. enteritidis* PT5c and PT6a infection are analysed together, the odds ratio is 22·35 (95% CI: 2·99–166·7, $P = 0·0024$).

Cohort study. Of the 31 household primary outbreak cases of *S. enteritidis* PT5c infection, 25 had eaten food from Chinese food businesses during the relevant period. During interviews with these cases we identified a further 23 people who had eaten food with them from Chinese food businesses. Of these 23, 2 were probable cohort cases. The 25 confirmed cases, the 2 probable cases, and 17 of the 21 other subjects reported their history of chicken consumption (Table 2).

Similarly of the 31 household primary outbreak cases of *S. enteritidis* PT6a infection, 20 had eaten food from a Chinese food business during the relevant period. During interviews with these cases we identified 25 people who had eaten food with them from Chinese food businesses. Of these 25, 7 were probable cohort cases. Nineteen of the 20 confirmed cases, the 7 probable cases, and 15 of the 18 other subjects reported their history of chicken consumption (Table 2).

Analyses addressing the cohorts of confirmed cases of *S. enteritidis* PT5c and PT6a infection and their asymptomatic eating companions, singly and combined, failed to show any statistically significant association between being a case and the consumption of chicken. This remained true when probable cohort

Table 2. *Results of cohort study*

Organism	Ate chicken: Confirmed case	Ate chicken: Probable case	Ate chicken: Non-case	Ate chicken: Attack rate (%)	Did not eat chicken: Confirmed case	Did not eat chicken: Probable case	Did not eat chicken: Non-case	Attack rate	Odds ratio	*P*-value
S. enteritidis PT5c	19	2	11	63* 66†	6	0	6	50* 50†	1·7* 1·9†	0·3* 0·3†
S. enteritidis PT6a	12	3	7	63* 68†	7	4	8	47* 58†	2·0* 1·6†	0·3* 0·5†
S. enteritidis PT5c & PT6a	31	5	18	63* 67†	13	4	14	48* 56†	1·9* 1·7†	0·2* 0·3†

* Calculated excluding probable cases.
† Calculated including probable cases.

cases were included in the cohorts (Table 2). As all of the odds ratios' 95% confidence intervals included 1·0, they are not presented.

Environmental

Three out of 20 Chinese outbreak food businesses were found to have important defects in their practices, processes, equipment or premises that could have led to cross-contamination of foods to be consumed [4]. No common source was identified for any food. No common primary or secondary food supplier was identified. No common poultry farm was identified, nor were Port Authorities able to specify any particular source of poultry to Chinese food businesses in Scotland.

Microbiological

S. enteritidis PT5c was isolated from three food samples and two environmental samples. The food isolates were from raw chicken, cooked chicken and raw eggs, from three different food businesses. The environmental isolates were from a floor swab and a dish-cloth, again from different food businesses.

The isolates of *S. enteritidis* PT5c from raw egg and from a dish-cloth were obtained from the same premises, those of a food business associated with cases of *S. enteritidis* PT6a infection which had been investigated prior to the national outbreak being identified. Isolates of *S. enteritidis* PT4 were also obtained from the same premises.

The review of recent surveillance data on the incidence of salmonella in animals and food in Scotland and the rest of the UK revealed no reports of either *S. enteritidis* PT5c or PT6a.

DISCUSSION

This investigation convincingly linked infection to eating from one of a number of apparently unconnected Chinese food businesses. The outbreak was unusual in being of two different and microbiologically distinct phage types of *S. enteritidis*, each of which was responsible for about half the cases, and which were epidemiologically very similar. Environmental and microbiological investigations were compatible with the hypothesis that was confirmed by a case-control study, which showed a highly statistically significant association between becoming a case and eating from a Chinese food business.

The cohort study consisted of identifying groups of people on the basis of their having eaten from a Chinese food business with a confirmed case. These groups were then considered as a single cohort, and unconfirmed, or probable, cases were identified within this single cohort. Because of this novel design, which may be deemed to have characteristics more akin to a case-control study, we employed odds ratios, a measure which is applicable to both designs, rather than relative risk which is applicable only to cohort studies.

The investigation did not identify chicken from Chinese food businesses as a specific food vehicle, possibly because the attack rate among those exposed to chicken was so low as not to result in a statistically significant difference between cases' and non-cases' consumption. Alternatively sample size, which was outside our control, may have been too small. We believe, however, that the likely explanation is that chicken was not the only vehicle. As cross-contamination in food businesses' kitchens is possible, and was indeed deemed to have been likely in some of them, the inability to identify a single food vehicle is unsurprising. While there was probably a single source of contamination, there may well have been numerous vehicles of infection. The epidemiological method is at its most powerful when seeking to identify a vehicle or vehicles of infection. The method is severely limited in its ability to identify the source from which the vehicle was contaminated, unless a link can be established with a number of vehicles containing a common component. In this outbreak environmental and microbiological investigations provided no direct evidence of such a source. Nonetheless we think it reasonable to conclude on the basis of biological plausibility that other vehicles were contaminated in the food businesses' kitchens from a raw, or inadequately cooked source, probably chicken. Whatever the explanation, the case-control study linking infection to Chinese food businesses supports the conclusion that defects in catering practice allowed salmonellas which entered the kitchens to contaminate a food or foods which were then served to customers.

Environmental and microbiological investigations were unable to identify any characteristics which distinguished Chinese food businesses that were associated with cases, from those which were not. Food businesses from which cases ate had no identifiable common supplies or practices. It remains unclear whether those Chinese food businesses at which cases ate were especially deficient in some respect or were merely unfortunate. It appears however that a source of salmonella entered their premises and was insufficiently heat treated, or was allowed to contaminate another vehicle or vehicles, or both. Whether the cause of the outbreak was the defect in catering practice or the entry of the salmonella into the kitchen on the source, which was probably raw chicken, is a matter of conjecture rather than fact.

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REFERENCES

1. Browning LM, Cowden JM, Edwards GFS, Reilly WJ, Mather H. Salmonella in Scotland 2000. SCIEH Weekly Report 2001; **35**: 306–9. http://www.show.scot.nhs.uk/scieh/PDF/pdf2001/0149.pdf accessed 3 April 2002.
2. Anonymous. Surveillance report: gastrointestinal infections. SCIEH Weekly Report 2000; **34**: http://www.show.scot.nhs.uk/scieh/PDF/pdf2000/0002.pdf accessed 3 April 2002.
3. Cowden J, Locking M. Surveillance report: gastrointestinal infections. SCIEH Weekly Report 2000; **34**: 82–3. http://www.show.scot.nhs.uk/scieh/PDF/pdf2000/0014.pdf accessed 3 April 2002.
4. Donaghy M (Chairman). Report of the outbreak control team with responsibility for managing the national outbreak of *Salmonella enteritidis* phage type 5c and *Salmonella enteritidis* phage type 6a, May–August 2000. Food Standards Agency (Scotland), Aberdeen, 2000.
5. SPSS for windows. Chicago: SPSS Inc., 2000.
6. EGRET for windows. A software package for the analysis of biomedical and epidemiological studies. Cambridge, MA: Cytel Software Corporation, 2001.