National outbreak of *Salmonella* Java phage type 3b variant 9 infection using parallel case–control and case–case study designs, United Kingdom, July to October 2010

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Between July and October 2010, a national outbreak comprising 136 cases of *Salmonella* Java phage type 3b variant 9 was identified by the Health Protection Agency. Most cases were female. Cases had a median age of 39.5 years and lived in London, the South East and East of England. Parallel case–control and case–case study designs were undertaken to test the generated hypotheses. The case–case study aimed to examine if the infection was associated with eating food items purchased from commercial catering settings, and the reference group comprised non-travel related cases of *S*. Enteritidis infected during the same time period as the cases. The case–control study was designed to examine if the infection was associated with specific food items purchased from commercial catering settings, and recruited case-nominated controls. However, in response to poor recruitment we adapted our methods to investigate food exposures in the same way. Results of epidemiological investigations are compatible with salad vegetables as the potential source, but no common suppliers of salad were identified and no organisms were isolated from environmental and food samples. Limitations in the case–control study highlight the potential value of using a combination of epidemiological methods to investigate outbreaks.

Introduction

Salmonella enterica Paratyphi B variant Java shares the same somatic and flagellar antigens as other *S*. Paratyphi B variants, but utilises d-tartrate as a carbon source. *S*. Java is thought to be less virulent than non d-tartrate utilising *S*. Paratyphi B, with infections characterised by watery diarrhoea, abdominal pain and fever. However, infection can also be invasive, producing typhoid-like clinical symptoms [1].

S. Java has an animal reservoir. It is present in poultry flocks in the European Union and is the most common serovar reported in poultry in the Netherlands [2,3]. A recent increase in the incidence in poultry has also been reported in Germany [4]. Outbreaks of *S*. Java have been reported in the past, associated with salad vegetables, goat's milk cheese, poultry, reptiles and tropical fish aquariums [4-8]. *S*. Java is an uncommon cause of salmonellosis in the United Kingdom (UK), with 151, 112 and 130 cases reported in 2007, 2008 and 2009 respectively according to the national database.

In 2007, a multi-country outbreak of *S*. Java phage type (PT) 3b variant 9 (var9) involved cases in Denmark, Finland, the Netherlands, Norway, the UK and the United States (US). Epidemiological evidence suggested an association with salad vegetables [9].

Outbreak description

Between 27 July and 1 October 2010, 136 cases with *S*. Java PT 3b var9 were reported for the UK by the Laboratory of Gastrointestinal Pathogens (LGP) at the Health Protection Agency (HPA), compared to five in 2009 and one in 2008 (Figure). The LGP routinely receives isolates of *Salmonella* species for testing from local laboratories in England and Wales, and this is the basis of routine national surveillance. The outbreak strain was fully susceptible to the LGP panel of antimicrobial agents and had the pulsed-field gel electrophoresis (PFGE) profile SPTJXB.0001.

Cases were non travel-related. Isolates had been submitted to the LPG from most regions, with predominance in the East of England, London and the South East. The majority of cases were female (82/130) and the median age was 39.5 (interquartile range: 24–53). The on-going and widespread nature of the outbreak

indicated exposure to the outbreak strain of *Salmonella* through a widely distributed source. The outbreak of Java PT 3b var9 was notified by the LPG on 18 August 2010 and an immediate investigation was launched to identify the source.

Methods

Microbiological investigation

Local clinical microbiology laboratories referred all presumptive isolates of *S. enterica* to the LGP in the HPA Department of Gastrointestinal, Emerging and Zoonotic Infections (GEZI) for confirmation and characterisation. Isolates were sero-typed, phage-typed and screened for antimicrobial susceptibility. Pulse Field Gel Electrophoresis (PFGE) was performed on all *S.* Java PT 3b var9 isolates to reveal if the strain type identified was the same in all isolates.

Epidemiological investigation

Hypothesis generation

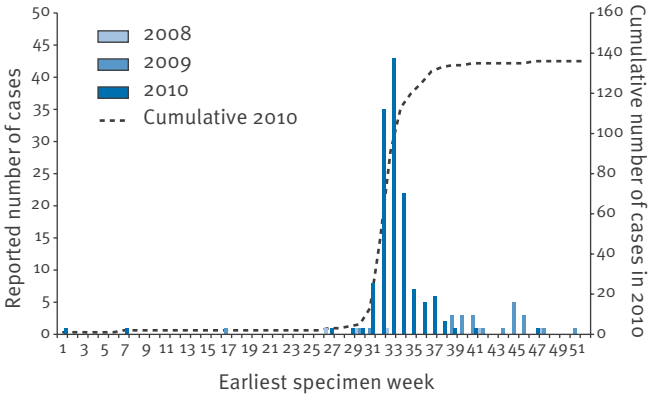
Trained interviewers (medical registrars and epidemiological scientists) based at the HPA Centre for Infections (CfI) interviewed 11 non travel-related cases of fully sensitive *S.* Java PT 3b var9, using a detailed standard *S. enterica* trawling questionnaire between 20 and 24 August. The 29 page long trawling questionnaire conducted over the phone collected an extensive food history for the five days before the onset of illness and comprised also detailed questions about salad vegetables, including sprouted seeds, herbs, salad dressing and pickles. Any exposures reported by eight or more cases were considered eligible for inclusion in an analytical study.

Analytical epidemiology

The generated hypotheses were tested using a case–control study with case-nominated controls. A parallel case–case study was also carried out using laboratory-confirmed cases of *S.* Enteritidis infected at the same time period as the *S.* Java PT 3b var9 cases.

Figure

Weekly reported cases of non-travel related fully sensitive *Salmonella* Java phage type 3b variant 9 from 2008 to 2010 and cumulative incidence for 2010, United Kingdom, 1 January 2008–31 December 2010



The two strategies were developed to test different aspects of the generated hypotheses. The case–case study was designed to examine if the risk of infection was associated with eating food obtained from commercial catering settings referred to as 'eating away from home', while the aim of the case–control study was to examine if the infection was associated with the consumption of specific food items eaten away from the home.

A secondary aim of undertaking the case–control and case–case studies was to compare and contrast the usefulness of these two methods in recruiting controls for the investigation of national outbreaks.

Case definition and controls

For the analytical epidemiological investigation a case of *S.* Java PT 3b var9 was defined as a primary non travel-related symptomatic adult of 18 years of age or older, resident in England, infected with *S.* Java PT 3b var9 (PFGE: SPTJXB.0001) confirmed by LGP since 27 July 2010 and fully sensitive to the LGP panel of antimicrobial agents.

Note that the study was restricted to people aged 18 years and older to reflect the age distribution of cases (less than 15% of all cases were under 18 years old).

Case-nominated controls were sought, and were defined as case-nominated individuals 18 years or older, and who had not: (i) experienced an episode of gastrointestinal illness in the seven days before interview, (ii) travelled outside the UK in the seven days prior to the date of the interview, (iii) shared a household with an individual with any gastrointestinal illness.

A *S.* Enteritidis case was defined as a primary non travel-related symptomatic adult of 18 years of age or older, infected with *S.* Enteritidis as confirmed by LGP since 27 July 2010, and resident in England.

Note that *S.* Enteritidis cases were used as a comparison group because there is no reason to believe that the eating habits of *S.* Enteritidis cases are different to those of the general population and it is unlikely that the exposures of interest would be under- or over-represented in these cases.

Case–case and case–control questionnaires

Standard structured case–control and case–case questionnaires were designed and administered to all subjects by telephone interview. The case, case–control and case–case questionnaires contained questions related to the same exposures identified in the hypothesis generation.

All cases were interviewed by trained staff from CfI and all interviewers were fully briefed on the questionnaire and interviewing technique. Up to three attempts to contact subjects were made at different times of the

day or evening. One case-nominated control and one *S*. Enteritidis reference cases were sought per case.

Statistical analysis

The case–control and case–case studies were analysed separately. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for all exposure associations with the outcome variable (caseness) tested in univariable analysis using the Chi-squared and Fisher's exact tests. Exposures were tested singularly and also grouped into broader categories.

Exposures with an estimated OR>1 and a p<0.2 were deemed eligible for inclusion in the multivariable analyses: multivariable logistic regression analysis for the case–case study and a multivariable conditional logistic regression analysis for the case–control study. Age and sex were controlled for in the multivariable analysis.
This conservative inclusion criterion for the multivariable analysis was selected to avoid the exclusion of exposures that are falsely non-significant.

Results

Epidemiological Investigation

Hypothesis generation

Analysis of the trawling questionnaires identified the following common (identified in eight or more cases) exposures: contact with domestic cats, eating food obtained from commercial catering settings (i.e. eating away from home), eating lettuce/salad leaves, tomatoes, cucumbers and prawns/scampi, buying food from a given supermarket chain "F".

On the basis of this evidence, analytical epidemiological studies were designed to test the null hypotheses that infection with *S*. Java PT 3b var9 was not associated with:

- Contact with domestic cats,
- Eating food obtained from commercial catering settings (restaurants: table and take away, hotels, pubs etc),
- Eating lettuce/salad leaves,
- Eating tomatoes,
- Eating cucumbers,
- Eating prawns/scampi,
- Buying food from a given supermarket chain "F";
- Buying lettuce/salad leaves from a given supermarket chain "F".

Analytical Investigation

One hundred and thirty six cases with the outbreak strain *S*. Java PT 3b var9, PFGE profile SPTJXB.0001, were reported by the LGP. Of these, 11 were interviewed during trawling and therefore excluded from the analytical investigation. A further 29 cases did not meet the case definition (12 were too young, nine were not resident in England, seven had a history of foreign travel and one case was a secondary case). Of the remaining 96 cases, four did not want to participate, contact could not be made with 25 cases and a further 19 were awaiting follow up information from local Health Protection Units including confirmation of name and contact details and consent for inclusion in the study.

Forty eight cases of *S*. Java were successfully interviewed, the median age was 44.5 years (interquartile range: 31–53), 34/48 cases were female. The median age of individuals who met the case definition but did not participate in the study (excluding cases already interviewed for the hypothesis generation) was 36 years (interquartile range: 22–56) and 29/48 were female. There was no difference in the age distribution of the two groups (p=0.52).

The most common symptoms in the cases were diarrhoea (48/48 of cases questioned), abdominal pain (42/48) and fever (37/48) and eight cases questioned were hospitalised during their illness. The date of onset of illness for interviewed cases ranged from 10 July to 31 August with the majority cases reporting an onset date between 16 and 22 August.

Twenty nine case-nominated controls were successfully interviewed, the median age of 49 years (interquartile range: 33–56 years) was similar to that of cases and 20/29 were female.

One hundred and twenty two cases of *S*. Enteritidis were identified for the same time period. Fifty cases were travel-related, 33 could not be contacted and 10 cases did not meet the eligibility criteria; the remaining 29 were interviewed. The median age of *S*. Enteritidis cases was 45 years (interquartile range: 29.5–59 years), 17/29 cases were female.

We initially intended to carry out the case–control and case–case study designs to investigate different hypotheses. In response to the poor recruitment of case-nominated controls however, we adapted our methods to investigate food exposures in the same way.

Statistical analysis

Case–control study

Based on the results of the crude analysis, no exposure was found to satisfy the criteria of an odds ratio higher than 1 and p<0.2 and so multivariable analysis was not undertaken. Furthermore, no grouped exposure satisfied these criteria. The exposures with OR>1 were 'eating out – cucumber' (OR: 1.65, 95% CI: 0.53–5.06) and 'takeaway – salad leaves'(OR: 1.81, 95% CI: 0.58–5.55).

Case–case study

Single variable analysis found 12 single and seven grouped exposures that had an odds ratio higher than 1 and p<0.2 (Table 1 and 2 respectively).

The multivariable analysis of single exposures from the case–case study indicates a significant association

between symptomatic infection of *S.* Java PT 3b var9 and eating out at restaurants, eating pre-packaged mixed salad leaves at home as well as consumption of salad leaves from takeaway restaurants (Table 3).

The multivariable analysis of the grouped food exposures from the case–case study indicates the only exposure associated with being a *S.* Java case was 'eat home or out – any salad leaves' (Table 3), whereas there was no evidence of association for 'eat home or out – scampi' (OR: 7.38, 95% CI: 0.70–78.38, p=0.057).

Discussion

On 27 July 2010, a national outbreak of *S.* Java PT 3b var9 took place in the UK and an investigation was initiated on 18 August. The cases were distributed across the country with initial analysis of stool samples undertaken by independently operated local clinical microbiology laboratories. *Salmonella* isolates were then referred to the HPA LGP reference laboratory for further typing. There is a necessary delay between a patient experiencing symptoms and the HPA becoming aware of the case. This delay is dependent on how quickly a case presents to healthcare, how quickly samples are taken and the isolation, referral and typing of *Salmonella* samples. However, the centralised laboratory and national surveillance system provided prompt identification of a nationwide increase in cases and enabled a timely nationally coordinated response.

The number of new cases of *S.* Java PT 3b var9 and *S.* Enteritidis diminished considerably over the course of the study restricting the recruitment of new cases and reference cases for the investigation and the outbreak control team closed the investigation on the 8 October 2010, 11 weeks after the first case was reported by LGP.

In total only half (48/96) of the eligible cases of *S.* Java PT 3b var9 were included in the analysis and there is

Table 1

Single variable analysis for single exposures with odds ratio>1 and p<0.2, case–case study, *Salmonella* Java phage type 3b variant 9 outbreak, United Kingdom, July–October 2010 (n=77)

Exposure	Exposed		Not exposed		Odds ratio	95% confidence interval	p-value
	Salmonella Java n (%)	*Salmonella* Enteritidis n (%)	*Salmonella* Java n (%)	*Salmonella* Enteritidis n (%)			
Travelling within United Kingdom	12 (26)	0 (0)	35 (74)	28 (100)	1.00	2.39–∞	0.003
Daytrips	7 (16)	1 (4)	37 (84)	27 (96)	5.11	0.76–∞	0.139
Cat at home	16 (34)	5 (17)	31 (66)	24 (83)	2.48	0.82–7.43	0.112
Other pets at home	9 (20)	2 (7)	35 (80)	27 (93)	3.47	0.77–∞	0.182
Eating out – restaurant	32 (67)	13 (45)	16 (33)	16 (55)	2.46	0.97–6.28	0.060
Eating out – salad leaves	17 (36)	4 (14)	30 (64)	25 (86)	3.54	1.09–11.3	0.039
Eating out – tomatoes	13 (28)	3 (10)	33 (72)	26 (90)	3.41	0.93–12.29	0.085
Eating out – cucumber	12 (26)	3 (10)	35 (74)	26 (90)	2.97	0.81–10.75	0.142
Takeaway – salad leaves	13 (28)	3 (11)	33 (72)	24 (89)	3.15	0.86–11.38	0.142
Eat at home – mixed salad	14 (30)	3 (11)	33 (70)	25 (89)	3.54	0.97–12.66	0.086
Eat at home – scampi	7 (15)	1 (3)	39 (85)	28 (97)	5.03	0.75–∞	0.141
Other supermarket chains than chain "F"	9 (22)	2 (7)	31 (78)	27 (93)	3.92	0.86–∞	0.104

Table 2

Single variable analysis for grouped exposures with odds ratio>1 and p<0.2, case–case study, *Salmonella* Java phage type 3b variant 9 outbreak, United Kingdom, July–October 2010 (n=77)

Exposure	Exposed		Not exposed		Odds ratio	95% confidence interval	p-value
	Salmonella Java n (%)	*Salmonella* Enteritidis n (%)	*Salmonella* Java n (%)	*Salmonella* Enteritidis n (%)			
Eating out – salad leaves	25 (56)	7 (26)	20 (44)	20 (74)	3.57	1.28–9.91	0.014
Eating out – tomatoes	18 (41)	6 (22)	26 (59)	21 (78)	2.42	0.83–6.98	0.106
Eating out – cucumber	19 (45)	4 (13)	23 (55)	26 (87)	4.20	1.29–13.47	0.019
Eat home or out – any salad leaves	40 (87)	14 (50)	6 (13)	14 (50)	6.67	2.19–20.18	0.001
Eat home or out – tomatoes	29 (67)	13 (45)	14 (33)	16 (55)	2.55	0.98–6.67	0.056
Eating home or out – cucumber	25 (57)	8 (30)	19 (43)	19 (70)	3.13	1.14–8.51	0.026
Eating home or out – scampi	13 (29)	3 (10)	32 (71)	26 (90)	5.28	1.2–∞	0.036

potential that the individuals who did not participate were systematically different from those who did. The age and sex distributions of the cases of *S.* Java PT 3b var9 included in the study were similar to the distributions in the total population of cases, however, it is possible that the study population was not representative of the total population of cases for reasons not considered in this study.

The recruitment of controls for both studies was also challenging, which may have resulted in biases in the individuals included in the study.

Comparison of case–control and case–case study designs

The case-nominated control design is considered a useful way of rapidly recruiting matched controls [10]; however, this method was not successful in this study for a number of reasons. Many cases were reluctant to provide contact details for friends and colleagues without prior consent from these individuals. In some instances, cases were willing to participate in the study but did not have any friends or colleagues to nominate as controls.

Four cases were only able to nominate controls from the same household, a potential for bias as the case and control may have shared the activities/exposures under investigation [11]. This may have resulted in the cases and controls being overmatched. There is also the possibility of recall bias amongst case-nominated controls that may have been aware of the hypothesis under investigation.

The finding that case-nominated controls may be hard to recruit is in keeping with experience from previous studies [12,13]. This suggests that other strategies need to be employed for selecting controls and that case-nominated controls should only be used where alternative methods cannot be readily identified.

The case–case comparison has previously been developed from the case–control methodology, and in this study we found that it was quicker and easier to recruit reference case–controls as compared with the case-nominated controls [14].

The case–case study was more advantageous than the case–control study in the investigation of this outbreak for a number of reasons. The demographic details of *S.* Enteritidis cases were already available from laboratory reporting, allowing the case–case study to be undertaken much faster than the case–control study.

The use of reference cases allows investigators to select controls randomly from the total population of controls as opposed to the selection of case-nominated controls which is prone to selection bias.

The inclusion of previously ill controls may introduce potential bias in the study and selection bias may have occurred with this study design if historical reference cases were recruited because exposures such as dietary habits and behaviour may have changed with time. This was avoided through the recruitment of reference cases that were infected in the same period of time as the *S.* Java PT 3b var9 cases.

There is the potential for overmatching of cases and reference cases in the case–case study design. This could lead to type II error i.e. high number of false negative associations. To avoid this, the choice of controls was carefully considered to ensure that the exposures under investigation would not be over-represented in the control group.

Conversely bias may be introduced if the reference cases selected are less likely to be exposed to food items under investigation. This can cause type I error i.e. false positive associations. However, given that *S.* Enteritidis has been isolated in a wide variety of food items we believe it is unlikely that reference cases can have different dietary patterns than the rest of the population.

For these reasons it is unlikely that the recruitment of reference cases would have produced a bias in the investigation of this outbreak.

Table 3

Multivariable analysis for single and grouped exposures with odds ratio>1 and p<0.05, *Salmonella* Java phage type 3b variant 9 outbreak, United Kingdom, July–October 2010

Variable	Multiple variable analysis		
	Odds ratio	95% confidence interval	p-value
Eating out – restaurant	3.72	1.03–13.39	0.038
Takeaway – salad leaves	6.92	1.08–44.2	0.021
Eat home – pre-packaged mixed salad	7.70	1.31–45.38	0.012
Eat home or out – any salad leaves	5.87	1.31–30.89	0.030

An odds ratio of one, and baseline 95% confidence interval were considered in the absence of exposure.

Salad vegetables

The results of the case–case study confirmed a significant association between symptomatic infection of *S.* Java PT 3b var9 and eating out at restaurants, eating pre-packaged mixed salad leaves at home, consumption of salad leaves from takeaway restaurants and eating any salad leaves either at home or purchased from commercial catering settings. Since salad is often used as a garnish in meals eaten in commercial catering settings, it is possible that the model underestimated the proportion of cases who consumed salad leaves away from home.

We cannot exclude the possibility that the study may have missed the right vehicle of the outbreak such as

sprouted seeds which have been implicated in two recent outbreaks in Europe [15,16]. It is likely that the consumption of smaller food items (seeds, sprouted seeds and herbs) in salads prepared by commercial caterers was not remembered or was not noticed by cases. None of the smaller salad items were found to be associated with cases during the hypothesis generation. It is possible that salad leaves were a confounding factor in this investigation and smaller, less memorable items should be considered in outbreaks where salad vegetables appear to be implicated.

Environmental investigations did not identify common suppliers of salad vegetables and the short shelf life of salad vegetables limited the ability to acquire any suspect foods for microbiological analysis.

The consumption of fresh and bagged salad vegetables across the globe has increased in the last twenty years. In the US there was a 9% increase between 1996 and 2005 as compared with the previous decade, however, outbreaks associated with these food items have increased by 38.9% during this the same time period [17]. In Europe there have been a number of country-wide and region-wide *Salmonella* outbreaks attributed to locally produced and imported salad greens [18].

The contamination of salad leaves and salad vegetables during their production and processing has been implicated in a number of geographically widespread outbreaks [19]. High risk practices during production and processing include the use of contaminated water either to irrigate the crops, to apply pesticides or other dressings, or to wash the crop once harvested; the use of human or animal sewage as a crop fertiliser; and the transport of the harvested crop in a contaminated vehicle/storage system, e.g. trucks previously used for transporting waste [20]. Crops growing in the field are also vulnerable to contamination from sources such as wild animals and birds [21].

The mild processing and packaging of these food items produce an environment that encourages the proliferation of bacteria transferred onto the vegetable surface during the growing period [22,23].

Gastrointestinal infection associated with salad vegetables may also be the result of cross-contamination from poultry, meat or meat products or contamination by the food handler during food preparation in the home or in catering establishments. A review of more than 2,000 general food-borne outbreaks from 1992 to 2006 undertaken by the HPA found that 4% of them were associated with prepared salads. The review found that most of the outbreaks linked to salads occurred in the catering sector and were associated with infected food handlers, cross-contamination and poor storage [24]. A study of sporadic cases of campylobacter infections in Wales found that infection was associated with specific salad vegetables because extensive handling required during preparation and use of a chopping board increased their likelihood of becoming contaminated [25].

The increase in illness and outbreaks associated with the consumption of fresh ready to eat salad vegetables indicates the ongoing need to improve methods in the production and preparation of these foods to reduce the potential for contamination with *Salmonella* and other enteric pathogens [26-28].

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