# FOODBORNE OUTBREAK OF *SALMONELLA* BAREILLY IN THE UNITED KINGDOM, 2010

# REPORT OF THE UK OUTBREAK CONTROL TEAM

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JULY 2011

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## TABLE OF CONTENTS

## EXECUTIVE SUMMARY

We report the findings of the investigation of an outbreak of food borne *Salmonella* Bareilly infection in the United Kingdom in 2010. Between August and mid December 2010 (weeks 30 to 50), 220 laboratory confirmed outbreak cases of *S.* Bareilly infection (excluding travel related cases and infections with non outbreak strains of *S.* Bareilly) were identified across England, Wales, and Northern Ireland. An additional 21 cases were identified in Scotland, bringing the UK total to 241 cases.

Epidemiological and microbiological investigations implicated bean sprouts as a vehicle for *S.* Bareilly transmission, consistent with previous outbreak investigations which identified bean sprouts as a vehicle for *Salmonella* transmission.[1,2,3,4] A case control study showed that consumption of bean sprouts was significantly associated with being a case (odds ratio 6.8, 95% confidence interval 1.4 to 33.0). *S.* Bareilly with an indistinguishable pulsed field gel electrophoresis (PFGE) pattern from outbreak cases was also detected in a number of samples of commercially produced bean sprouts.

As in previous outbreaks, this investigation concluded that the seeds were likely to have been contaminated at source prior to importation.[5] The investigation found that the method used for routine microbiological quality control testing of bean sprouts may not be sensitive to low levels of *Salmonella* contamination. This may have implications for future testing protocols.

Public health interventions resulting from this investigation focused mainly on communications to the general public and to environmental health professionals advising of the correct preparation of bean sprouts, and on improving information on food labels where this was ambiguous. The investigation concluded that raising public awareness to ensure the correct preparation of raw bean sprouts during cooking was the principal means of minimising risk to consumers and reducing further cases.

The outbreak was declared over on the 21st January 2011, when reports of cases of *S.* Bareilly had fallen to background levels.

## INTRODUCTION

Between August and December 2010, there was a large outbreak of food borne *S.* Bareilly infection affecting all parts of the United Kingdom, with a total of 241 outbreak cases, including one death. This document describes the findings of the investigation and the measures taken to protect public health.

## BACKGROUND

*Salmonella* is a genus of Gram negative bacteria from the family Enterobacteriaceae and has two species, of which *Salmonella enterica* is the commoner. Serological characterisation of bacterial somatic (O) and flagellar (H) antigens allows *Salmonella enterica* to be subdivided into over 2,400 different subtypes known as serovars.[6] Individual strains of a particular *Salmonella* serovar can be identified using a molecular technique known as pulsed field gel electrophoresis (PFGE).

*Salmonella enterica* subsp. *enterica* serovar Bareilly (O antigens 6, 7, 14; H antigens y: 1, 5), usually referred to as *Salmonella* Bareilly, is a group C1 serovar which was first identified in India in 1928.[7] From 2005 to 2009, an average of 53 cases were reported each year in England, Wales and Northern Ireland, of which 31% were attributable to travel to India. In Scotland in a typical year three to five cases occur. The proportion of cases related to travel is likely to be an underestimate because of underreporting.

*Salmonella* infection is one of the commonest causes of gastroenteritis in humans and may cause sizable outbreaks, particularly when transmitted in contaminated foodstuffs. The infective dose is usually between 1,000 and 100,000 organisms (but may be lower for some foodstuffs and for vulnerable people) and most cases occur within 12-36 hours of ingestion of bacteria. The clinical features typically include diarrhoea, with or without nausea, vomiting, abdominal pain and fever. Groups at greatest risk of infection include the young, the old, and patients with low gastric acidity. Although usually confined to the gastrointestinal tract, systemic infection may occur. Infection may be severe or fatal in elderly, pregnant and immunosuppressed cases.

Contaminated foods which have been associated with the transmission of *Salmonella* include meat (especially poultry meat), eggs, imported foods and fresh produce such as salad vegetables. Cross contamination of other foods may occur. Of food samples sent to the Salmonella Reference Unit since 2004, *Salmonella* Bareilly has been most commonly isolated from spices.

Consumption of bean sprouts has previously been associated with outbreaks of *Salmonella* infection (including *S.* Bareilly).[1,2,3,4] In 1988 there was an outbreak of 143 confirmed cases of *Salmonella* Saint-Paul infection in the United Kingdom.[1] A case control study provided evidence of an association with the consumption of bean sprouts. The outbreak strain of *S.* Saint-Paul was also isolated from samples of bean sprouts on retail sale in different cities in the UK, as well as from mung bean seeds sampled from the premises of the producer most strongly linked to cases. The investigation concluded that the imported seeds were contaminated with *S.* Saint-Paul and that measures taken during the sprouting process did not reliably eliminate the organism. This UK

outbreak strain was indistinguishable from that responsible for another outbreak in 1988 associated with the consumption of bean sprouts which was reported from Sweden.

Sprouted seeds (mainly alfalfa) were implicated in several outbreaks of salmonellosis between 1973 and 1998. Between 2000 and 2002, seven such outbreaks were reported from the US and Canada.[5] Epidemiological investigations identified associations between infection and consumption of raw mung bean sprouts. In two investigations the outbreak strain was isolated from environmental specimens: from spent irrigation water and a drain in one outbreak; and from harvested sprouts in another. In four outbreaks the seeds were traced to China and in three to either China or Australia. The investigations found no evidence that either sprout growers or their employees were the source of contamination. The findings in two outbreaks were consistent with contaminated seed rather than environmental contamination at the premises of the sprout growers.

The probable vehicle of infection in an outbreak of salmonellosis in a restaurant in Sweden in 2006 was mung beans, soaked in lukewarm water for 24 hours to give them a soft consistency before serving. *S.* Bareilly and *Salmonella* Virchow were isolated from cases. Mung beans had been included in all dishes served at the restaurant. The outbreak ended after bean sprouts were excluded from the menu.[8]

Bean sprouts follow a complex path from farm to table that includes growing, harvesting, processing and exportation of seeds to the country in which sprouting and distribution of the finished product takes place. Contamination can occur at any point of production and distribution. Bean sprouts are produced at temperatures of 20-30$^{o}$ Celsius under intermittent irrigation and the sprouting process is a potent bacterial amplification step that occurs shortly before marketing and consumption. Disinfection of seeds reduces bacterial load, but *Salmonella* can be internalised in sprouts during sprouting and external cleaning of sprouts may not eliminate contamination with *Salmonella* and other organisms.[9,10,11,12,13] As a result, many bean sprouts purchased commercially are raw products which are not ready to eat although they are safe to eat if prepared appropriately. Those bean sprouts which are sold as ready to eat are required to have been produced from good quality seeds with good husbandry.

## INVESTIGATION OF THE OUTBREAK

### OUTBREAK NARRATIVE INCLUDING KEY DATES AND EVENTS

On the 17$^{th}$ August 2010, Cumbria and Lancashire Health Protection Unit (CLHPU) informed Greater Manchester Health Protection Unit (GMHPU) of a possible outbreak of *Salmonella* infection among guests who had attended a Jewish wedding reception at a function facility in the Greater Manchester area of the North West region of England on the 8$^{th}$ August 2010. One guest had become unwell on the day following the reception and had been admitted to hospital in Blackburn with a diagnosis of *Salmonella* infection. According to initial reports, 12 guests were symptomatic, three of whom had been hospitalised. Three of these cases had been diagnosed with *Salmonella* infections, of whom two had preliminary microbiology results indicating that a group C *Salmonella* had been isolated (later further characterised as *S.* Bareilly).

GMHPU convened a local outbreak control team (OCT) to manage and investigate the incident and declared a level 2 incident on the Health Protection Agency (HPA) Incident Reporting Information System (IRIS). Subsequent investigations identified a total of 10 microbiologically confirmed cases of *S.* Bareilly among wedding guests, and over 30 guests with symptoms fitting the probable case definition (see page 13) but without microbiological confirmation of *S.* Bareilly infection. Four confirmed cases were admitted to hospital, one of whom died.

The wedding reception was externally catered for by a caterer based in the North West of England (hereafter referred to anonymously as Caterer 9) consisting of a mini market and delicatessen for which food was cooked on the premises. Local environmental health officers (EHOs) had not previously been aware that the delicatessen was also catering for large functions. Although assurances were given that food safety checks had been carried out, no documented food safety management system was in place at the premises. EHOs did not however identify any food hygiene breaches likely to have caused the outbreak. At the time of writing, completion of the investigation of the premises has been delayed by ongoing consideration of legal issues, but interim results of the investigation show that no *Salmonella* was detected from environmental swabs taken from the premises. At the time the swabs were taken, cleaning would have taken place at the premises a number of times since the food had been produced for the wedding. No leftover food from the wedding remained for microbiological analysis. No illness had been reported among food handlers at the premises and so none were screened for *Salmonella* infection.

The caterer had been scheduled to cater for a further function at the end of August. Although it was of concern to the local OCT that the cause of the outbreak had not yet been identified, the caterers had complied with all measures advised by the investigating EHOs and there were no grounds to issue a prohibition notice to prevent catering for the upcoming function. Local EHOs had extensively assessed the premises, food hygiene processes and suppliers prior to this event and continued to closely monitor food practices at the caterers, particularly over the weekend during the preparation of food for the function.

Food was also supplied to the wedding reception on the 8$^{th}$ of August from two other sources. Caterer 13, an unregistered home caterer, had supplied sandwiches with a variety of fillings. EHOs from the local authority inspected the facilities of this business and took environmental swabs from a number of areas, from which no *Salmonella* was detected. This business had neither a documented food safety management system nor adequate evidence of training in food hygiene. During the investigation of these premises, a Salford EHO obtained a list of several other unregistered local caterers to Jewish functions. Subsequent visits to several such caterers by Salford Environmental Health identified a lack of awareness of statutory food hygiene requirements of catering for large functions and a briefing session was planned to address this.

A registered home caterer in Salford, Caterer 7, supplied platters of vegetable crudités with dips to the wedding. EHOs inspected the kitchen facilities of Caterer 7 and took swabs from food samples and the environment, all of which were negative for *Salmonella*.

The function facility premises were also inspected by EHOs, but there were no findings of concern.

Information from initial interviews with cases of disease indicated that all had eaten the menu items teriyaki salmon, chicken soup, roast chicken, roast potatoes, and roast vegetables and so the local OCT hypothesised that one of these foods could be the common food vehicle of the outbreak. A large amount of chicken had been prepared and cooked for the reception in a small kitchen on the premises of Caterer 9, raising the possibility of cross contamination. A large number of eggs had been broken on the premises by a member of the Beth Din (rabbinical court of Judaism) in accordance with the requirements for kosher food. The chicken used at Caterer 9 was sourced from a Meat Hygiene Service approved premises which tested regularly for *Salmonella* and from which samples taken on the 9th August 2010 were negative for *Salmonella*. All eggs used at Caterer 9 were British Lion marked, indicating flocks were vaccinated against *Salmonella* Enteritidis.

The salmon teriyaki dish contained noodles, pak choi and bean sprouts, which had been added at a late stage in the cooking and warmed through rather than cooked. The bean sprouts had originated from an established producer in the North West (Producer 1; see Figure 7).

A case of *S.* Bareilly infection was identified who had not attended the wedding, but who prior to illness had purchased food from Caterer 9 including chicken and a side dish described as containing noodles, which may also have contained bean sprouts.

It was hypothesised that teriyaki salmon, chicken soup, roast chicken, roast potatoes, and roast vegetables were possible vehicles of infection and a cohort study was undertaken with the cooperation of the father of the bride. The results of the cohort study (see page 14) provided some evidence of an association of illness with consumption of roast chicken, but no evidence of an association of illness with the teriyaki salmon dish. The study was regarded as possibly biased due to the difficulties of data collection and was therefore regarded as inconclusive as to the precise food vehicle of infection, although food borne infection at the wedding reception was the only plausible cause of the outbreak.

The cause of death of the case who died was certified as pneumonia with *Salmonella* gastroenteritis as a contributory cause. The Manchester coroner opened and adjourned an inquest into the death which at the time of writing has now reopened. On the advice of the coroner the police were offered the opportunity to attend outbreak control team teleconferences as observers. The death received some coverage in the regional media.

On the 2nd and 3rd September 2010, the Scottish Salmonella, Shigella and Clostridium Difficile Reference Laboratory (SSSCDRL) reported to Health Protection Scotland the identification of 10 confirmed cases of *S.* Bareilly infection of indistinguishable PFGE type across three NHS Board areas in the central belt of Scotland. These were the first reports of this serovar in Scotland during 2010. The PFGE profile for the Scottish cases was indistinguishable from that of the English cases. The onset dates of the Scottish cases ranged between the 3rd and the 24th of August 2010. EHOs from Edinburgh City Council established that three of five cases reported

from Lothian NHS Board area had eaten at a canteen in Edinburgh (Caterer 14). All three had eaten salad leaves supplied by Wholesaler 5, based in the North West of England.

Because of the increase in Scotland of the number of reports of *S.* Bareilly in cases with no point source exposure HPS convened a Scottish national outbreak control team on the 6th September 2010, including a representative from Health Protection Agency North West (HPA NW). As cases of *S.* Bareilly infection had now been detected in both England and Scotland, a joint UK outbreak control team with representatives from both nations was convened and first met via teleconference on the 10th September 2010. The Gastrointestinal, Emerging and Zoonotic Infections Department at HPA Colindale reported an increase in identifications of *S.* Bareilly unassociated with the Jewish wedding or any other point source exposure from elsewhere in England. A total of 44 cases had been reported in England since the beginning of August, when in a typical year far fewer cases would be expected for the same time period (see Figure 1).

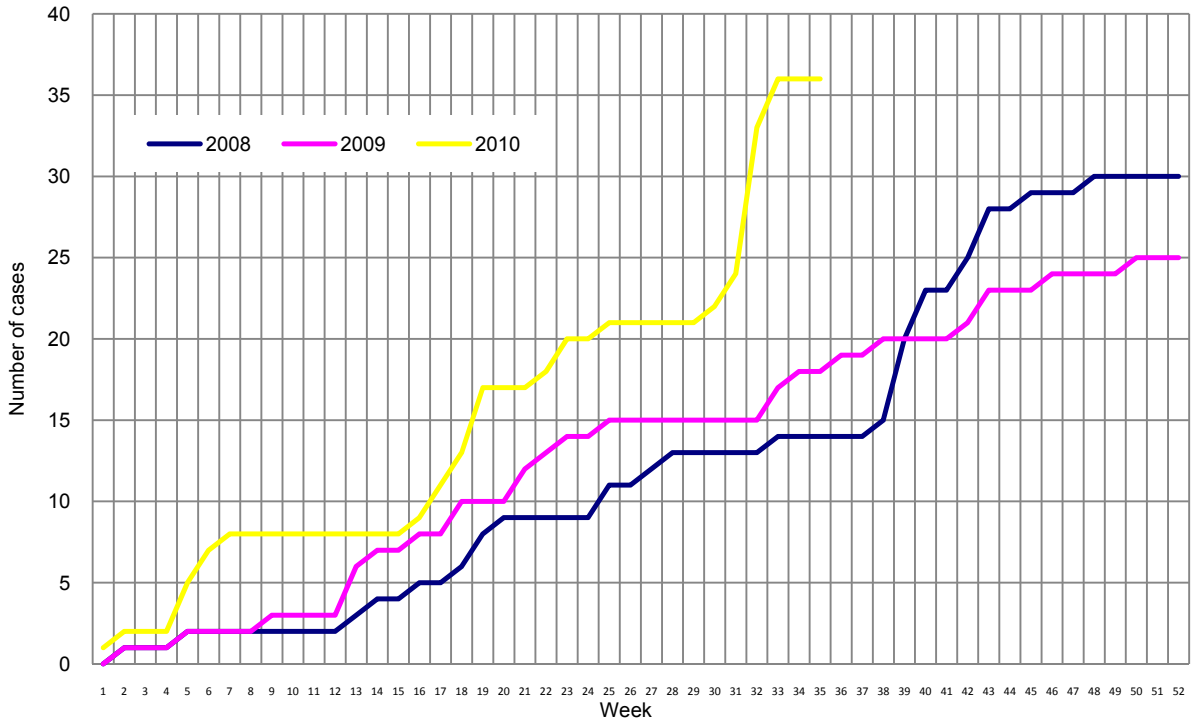



**Figure 1: Cumulative incidence (weekly) of non travel-related fully sensitive *S.* Bareilly, England and Wales, 2008 - 2010**

The UK OCT agreed that the coordination of the public health response to the incident would be led by the HPA Local and Regional Services (LaRS) division, with support from HPA Colindale and HPS. The HPA declared the event a level 3 incident on the 10th September 2010 and submitted an alert to the European Centre for Disease Control Epidemic Intelligence Information System (ECDC EPIS). No reports of similar outbreaks in other European nations were received.

Trawling questionnaires were administered to 12 cases in England and four cases in Scotland to generate hypotheses for an analytical study. In decreasing order, the most frequently reported foods consumed were

milk, tomatoes, breakfast cereal, cheese, lettuce, onions, bread, mixed salad leaves, cucumber, and snack foods. Only three cases recalled eating sprouted seeds. No particular supermarket chain was overrepresented.

Less detailed questionnaires, which EHOs routinely administer to cases of salmonellosis, were also collated from Health Protection Units (HPU) across England throughout the outbreak, and indicated that salad, chicken, vegetables and eggs were the most commonly recalled foods consumed. Few cases were specifically asked about bean sprout consumption in spite of a request to do this, and no case spontaneously reported having eaten them. No link to any particular supplier was identified.

The Food Standards Agency Scotland (FSAS) and Edinburgh City Council EHOs traced the suppliers of salad vegetables to catering establishments associated with cases in Scotland, and discovered that on the 16th August 2010, a wholesaler in Kent (Wholesaler 23), who supplied an implicated catering establishment in Scotland, had identified a group C *Salmonella* in bean sprouts supplied to them by a supplier in Lancashire (Wholesaler 15) who had in turn been supplied by a producer in the North West of England (Producer 1). No samples had been retained for further typing. Wholesaler 15 had themselves also detected contamination of bean sprouts with a group C *Salmonella* on quality control testing, but again without retaining samples for further typing. Producer 1 performed regular quality control testing of samples of bean sprouts but had not detected *Salmonella* contamination for several years.

On the basis of the epidemiological and microbiological information, it was hypothesised that bean sprouts were the most likely source of contamination and bean sprouts or salad leaves the most likely food vehicles of infection of *S.* Bareilly. The UK OCT recommended intensive sampling of bean sprouts to test for *S.* Bareilly contamination. Samples were taken from Producer 1 and Wholesaler 15 daily for one week. *S.* Bareilly with an indistinguishable PFGE profile to the outbreak strain was subsequently detected at low levels in a sample of bean sprouts obtained from Wholesaler 15 (who had obtained them from Producer 1) using a sensitive testing methodology and a larger sample of bean sprouts than that used conventionally for quality control testing. *S.* Bareilly of an indistinguishable PFGE type to the outbreak strain was also detected in bean sprouts supplied to Caterer 14 in Scotland by Wholesaler 13 (again originally sourced from Producer 1). None of the environmental or food samples obtained from Producer 1 as part of the UK OCT investigation were positive for *S.* Bareilly but a group C *Salmonella* (which was not further characterised) was detected in an outdoor husk waste skip as part of a joint investigation between Wholesaler 15 and Producer 1. No microbiological evidence was found to link cases with another producer of bean sprouts (Producer 2; see Figure 8).

A case control study with telephone interviews of English and Scottish cases (and controls) was undertaken to test the hypothesis that bean sprouts and/or salad leaves were the most likely food vehicles of infection of *S.* Bareilly (see page 24). The analysis of the case control study concluded that consumption of bean sprouts was significantly associated with being a case.

On the 17th of September the HPA, HPS, and the Food Standards Agency (FSA) posted statements on the national *S.* Bareilly investigation on their respective websites, advising that bean sprouts should be washed and

thoroughly cooked. On the 29th of September, the FSA updated the advice on its website and drafted a letter for Environmental Health Officers and food inspectors, reminding them of the guidance for the safe preparation and cooking of bean sprouts. The HPA issued a statement proactively on behalf of the OCT to the news media, generating coverage by the Daily Telegraph, Scotsman and various trade and medical journals in the UK and abroad.

In addition to growing bean sprouts, Producer 1 also grew mustard and watercress and received some vegetable and salad items for wholesale distribution. Inspection by a technical enforcement officer from Fylde Borough Council of the premises of Producer 1 showed a very low likelihood of cross contamination between bean sprouts and other fresh produce. Wyre Council Environmental Health Department inspected the premises of Wholesaler 15 and found the likelihood of cross contamination there to be very low.

In early October, the outbreak strain of *S.* Bareilly was also detected in samples of bean sprouts from two supermarkets in the North West of England (Retailers 1 and 2). The bean sprouts had been sourced from Producer 1, who had sourced mung bean seeds from a Danish importer/broker (Importer/broker 1). Importer/broker 1 distributes seeds which originate from a source in the Guangdong region of China (Exporter (China) 1; see Figure 7), which is likely to be the origin of contaminated seed.

In the UK, the outbreak peaked in week 37 of 2010, tailing off during October 2010. The epidemic curve of cases of *S.* Bareilly had further small peaks and did not fall back to background levels until late January 2011, when the UK OCT declared the outbreak to be over. Small numbers of *S.* Bareilly cases continued to be reported at the time of writing and were monitored by the HPA and HPS.

# EPIDEMIOLOGICAL INVESTIGATIONS

## GREATER MANCHESTER WEDDING RECEPTION OUTBREAK

### DESCRIPTIVE EPIDEMIOLOGY - METHODS

The definition agreed for a probable case for the wedding outbreak in Greater Manchester was: acute diarrhoea (three or more stools in 24 hours) or vomiting or abdominal pain or fever in a wedding guest, with onset during the seven days following the wedding reception. The definition of a confirmed case was someone who fulfilled the criteria of a probable case and from whose stool *S.* Bareilly had been cultured.

### DESCRIPTIVE EPIDEMIOLOGY - RESULTS

Data were collected on 36 guests fitting the probable case definition (of whom seven were confirmed), with onset dates ranging from the 8th to the 13th August 2010 (see Figure 2) and on 66 non-cases who attended the wedding reception.

Cases were slightly younger with a narrower range of ages than non-cases. There was a female predominance among cases but not among non-cases (see Table 1). Neither difference was statistically significant.

| Variable | Cases | Non-cases | Total |
|---|---|---|---|
| Mean (standard deviation) age | 51 (14.9) | 53 (21.0) | 52 (19.0) |
| Female proportion | 22/35 (63%) | 31/62 (50%) | 54/97 (55%) |

**Table 1: Age and sex distribution of cases and non-cases**

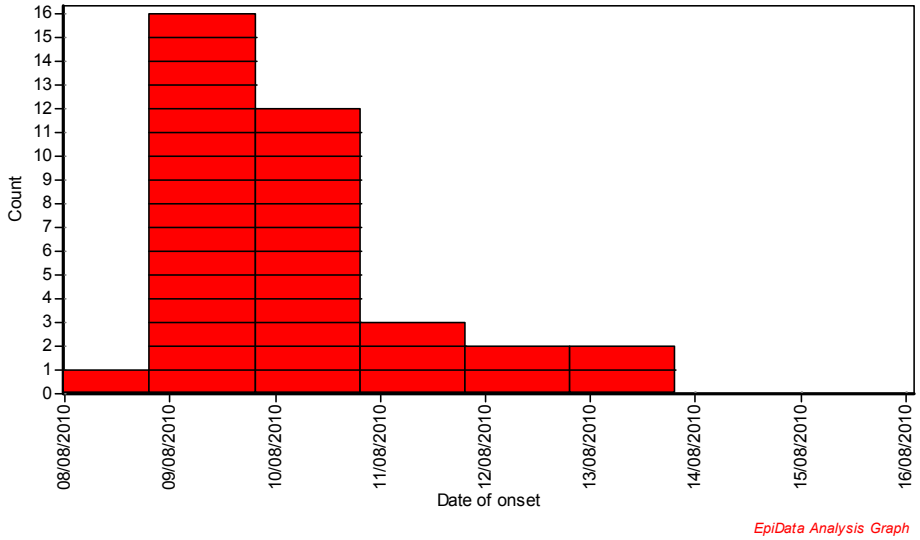



**Figure 2: Epidemic curve of dates of onset of probable cases**

The most commonly reported symptoms were diarrhoea, abdominal pain or cramps and nausea (see Table 2). Cases reported a median duration of illness of 5 days (range 1-16 days).

| Symptom | Number of probable cases affected |
|---|---|
| Diarrhoea | 36/36 (97%) |
| Abdominal pain/cramps | 30/36 (83%) |
| Nausea | 26/36 (72%) |
| Muscle aches | 21/36 (58%) |
| Fever | 18/36 (50%) |
| Vomiting | 8/36 (22%) |
| Blood in stools | 3/36 (8%) |

**Table 2: Symptoms reported by probable cases**

## COHORT STUDY

After the report of the NW wedding outbreak, the Greater Manchester OCT agreed to conduct a cohort study to identify possible food vehicles of infection.

## METHODS

It was initially envisaged that data for the cohort study would be collected by postal questionnaires to wedding guests (with emailed questionnaires to guests currently living abroad), but this approach was modified at a later stage to include data collection via telephone interviews, in order to expedite data collection, improve the response rate and to clarify food items consumed. Data collection was hampered by delays in obtaining a complete guest list (until the bride had returned from honeymoon) and delays in obtaining complete information on foods served at the wedding which necessitated repeated modifications to the questionnaire used.

The initial power calculation was based on initial reports of around 25 probable or confirmed cases, which estimated that with 75 non-cases (i.e. three non-cases per case) and an exposure rate in non-cases of 10%, there would be adequate power to detect an odds ratio of 5. This would also equate to a response rate of 60%, which would reduce non-response bias.

Local investigators developed a questionnaire for use by cases and non-cases. Contact details of wedding guests were obtained from the father of the bride.

Questionnaire data were double entered into an EpiData[14] database. Interim analyses were conducted to detect data errors. Data cleaning and descriptive analysis were conducted using EpiData Analysis. Backward stepwise unconditional logistic regression was conducted using the statistical software R.[15] After assessment for multicollinearity, possible risk factors with a p value of <0.2 in univariate analysis were combined into a single model and terms were removed sequentially based on the results of likelihood ratio testing.

## RESULTS

Univariate analysis identified several possible risk factors for illness (see Table 3).

In the final multivariate (logistic) regression model, three food variables were significantly associated with illness: consumption of roast chicken, consumption of vegetable crudités and consumption of pinwheel bread rolls with smoked salmon mayonnaise. There was no evidence of an association between the teriyaki salmon dish and illness. Wedding guests who were 65 years or above had a significantly reduced odds of illness compared to younger guests (see Table 4).

| Possible risk factors | | Exposed | | | | Not Exposed | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | n | Ill | AR (%) | (95% CI) | n | Ill | AR (%) | (95% CI) | RR | (95% CI) | Chi2 (df=1) | p value | Exact p value |
| Age 65+ | 97 | 20 | 4 | 20 | (8-42) | 77 | 31 | 40 | (30-51) | 0.50 | (0.20-1.24) | 2.826 | 0.0928 | 0.1197 |
| Sex | 102 | 46 | 14 | 30 | (19-45) | 56 | 22 | 39 | (28-52) | 0.77 | (0.45-1.34) | 0.866 | 0.3520 | 0.4082 |
| Potato rosti | 91 | 54 | 24 | 44 | (32-58) | 37 | 10 | 27 | (15-43) | 1.64 | (0.90-3.02) | 2.846 | 0.0916 | 0.1231 |
| Beef pastrami* | 93 | 49 | 24 | 49 | (36-63) | 44 | 11 | 25 | (15-39) | 1.96 | (1.09-3.52) | 5.680 | 0.0172 | 0.0198 |
| Vegetable Spring Rolls | 95 | 52 | 23 | 44 | (32-58) | 43 | 13 | 30 | (19-45) | 1.46 | (0.85-2.53) | 1.960 | 0.1615 | 0.2040 |
| Chilli Dip* | 93 | 24 | 16 | 67 | (47-82) | 69 | 20 | 29 | (20-41) | 2.30 | (1.44-3.66) | 10.656 | 0.0011 | 0.0016 |
| Soy Sauce Dip | 92 | 23 | 12 | 52 | (33-71) | 69 | 23 | 33 | (23-45) | 1.57 | (0.94-2.62) | 2.598 | 0.1070 | 0.1382 |
| Mini lamb sausages with mint jelly* | 98 | 48 | 24 | 50 | (36-64) | 50 | 12 | 24 | (14-37) | 2.08 | (1.18-3.68) | 7.123 | 0.0076 | 0.0115 |
| Cocktail fish balls | 93 | 56 | 23 | 41 | (29-54) | 37 | 9 | 24 | (13-40) | 1.69 | (0.88-3.23) | 2.769 | 0.0961 | 0.1206 |
| Fish goujons with tartar sauce | 94 | 57 | 24 | 42 | (30-55) | 37 | 10 | 27 | (15-43) | 1.56 | (0.85-2.87) | 2.209 | 0.1372 | 0.1878 |
| Pinwheel bread rolls with egg mayonnaise | 77 | 20 | 8 | 40 | (22-61) | 57 | 22 | 39 | (27-52) | 1.04 | (0.55-1.94) | 0.012 | 0.9118 | 1.0000 |
| Pinwheel bread rolls with tuna mayonnaise | 94 | 12 | 8 | 67 | (39-86) | 82 | 27 | 33 | (24-44) | 2.02 | (1.22-3.36) | 5.099 | 0.0239 | 0.0508 |
| Pinwheel bread rolls with smoked salmon mayonnaise* | 93 | 34 | 20 | 59 | (42-74) | 59 | 15 | 25 | (16-38) | 2.31 | (1.38-3.89) | 10.252 | 0.0014 | 0.0019 |
| Vegetable crudités* | 92 | 32 | 19 | 59 | (42-74) | 60 | 16 | 27 | (17-39) | 2.23 | (1.34-3.70) | 9.472 | 0.0021 | 0.0032 |
| Melon baskets with fruit | 98 | 7 | 1 | 14 | (3-51) | 91 | 33 | 36 | (27-47) | 0.39 | (0.06-2.47) | 1.386 | 0.2391 | 0.4160 |
| Peanuts | 99 | 35 | 15 | 43 | (28-59) | 64 | 20 | 31 | (21-43) | 1.37 | (0.81-2.32) | 1.334 | 0.2481 | 0.2770 |
| Crisps* | 99 | 45 | 23 | 51 | (37-65) | 54 | 13 | 24 | (15-37) | 2.12 | (1.22-3.69) | 7.754 | 0.0054 | 0.0067 |
| Teriyaki salmon on Chinese noodles & bean sprouts | 99 | 89 | 35 | 39 | (30-50) | 10 | 1 | 10 | (2-40) | 3.93 | (0.60-25.70) | 3.341 | 0.0676 | 0.0884 |
| Chicken soup with lokshen & kneidles | 89 | 80 | 24 | 30 | (21-41) | 9 | 2 | 22 | (6-55) | 1.35 | (0.38-4.79) | 0.237 | 0.6266 | 1.0000 |
| Butternut squash soup | 100 | 8 | 3 | 38 | (14-69) | 92 | 33 | 36 | (27-46) | 1.05 | (0.41-2.66) | 0.008 | 0.9266 | 1.0000 |
| Potato salad | 83 | 21 | 8 | 38 | (21-59) | 62 | 18 | 29 | (19-41) | 1.31 | (0.67-2.56) | 0.599 | 0.4390 | 0.5868 |
| Moroccan carrot salad | 98 | 32 | 15 | 47 | (31-64) | 66 | 21 | 32 | (22-44) | 1.47 | (0.88-2.45) | 2.102 | 0.1471 | 0.1819 |
| Tabbouleh salad | 91 | 38 | 18 | 47 | (32-63) | 53 | 17 | 32 | (21-45) | 1.48 | (0.88-2.47) | 2.187 | 0.1392 | 0.1901 |
| Red pepper salad | 91 | 29 | 15 | 52 | (34-69) | 62 | 20 | 32 | (22-45) | 1.60 | (0.97-2.65) | 3.163 | 0.0753 | 0.1054 |
| Roast chicken in lemon and thyme sauce | 99 | 89 | 35 | 39 | (30-50) | 10 | 1 | 10 | (2-40) | 3.93 | (0.60-25.70) | 3.341 | 0.0676 | 0.0884 |
| Chickpea and bean tagine | 99 | 9 | 1 | 11 | (2-44) | 90 | 35 | 39 | (29-49) | 0.29 | (0.04-1.85) | 2.728 | 0.0986 | 0.1495 |
| Paprika roasted potatoes | 97 | 91 | 34 | 37 | (28-48) | 6 | 1 | 17 | (3-56) | 2.24 | (0.37-13.68) | 1.045 | 0.3066 | 0.4136 |
| Roasted seasonal vegetables | 98 | 88 | 33 | 38 | (28-48) | 10 | 3 | 30 | (11-60) | 1.25 | (0.47-3.35) | 0.217 | 0.6411 | 0.7414 |
| Chocolate brownies with summer fruits | 97 | 80 | 30 | 38 | (28-48) | 17 | 3 | 18 | (6-41) | 2.13 | (0.73-6.17) | 2.462 | 0.1166 | 0.1611 |
| Vanilla ice cream | 98 | 81 | 31 | 38 | (28-49) | 17 | 5 | 29 | (13-53) | 1.30 | (0.59-2.86) | 0.475 | 0.4909 | 0.5871 |
| Tea | 91 | 31 | 12 | 39 | (24-56) | 60 | 20 | 33 | (23-46) | 1.16 | (0.66-2.05) | 0.259 | 0.6107 | 0.6481 |
| Coffee | 90 | 47 | 17 | 36 | (24-50) | 43 | 14 | 33 | (20-47) | 1.11 | (0.63-1.97) | 0.130 | 0.7187 | 0.8252 |
| Mints | 94 | 68 | 27 | 40 | (29-52) | 26 | 7 | 27 | (14-46) | 1.47 | (0.73-2.96) | 1.331 | 0.2486 | 0.3383 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fruit kebab | 98 | 74 | 28 | | 38 | (28-49) | 24 | 7 | 29 | (15-49) | 1.30 | (0.65-2.58) | 0.593 | 0.4411 | 0.4751 |
| Ate any other food? | 100 | 15 | 7 | | 47 | (25-70) | 85 | 29 | 34 | (25-45) | 1.37 | (0.74-2.53) | 0.871 | 0.3506 | 0.3900 |
| Drinks | 101 | 93 | 33 | | 35 | (27-46) | 8 | 3 | 38 | (14-69) | 0.95 | (0.37-2.41) | 0.013 | 0.9090 | 1.0000 |
| Symptomatic member of household | 102 | 4 | 1 | | 25 | (5-70) | 98 | 35 | 36 | (27-46) | 0.70 | (0.13-3.90) | 0.193 | 0.6603 | 1.0000 |

**Table 3: Univariate analysis for possible risk factors for illness (food items significantly associated with illness in univariate analysis are asterisked)**

| Possible risk factors | Crude OR (95%CI) | Adjusted OR (95%CI) | p value |
|---|---|---|---|
| Age 65+ | 0.32 (0.1, 1.08) | 0.15 (0.04, 0.65) | 0.005 |
| Pinwheel bread rolls with smoked salmon mayonnaise | 3.49 (1.38, 8.81) | 4.42 (1.4, 14.02) | 0.008 |
| Vegetable crudités | 3.8 (1.48, 9.76) | 4.35 (1.42, 13.37) | 0.008 |
| Roast chicken | 6.1 (0.73, 51.24) | 9.94 (0.91, 108.12) | 0.026 |

**Table 4: Unconditional logistic regression model for risk factors for illness**

## DESCRIPTIVE EPIDEMIOLOGY OF CONFIRMED OUTBREAK CASES IN ENGLAND, WALES, SCOTLAND AND NORTHERN IRELAND

For the descriptive epidemiology, outbreak cases were defined as persons with microbiologically confirmed infection with *S.* Bareilly, fully sensitive to antimicrobials tested and reported from week 30 until the declaration of the end of the outbreak with no history of foreign travel and who did not have non-outbreak PFGE profiles.

Data on 272 cases of *S.* Bareilly between weeks 30 and 50 were available. Twenty seven cases were related to foreign travel (see Table 5). Of the remaining 245 cases, four had infections with non-outbreak strains of *S.* Bareilly.

The remaining 241 cases are henceforth referred to as outbreak cases. Of these, 106 had PFGE profile SBARXB.0016, two had PFGE profile SBARXB.0016+, and the remaining 133 did not have PFGE profiling. The outbreak peaked in week 37, with further smaller peaks in weeks 41 and 46 (see Figure 3). The first cases were located in the North West of England, related to the wedding, followed soon by cases in other regions and parts of Scotland, the numbers falling to lower levels after week 43 (see Figure 5). Cases were most commonly reported from the South East and North West regions of England, followed by London (see Table 6 and Figure 4).

Outbreak cases had a median age of 45 years (range <1 to 92 years) and 140 (58%) cases were female (see Figure 6).

One death and one severe complication (*Salmonella* meningitis complicated by spinal cord infarction and tetraplegia) are known to have occurred associated with *S.* Bareilly infections during this outbreak.

| Country | Frequency | Percent |
|---|---|---|
| India | 12 | 44.4 |
| Spain | 4 | 14.8 |
| Turkey | 2 | 7.4 |
| USA | 1 | 3.7 |
| Trinidad and Tobago | 1 | 3.7 |
| Sri Lanka | 1 | 3.7 |
| Niger | 1 | 3.7 |
| Maldives | 1 | 3.7 |
| Ireland | 1 | 3.7 |
| Indonesia | 1 | 3.7 |
| Egypt | 1 | 3.7 |
| Cyprus | 1 | 3.7 |
| Total | 27 | 100.0 |

**Table 5: Countries associated with travel related *S.* Bareilly infections (for cases from England, Wales and Northern Ireland)**

| Region | N | % |
|---|---|---|
| South East England (SE) | 45 | 18.7 |
| North West England (NW) | 43 | 17.8 |
| London (Lond) | 28 | 11.6 |
| South West England (SW) | 23 | 9.5 |
| Yorkshire & Humber (YH) | 22 | 9.1 |
| West Midlands (WM) | 21 | 8.7 |
| North East of England (NE) | 12 | 5 |
| East of England (EE) | 11 | 4.6 |
| Lothian (Scotland; LO) | 8 | 3.3 |
| East Midlands (EM) | 7 | 2.9 |
| Wales (Wa) | 6 | 2.5 |
| Lanarkshire (Scotland; LN) | 4 | 1.7 |
| Greater Glasgow & Clyde (Scotland; GGC) | 3 | 1.2 |
| Tayside (Scotland; TY) | 3 | 1.2 |
| Ayrshire & Arran (Scotland; AA) | 2 | 0.8 |
| Northern Ireland (NI) | 2 | 0.8 |
| Grampian (Scotland; GR) | 1 | 0.4 |
| | | |
| Total | 241 | 100 |

**Table 6: Geographical distribution of cases**

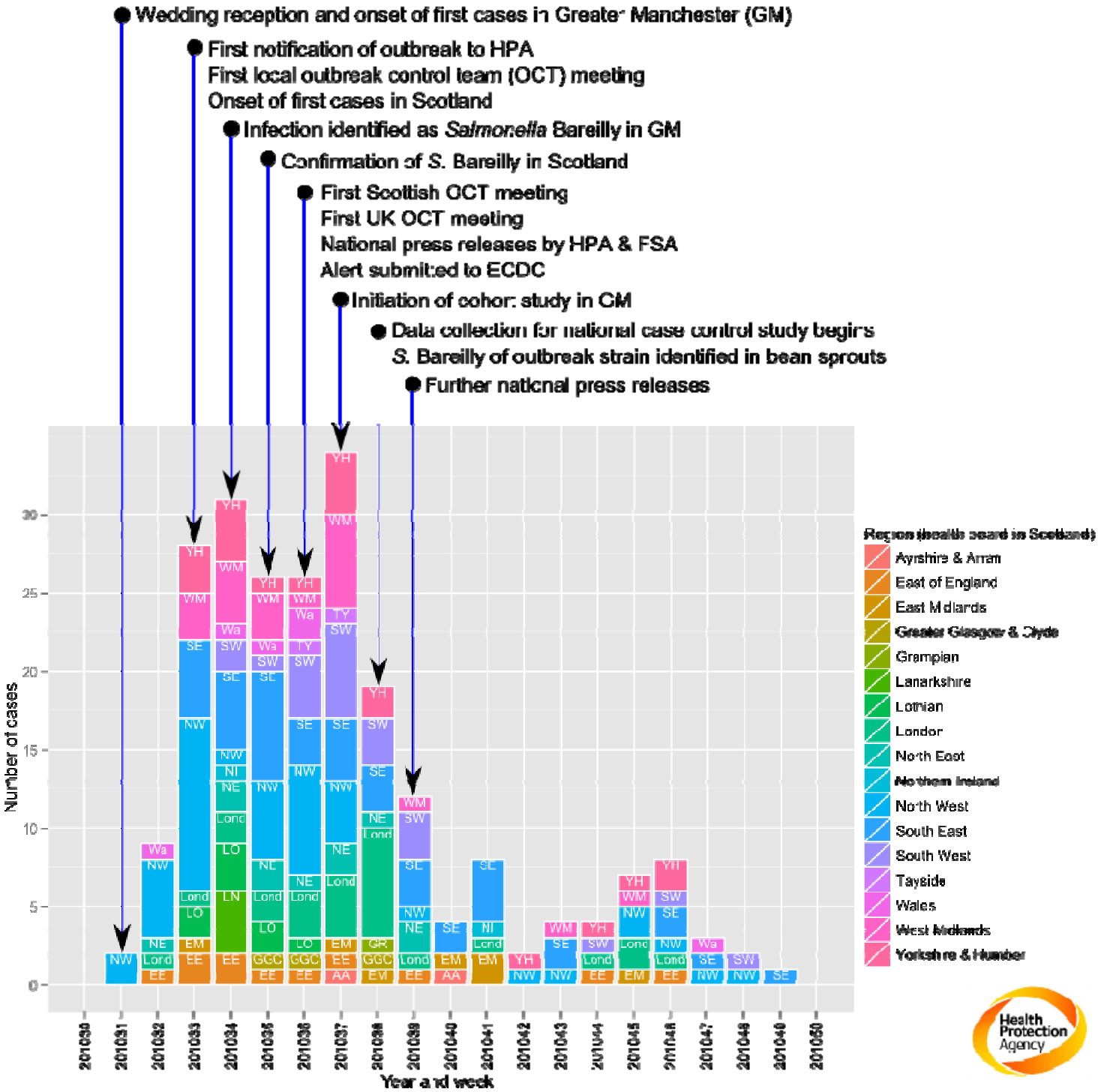
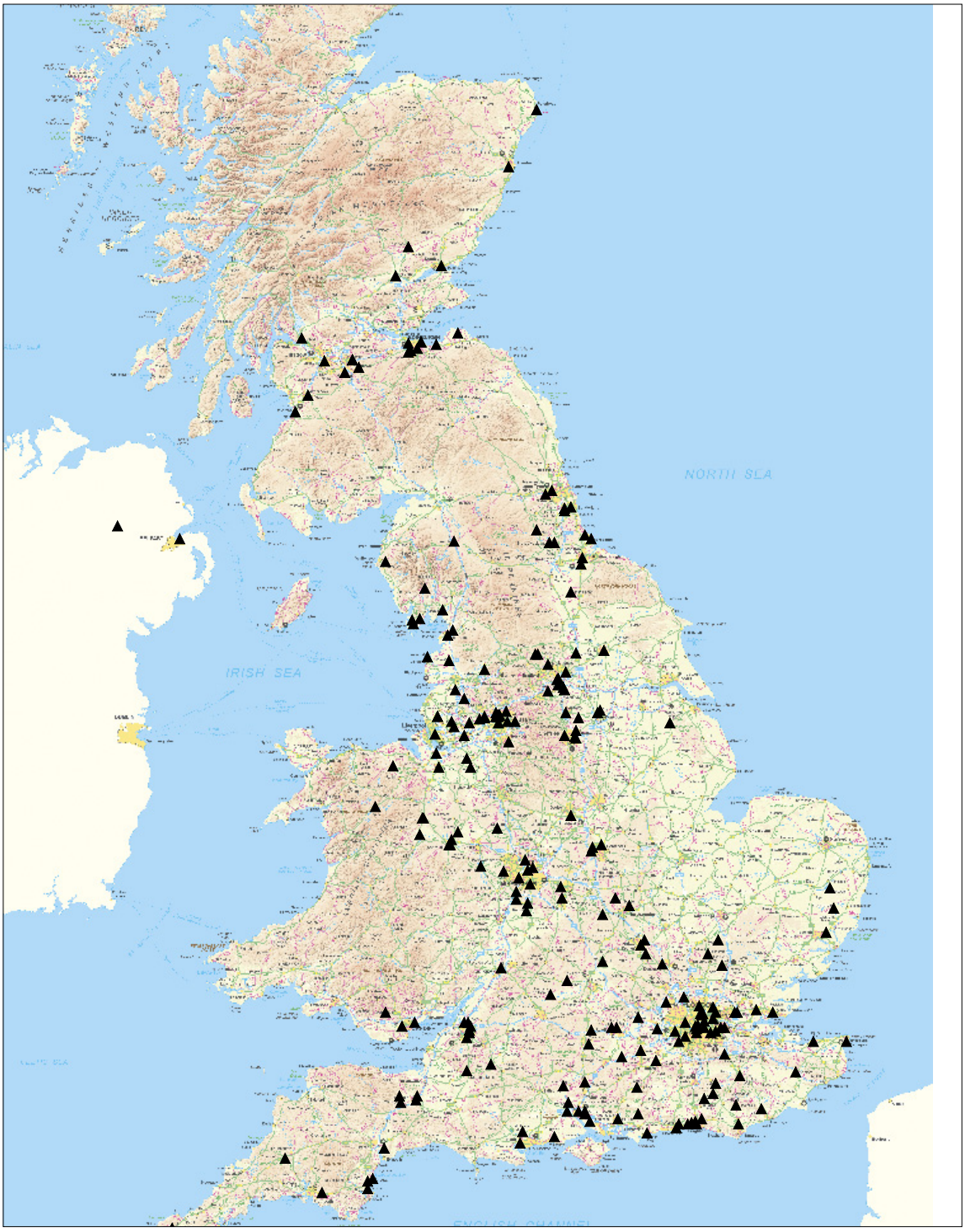



**Figure 3: Confirmed *S.* Bareilly cases in England, Wales and Northern Ireland, from the beginning of the NW outbreak until the outbreak was declared over, with key dates relating to public health response (n=240)**




**UK Salmonella Bareilly outbreak cases, weeks 30-50, 2010**



**Figure 4: *S.* Bareilly outbreak case distribution (by residential postcode) in England, Scotland, Wales and Northern Ireland (n=240)**

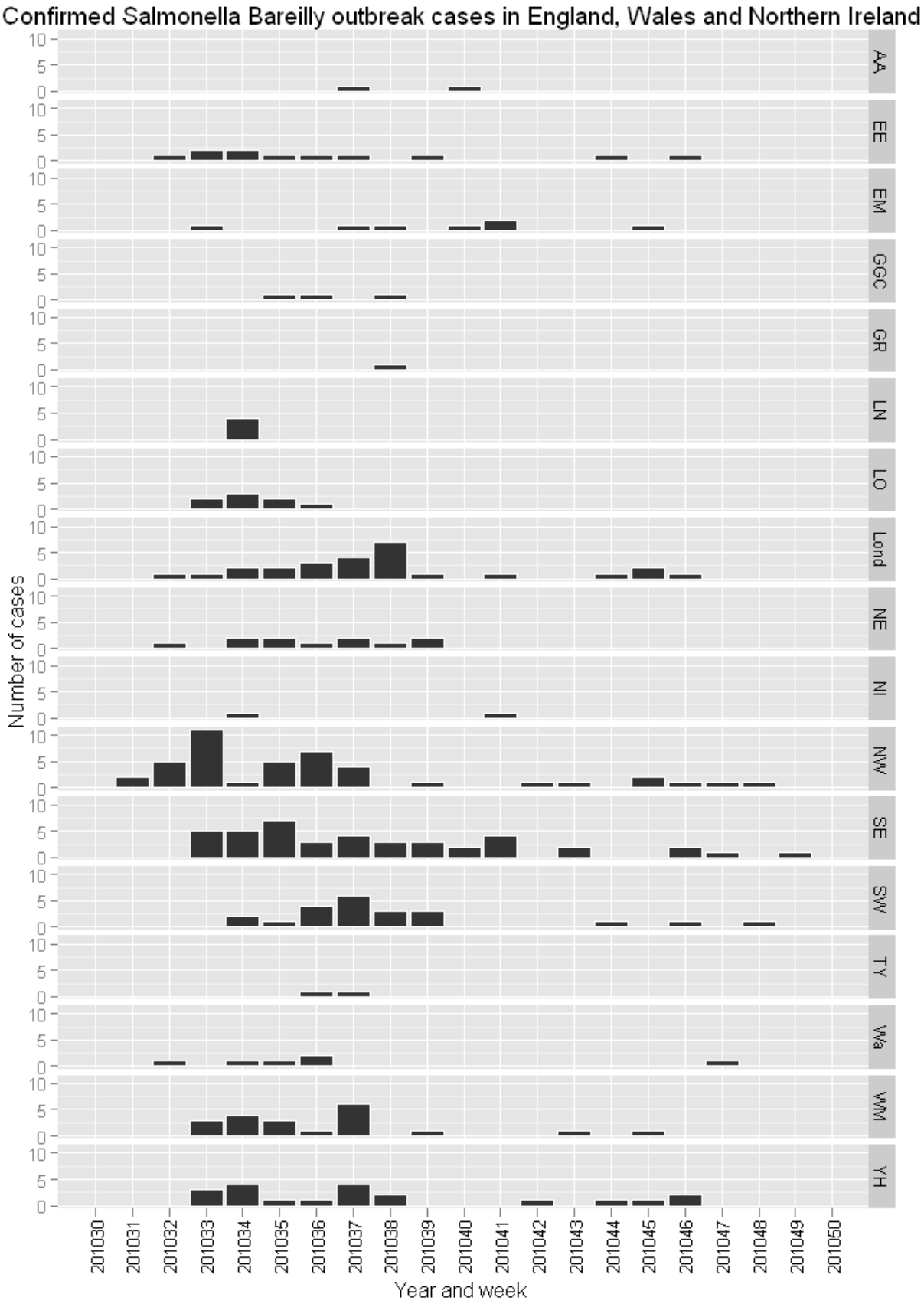



**Figure 5: Confirmed *S.* Bareilly cases in England, Scotland, Wales and Northern Ireland (n=240)**

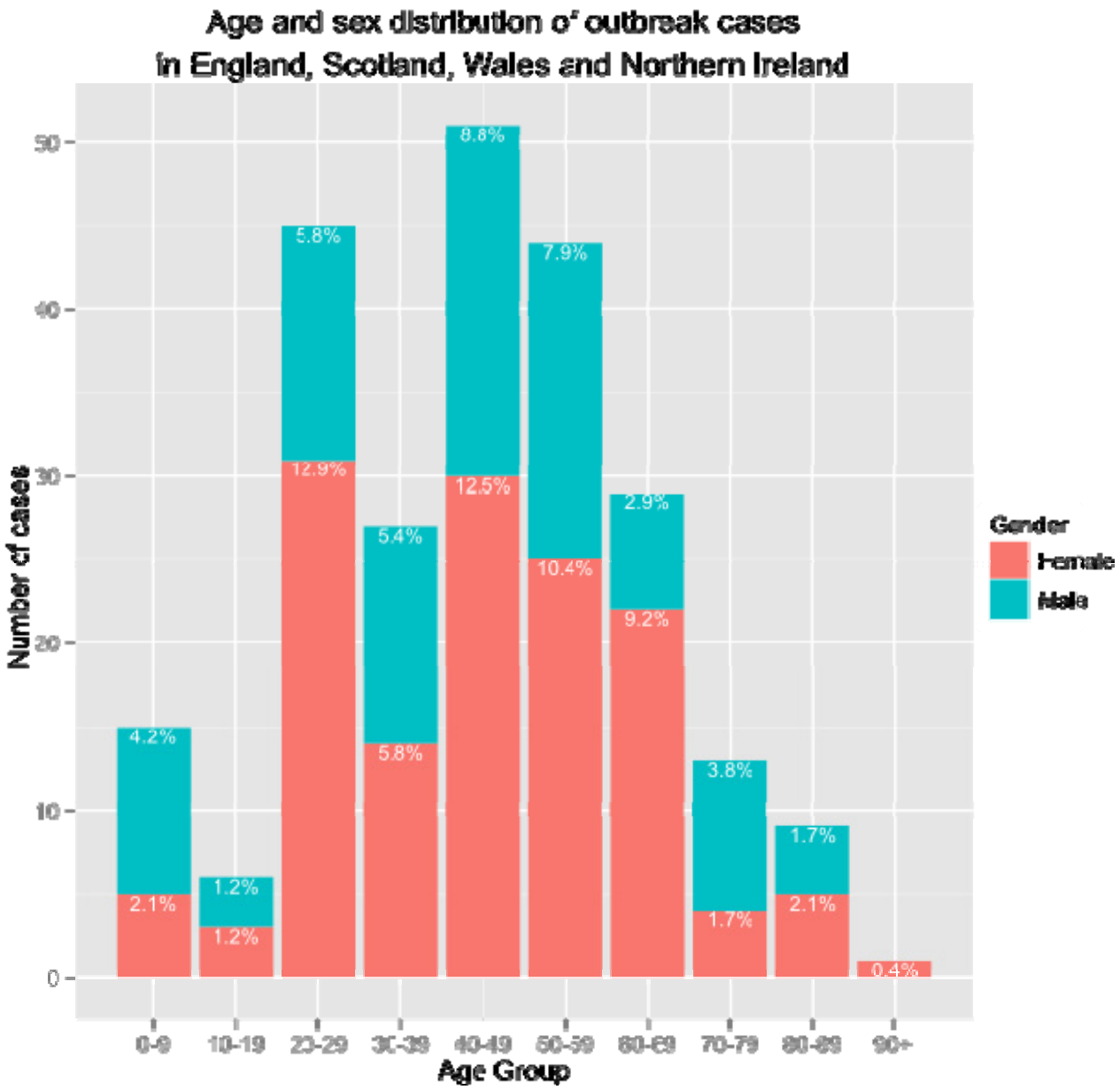



Figure 6: Age and sex distribution of outbreak cases in England, Scotland, Wales and Northern Ireland (n=2)

## UNITED KINGDOM CASE CONTROL STUDY

### METHODS

The case control study was conducted using telephone interviews to cases of *S.* Bareilly fitting the confirmed case definition and to controls selected by sequential digit dialling to match on location of residence.

For the case control study, cases were defined as persons with microbiologically confirmed infection with fully sensitive *S.* Bareilly; with a sample received at the HPA Salmonella Reference Unit or the SSSCDRL on or after the 1st August 2010; with no history of foreign travel or close contact with a case of diarrhoea in the seven

days prior to the onset of illness; who did not attend the wedding in the North West of England associated with the point source outbreak and who had not been previously interviewed as part of the preliminary investigation.

Controls were defined as residents of England, Wales, Northern Ireland or Scotland residing in the area covered by the same telephone exchange as their matched cases who had not had gastrointestinal symptoms (vomiting and/or diarrhoea) in the seven days prior to interview, and who had no history of foreign travel or of close contact with a case of diarrhoea in the seven days prior to interview. The case:control ratio was 1:2.

At the inception of the case control study in mid-September 2010 there had been 58 cases in England and Wales, of which 10 were part of the NW wedding outbreak, 12 had been interviewed in the trawling study, seven were related to foreign travel and two were of non-outbreak strains, leaving a potential of 27 cases for inclusion in the study. In Scotland there had been 15 cases of which four had been interviewed as part of the trawling study, leaving a potential of 11 cases for inclusion. There were therefore a possible 38 cases for inclusion in the case control study. Newly reported cases were recruited to the study prospectively.

Assuming that two controls per case could be recruited, and that 50% of the controls had been exposed to the exposure of interest, it was estimated that the study would have adequate power to detect an odds ratio of 4 with a target sample size of 33 cases and 66 controls.

Separate questionnaires for cases and controls were developed (see page 40). Contact details were obtained from laboratory records, from general practitioners, or from local HPUs.

Interviewers received training covering the background to the study, the study protocol, the correct use of the questionnaires for data collection, other study procedures and the overall logistics.

Data collection began on the 21st September 2010 and the target sample size was achieved by the 3rd October 2010. To select controls, a telephone number sequence was compiled by making serial additions of five to the telephone number of the case. Numbers were then called in turn until two eligible controls had been recruited and interviewed successfully.

Questionnaires were double entered into an EpiData database.[14] Interim analyses were conducted to detect data errors. Data cleaning and descriptive analysis was conducted using Epidata Analysis. Forward stepwise conditional logistic regression was conducted using the statistical software R.[9] Terms for risk factors with a p value of <0.2 in univariate analysis were added sequentially after assessment for multicollinearity.

## RESULTS

Data on 34 cases (with onset dates ranging from the 13th August 2010 to the 14th September 2010) and 64 controls were collected.

Cases were slightly younger with a narrower range of ages than controls. The sex ratio was similar for cases and controls (see Table 7).

| Variable | Cases | Controls | Total |
|---|---|---|---|
| Mean (sd) age | 49 (15.1) | 54 (24.0) | 52 (21.3) |
| Female proportion | 21/33 (64%) | 36/59 (61%) | 57/92 (62%) |

**Table 7: Age and sex distribution of cases and controls**

Cases reported a median duration of illness of 7.5 days (range 2-30 days). Five cases had been hospitalised. The majority of cases had diarrhoea (32 of 24; 94%) and some had vomiting (7 of 34; 21%).

Univariate analysis identified a number of possible risk factors associated with illness (see Table 8).

| Possible risk factor | Coefficient | Matched odds ratio | Standard error of coefficient | z | p value |
|---|---|---|---|---|---|
| Demographics | | | | | |
| Age 65+* | -1.37 | 0.26 | 0.79 | -1.74 | 0.08 |
| Male sex | -0.58 | 0.55 | 0.49 | -1.21 | 0.23 |
| | | | | | |
| Food items consumed | | | | | |
| Any salad leaves* | 1.06 | 2.88 | 0.54 | 1.95 | 0.05 |
| Bean sprouts* | 2.12 | 8.3 | 0.79 | 2.69 | 0.01 |
| Mixed salad leaves | 0.82 | 2.27 | 0.59 | 1.39 | 0.17 |
| Iceberg lettuce | 1.03 | 2.81 | 0.63 | 1.65 | 0.10 |
| Little Gem | 0.79 | 2.21 | 0.77 | 1.03 | 0.31 |
| Rocket | -19.70 | <0.01 | 15239 | <-0.01 | 1.00 |
| Salad cress | 2.08 | 8.00 | 1.12 | 1.86 | 0.06 |
| *(Only 1 person reported eating alfalfa sprouts)* | | | | | |

**Table 8: Univariate analysis of possible risk factors for *S.* Bareilly (food items significantly associated with illness in univariate analysis are asterisked)**

In the final conditional logistic regression model, only bean sprouts was strongly associated with illness and was the only statistically significant predictor of illness (see Table 9).

| Risk factor | Crude odds ratio (95% confidence interval) | Adjusted odds ratio (95% confidence interval) | p value (LR test) |
|---|---|---|---|
| Consumption of any salad leaves | 2.88 (1.00, 8.34) | 2.06 (0.63, 6.71) | 0.222 |
| Consumption of bean sprouts | 8.29 (1.78, 38.69) | 6.77 (1.39, 32.99) | 0.008 |

**Table 9: Conditional logistic regression model for risk factors for *Salmonella* Bareilly**

## ENVIRONMENTAL INVESTIGATIONS

### INSPECTION OF THE PREMISES AND PROCESSES OF PRODUCER 1

On 30 September 2010, a consultant in communicable disease control from the Cumbria and Lancashire Health Protection Unit and a Technical Enforcement Officer (TEO) from Fylde Borough Council, accompanied by the owner of the business, visited the premises of Producer 1 after the identification of the premises as a potential source of contaminated bean sprouts.

Producer 1 was a medium sized nursery which grew bean sprouts, mustard and watercress and also received some vegetable and salad items for wholesale distribution. The TEO assessed the business as being well managed. Although water cress and mustard cress were also produced, they were grown in separate areas and there was an extremely low potential for cross-contamination. The key control points were well managed with recording of disinfection and cleaning schedules.

Seeds were delivered in 25kg sealed plastic sacks. Producer 1 sourced mung bean seeds from two main suppliers: Importer/broker 3 and Importer/broker 1. They purchased 22 tonnes of beans every three to four months. Mung beans supplied to Producer 1 from Importer/broker 3 originated from China, with certificates which declared the seeds to be free of pests, pollution, and moisture and to have been fumigated with aluminium phosphate. Importer/broker 3 also supplies Producer 3, who grows bean sprouts which are ultimately supplied to Retailer 2 and Retailer 3.

Mung bean seeds supplied to Producer 1 by Importer/broker 1 originated from China, with certificates which declared the seeds to be free from bad smells, weevils, moisture and foreign matter including glass, stones and iron. There had been no deliveries to Producer 1 by Importer/broker 1 during 2010.

Bean sprout production was an entirely separate process from mustard and watercress production and operated from a separate building. The building used was modern and of good construction with asphalt floors. It was in a good state of repair and was easily cleanable with good drainage and no pools of water. The whole process from delivery and storage of the raw beans, through to growth of the sprouts and packaging, was totally enclosed. The only connection to the outside environment was via air vents to the 11 sprout growing rooms.

When the beans were required for use they were emptied into a large plastic bin that had been disinfected with disinfectant (which contained 80-120ppm chlorine). One retained sample was taken from each lot of 14 bins per growing room and retained for three weeks. The beans were washed in disinfectant, mixed using a paddle cleaner and left to stand for 30 to 40 minutes.

The beans were grown in 11 growing rooms. The growing room was disinfected before each batch of 14 bins entered the room. The growing rooms were kept in darkness at 20°C and had two air vents, one which drew air

in from inside the building into the growing room and one external air vent that drew air out from the growing room to the outside. Each air vent had a fan that was switched on once during the growing period to change the air in the growing room. When not in use the external air vent was kept closed. Each bin had its own irrigation pipes which delivered computer controlled quantities of mains water. Some of the external air vents were about two inches from the ground and opened into an untidy grassy area, which was the only potential for ingress from the exterior. The beans were irrigated three hourly with mains water. The duration of irrigation was increased daily from 10 seconds up to 50 seconds by day five, when the bean sprouts were harvested. On day five there was a final irrigation with disinfectant (chlorine at 70ppm) for 50 seconds.

Bins with mature sprouts were pushed from the growing room into a walk-in chiller and stored at 0-5°C for up to 12 hours. A small trough was made in the centre of the bin by hand to promote drying.

The bin was taken into the packaging area where the sprouts were tipped into a hopper and shaken mechanically to separate the husks. The husks went into a separate container for disposal and the sprouts continued along the conveyor belt. The bins were taken to an automated bin washer and washed in a cycle with boiling water and disinfectant (chlorine 80-120ppm).

After separation the conveyor split into two. On one channel the sprouts were put into 4kg bags and hand-tied and on the other channel they were partitioned into 250g portions. The conveyor took the 250g portion to the packaging machine where they were put into bags which were mechanically sealed. The bags were stored in a second walk-in chiller that was separated from the packaging room by a sliding door.

Before distribution, the bean sprouts were put in a distribution area, with the other produce, i.e. the cress, which was in open trays, and the bought in vegetables. There was no potential for contamination of the bean sprouts inside the packaging.

The packaging room was in use for about five hours per day. The room was then stripped down and the equipment dismantled as far as possible. The equipment was cleaned with compressed air and a chlorine based foaming agent. The room was similarly cleaned. There were cleaning protocols and signed recording forms on the wall, in accordance with HACCP.

The room was not used for any product other than bean sprouts. The staff wore white coats, gloves, hats and plastic overshoes and were asked to wash their hands before entering the packing room.

Producer 1 grew 15 to 20 tonnes of sprouts each week and directly distributed about three-quarters of this to destinations in Preston, Liverpool, Manchester and Newcastle. The remainder went to wholesalers for further distribution, mainly in Bristol and the Midlands.

Four random samples from every batch of mung bean seed were sent to an external laboratory, Laboratory 1, for culture. In addition, Producer 1 conducted finished product testing every two weeks. A retained sample was taken from each lot of 14 bins per growing room and retained for three weeks. This was sent for culture if

potential problems were identified later on. Random samples of water used in the growing process and irrigation water were taken approximately every two to three weeks. Random post-packaging bean sprout samples were sent for culture every two to three weeks. There had been no positive sample results for the preceding 18 years.

During this investigation, a *Salmonella* group C was isolated from a swab from the husk waste skip, as part of an investigation undertaken by Wholesaler 15, which was supplied by Producer 1. This was a joint investigation between Wholesaler 15 and Producer 1, instigated following the detection of *Salmonella* group C in a routine sample of prepacked bean sprouts that Wholesaler 15 had sent for testing from Producer 1. The husk waste skip was kept outdoors and was exposed to the atmosphere, birds and any pests. It was filled with husk every day, left full overnight, and collected every morning. The skip was not washed. Following the visit from the environmental health department and the HPA, the company decided to empty the skip each evening.

Producer 1 supplied a spring roll manufacturer, Wholesaler 27, who carried out external audit sampling at the premises of Producer 1. Wholesaler 15, supplied by Producer 1, sampled bean sprouts on arrival from Producer 1. Prior to this incident Wholesaler 15 had done environmental sampling from the packaging area at Producer 1 and had reported no positive isolates of *Salmonella*.

The overall operation at Producer 1 was compliant with the Campden and Chorleywood "Guidelines for the Hygienic Manufacture, Distribution and Retail Sale of Sprouted Seeds"[9] with particular reference to mung beans, with two exceptions. Firstly, there was no information on bean storage at source in China or Myanmar. There was no evidence as to whether disinfection or microbiological testing was undertaken other than from certification provided by these suppliers. Secondly, the guidelines recommend a pre-soak of the beans before surface decontamination. Producer 1 did not undertake a pre-soak stage (although this stage is more for foreign body decontamination than disinfection).

## FRESH PRODUCE DISTRIBUTION NETWORKS IN ENGLAND AND SCOTLAND

Part of the complex United Kingdom bean sprouts distribution network was delineated as part of the investigation by the Food Standards Agency in England and Scotland (see Figure 7 and Figure 8 for networks and putative links to cases and positive food or environmental samples).

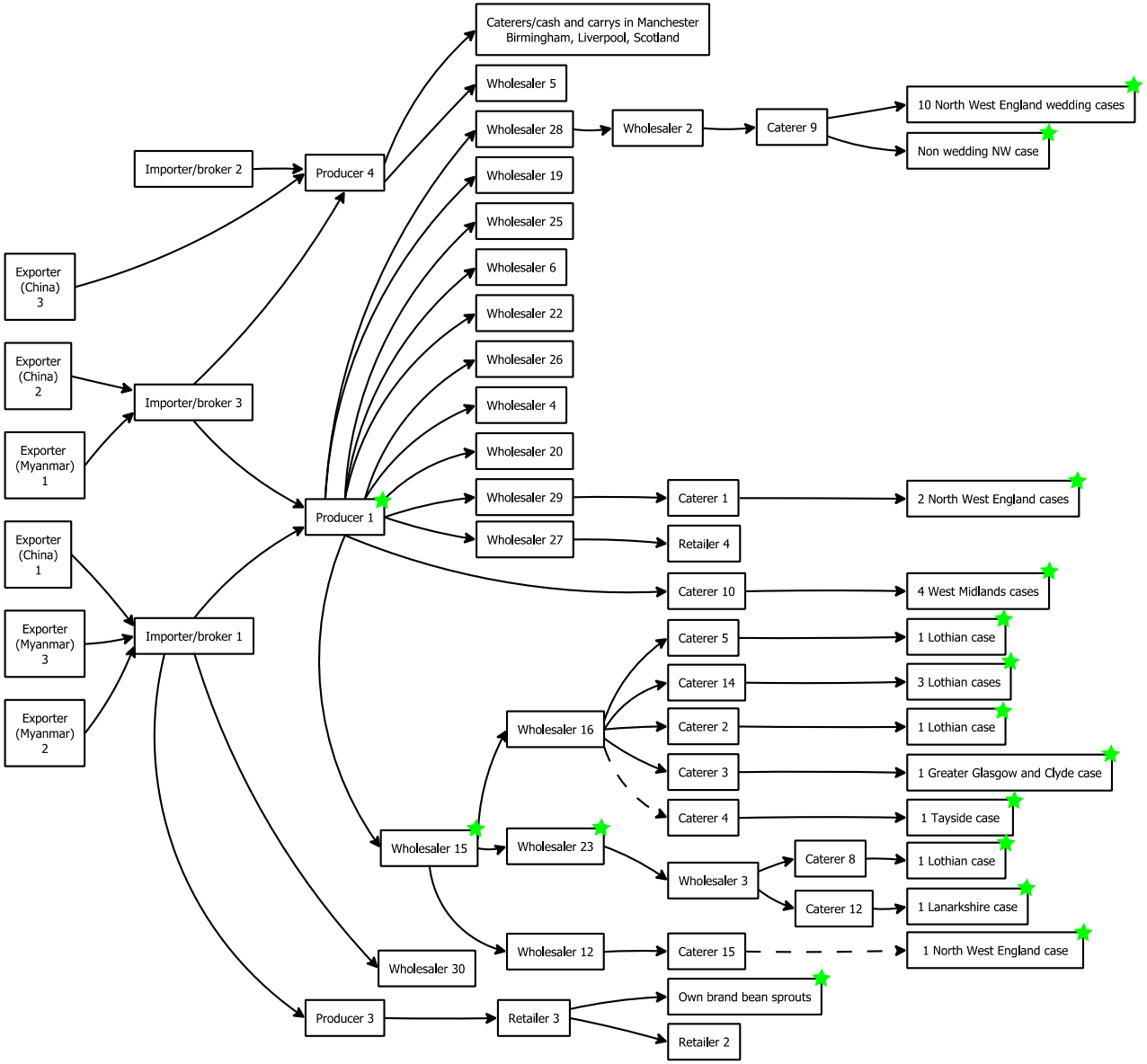



**Figure 7: Producer 1 bean sprout supply and distribution network (dotted lines indicate unconfirmed links; green stars indicate isolation of a group C *Salmonella* or *S.* Bareilly from food, environmental or clinical samples at that point of the network)**

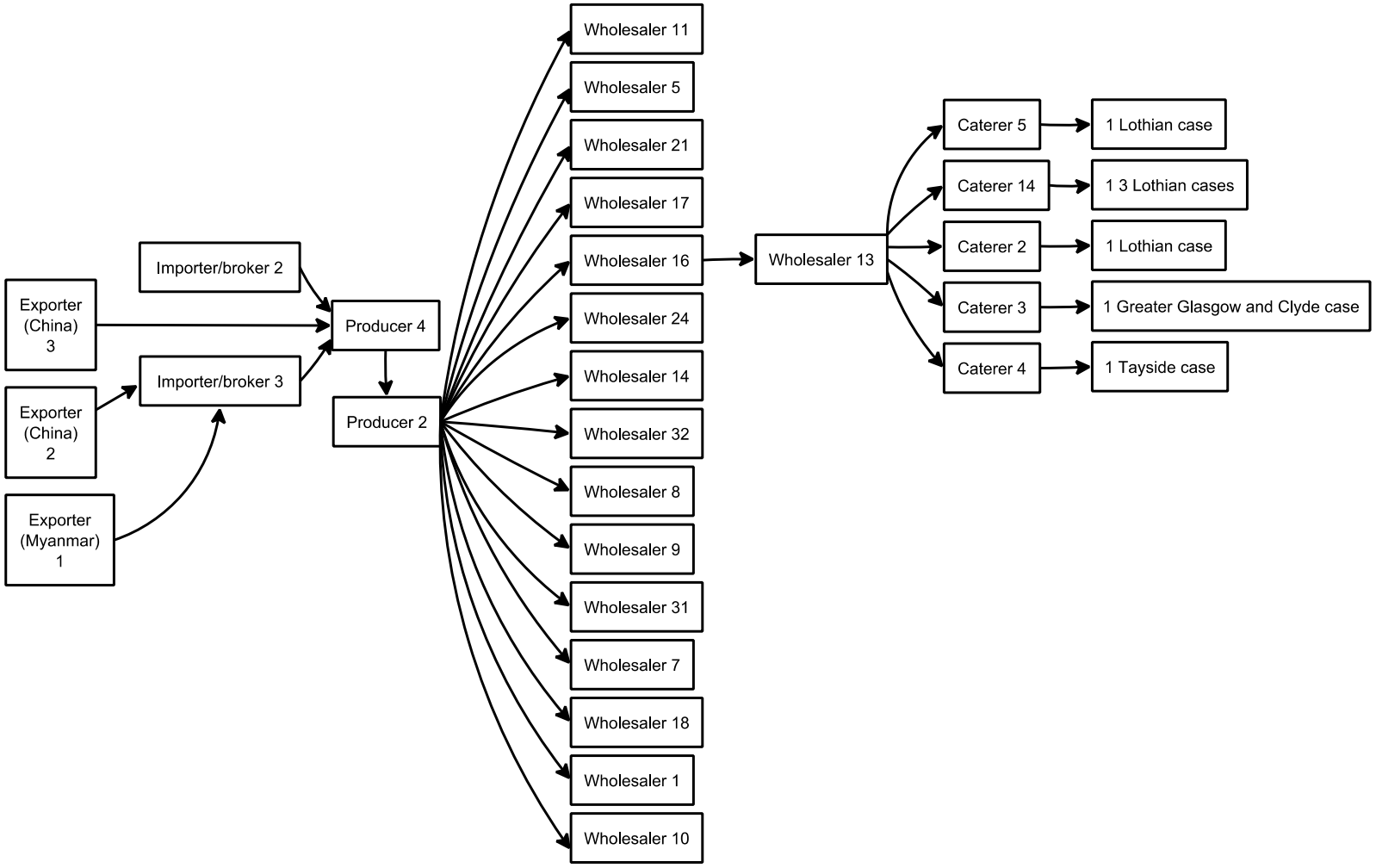



**Figure 8: Producer 2 bean sprout supply and distribution network**

The most parsimonious explanation of the findings of the investigation into the supply and distribution network is that contaminated seed imported from Exporter (China) 1 by Importer/broker 1 was supplied to Producer 1. Although Importer/broker 1 also imports mung bean seeds from Myanmar, these were not supplied to Producer 1. All investigations of bean sprouts eaten by cases of *S.* Bareilly, or positive on sampling for *S.* Bareilly, led back to Producer 1. There was no evidence to implicate other producers.

## MICROBIOLOGICAL INVESTIGATION

### MICROBIOLOGY OF HUMAN CASES

19 isolates from Scotland and 85 isolates from England and Wales were PFGE profiled as SBARXB.0016 or SBARXB.0016+, as defined in Pulse Net Europe, indicating that the strain of *S.* Bareilly in the Scottish and many of the English cases, including those linked to the Greater Manchester wedding, were indistinguishable and likely to have originated from a common source. A further 2 isolates from English cases had PFGE profiles that were distinct from the outbreak strain and are not considered to be related to the outbreak.

### MICROBIOLOGY OF FOOD AND ENVIRONMENTAL SAMPLES

Several samples of spices used in the preparation of food for the Greater Manchester wedding were tested for *Salmonella* at the HPA Food Water and Environmental (FWE) Laboratory and found to be negative. No samples of the chicken soup mix used were available but it was thought unlikely that this was the food vehicle.

As described above, routine testing for quality control purposes by two different fresh produce wholesalers (Wholesalers 15 and 23) in August and/or September 2010 had identified *Salmonella* group C from samples of bean sprouts which are intended to be cooked by consumers: from one sample on 16th August (Wholesaler 23) and from seven of 22 samples between 26th August and 9th September (Wholesaler 15). Both wholesalers are supplied by Producer 1; wholesaler 15 is an intermediary between Producer 1 and Wholesaler 23). None of the isolates from Wholesalers 15 or 23 were available for further testing.

A total of 67 samples were collected between the 21st September and the 28th October 2010 in connection with the national *S.* Bareilly outbreak investigation. Samples were collected from several producers of bean sprouts from sprouted mung beans in the North West of England which were linked to the national outbreak via distribution chains involving producers and intermediaries linked to cases in Scotland or Greater Manchester. Samples also included items submitted as part of investigations of Caterer 1 and Caterer 6. Most samples tested were from the bean sprout producer Producer 1 and included dried mung beans, bean sprouts from different stages of sprouting and environmental samples from the production facility, including spent irrigation waters. For one week, daily samples of bean sprouts from Wholesaler 23 and Producer 1 were tested at the HPA FWE Laboratory at Preston, beginning on 21st September. The reason for sampling at Wholesaler 23 and Producer 1 was to focus, at least initially, at or close to the beginning of the supply chain as it was known to the

OCT at the time, given the pivotal roles of Producer 1 and Wholesaler 23 in the supply/distribution chain. A positive result would strengthen the evidence that bean sprouts are the vehicle of infection.

For the detection of *S.* Bareilly in bean sprouts the HPA FWE laboratory used the UKAS accredited Preston Standard Operating Procedure FM10 for the detection of *Salmonella* species in food. This procedure is a documented in house version of the HPA Standard Operating Procedure F13 and is based on BS EN ISO 6579:2002 with modifications for public health investigations and the investigation of food poisoning/*Salmonella* incriminated samples. These modifications include the testing of a larger sample (100g instead of 25g) and the reincubation of the Rappaport-Vassiliadis Soya Peptone broth (RVS) and Muller-Kauffmann tetrathionate novobiocin (MKTTn) broths for a further 24 hours followed by subculture on selective agars and the reincubation of negative selective agars for a further 24 hours. It is important to note that the standard method (ISO 6579:2002) for detection of *Salmonella* from food sources does not require testing a sample of 100g.

The HPA FWE laboratory identified *S.* Bareilly with an indistinguishable PFGE type to the outbreak cases from a sample of bean sprouts from Wholesaler 23 (which had been sourced from Producer 1) which was taken on the 24th of September 2010. The level of contamination was 0.07 per gram, which may not have been detected in a sample of only 25 grams.

*S.* Bareilly was not isolated from any other samples submitted to the Preston laboratory as part of the national *S.* Bareilly outbreak investigation.

On the 29th of September 2010, *S.* Bareilly, with an indistinguishable PFGE type to the outbreak strain was also isolated by Edinburgh Scientific Services from two samples of bean sprouts from an unopened packet supplied to Caterer 14 by Wholesaler 23.

The UK OCT recommended a survey of ready to eat bean sprouts, described below.

## NORTH WEST BEAN SPROUT SNAPSHOT SURVEY

### METHODS

In order to assist with the national *S.* Bareilly outbreak investigation, the UK OCT recommended a snapshot survey for the detection of *Salmonella* in bean sprouts in the North West of England. The *Salmonella* detection was again carried out using the modified Preston Food method for outbreak/incident investigation. The HPA FWE laboratory requested the 45 local authorities in the North West to submit samples with a minimum weight of 100g of ready-to-eat (RTE) bean sprouts from retail outlets including institutional canteens and salad bars.

### RESULTS

Ready-to-eat bean sprouts were difficult to obtain, and so only all but four of the 44 samples were raw (i.e. not ready to eat) bean sprouts. *S.* Bareilly with an indistinguishable PFGE type to the outbreak strain was isolated from two samples of bean sprouts, both of which were raw (from two supermarkets: Retailer 1 and Retailer 3). Incidentally, two different *Salmonella* serovars, *Salmonella* Kottbus and *Salmonella* Abaetetuba were detected in a ready to eat product of sprouted seeds including mung beans purchased at a supermarket in the North West.

*S.* Bareilly was isolated from a bean sprout sample from a supermarket in the North West of England, which allowed the identification of a further bean sprout producer (Producer 3), not previously connected with the outbreak, but which on investigation by the local authorities was found to be using the same batch of mung beans from the same source as that in use by Producer 1.

An estimate of the level of *Salmonella* contamination was performed for the three samples contaminated with *S.* Bareilly (of which two were raw and one was a ready-to-eat sample of sprouted seeds) using a Most Probable Number method. The level of *Salmonella* contamination was estimated to be between 1 and 7 *Salmonella* organisms per 100g for all three samples.

# CONTROL MEASURES

## OVERALL CO-ORDINATION AND MANAGEMENT OF THE OUTBREAK

A local OCT convened by GMHPU and chaired by Dr Marko Petrovic led the response to the Greater Manchester wedding aspects of the outbreak.

A Scottish OCT convened by HPS and chaired by Dr John M. Cowden led the response to the Scottish aspects of the outbreak.

A UK OCT convened by HPA Local and Regional Services, with support from HPA Colindale and HPS and chaired by Professor Qutub Syed and Dr. Joe Kearney at different stages of the investigation led the response to the UK aspects of the outbreak.

## INFORMATION PROVIDED TO THE GENERAL PUBLIC

The UK OCT alerted the public to the possibility of a link between consumption of raw bean sprouts and the increased occurrence of *S*. Bareilly immediately after it was identified that a laboratory undertaking routine testing of salad produce for quality control purposes for a wholesaler in Kent had isolated Group C *Salmonella* in a bean sprout sample.

The first alert issued on the afternoon of Friday the 17th of September took the line that although there was no conclusive proof at this point that bean sprouts were responsible for the national outbreak, they were one line of inquiry. The statements made clear that bean sprouts were safe to eat, provided they were labelled “Ready to eat” or were washed and thoroughly cooked before consumption.

On the 23rd of September, the UK OCT issued a modified press statement. In the weeks between the 17th of September and the 12th of November, the HPA issued five press statements on behalf of the UK OCT. Health Protection Scotland also issued a press release. The FSA also issued statements and both agencies posted updates on their websites as and when there were developments. After the findings of the national case control study and microbiological investigations had strengthened the possibility of a link with bean sprouts, the UK OCT sent out a stronger public message on the 29th September advising that bean sprouts must be cooked thoroughly before consumption. After microbiological evidence of bean sprouts contamination with *S.* Bareilly continued to accrue, subsequent public alerts (such as that on the 7th of October) were more definite about the link with bean sprouts.

Key press statements are collated in the appendix.

## INFORMATION PROVIDED TO PROFESSIONALS AND BUSINESSES

The HPA sent out a number of internal briefings to HPA staff advising of the *S.* Bareilly outbreak, cascaded details of the investigation to all frontline HPA staff and published an article in the HPA's weekly Health Protection Report (HPR), which is read by health protection doctors and nurses, Directors of Public Health in Primary Care Trusts and local authority colleagues. The HPR is also accessed and read by many health reporters on the national and trade press. On the 8th September 2010, an internal briefing note (briefing note 2010/45) was sent out to all parts of the HPA, asking local health protection units to collect additional information on cases of *S.* Bareilly in England which were not linked to the wedding in the North West. The HPA Colindale Travel Section liaised with national focal points in Australia and the United States in relation to guests from those countries who had attended the GM wedding. Regular situation reports (SitReps) were produced and widely disseminated.

On 29th September, the FSA updated the advice on its website and drafted a letter for EHOs and food inspectors, reminding them of the guidance for the safe preparation and cooking of bean sprouts. The FSA also issued letters to local authorities on the 25th October 2010 and to UK trade associations on 1st November 2010 to update them on the progress of the investigation and to cascade advice on clear labelling of bean sprouts.

An interim report of the early stages of the outbreak was published in Eurosurveillance in December 2010.[16] Following this, an enquiry about UK *S*. Bareilly testing methodology was received from a US-based member of the International Sprout Growers Association (ISGA).

## OTHER ACTIONS TAKEN TO PREVENT FURTHER CASES

The examples of bean sprout labelling in Table 10 show how the instructions for preparation and cooking on packaging of bean sprouts vary in the information provided and in how explicit they are about the absolute requirement to wash and cook bean sprouts before consumption.

The FSA advises that all bean sprouts not labelled "ready to eat" should be cooked thoroughly until piping hot all the way through. The UK OCT was keen to eliminate labelling such as "washed and ready to use", which could be misconstrued to mean "washed and ready to eat." The FSA sent a letter to EHOs and food inspectors in England and Scotland on October the 25th asking them to address the issue of ambiguous labelling on bean sprout packaging with local suppliers, followed up by a letter to UK trade associations on the 1st of November to encourage them to cascade advice on clear labelling to their members as appropriate.

| Supplier | Instructions for use of bean sprouts |
|---|---|
| Unattributed | "Washed and ready to use"; does not advise against eating raw |
| Unattributed | "Cook before use" |
| Retailer | "Keep refrigerated. Stir-fry: 3 minutes. Once opened, consume within 24 hours. Do not exceed the Use By date."; does not advise against eating raw |
| Wholesaler 5 | "Wash before use"; no cooking instructions; does not advise against eating raw |
| Wholesaler 15 (250g packages) | Instructions advise rinsing and cooking before consumption |
| Producer 1 | "Washed and ready to cook"; does not advise against eating raw |
| Producer 2 | "Wash before use, keep refrigerated, do not freeze; eat within 1 day of purchase"; does not advise against eating raw |
| Retailers 2 and 3 | "Ready for cooking"; cooking instructions on back; did not advise against eating raw NB: Voluntarily changed to "Washed and ready to cook; do not eat raw" after Salmonella detected in raw bean sprouts |
| Producer 4 | Recommendation to immerse the bean sprouts in boiling water for 15 seconds before consumption |

**Table 10: Examples of labelling of bean sprouts identified during the investigation**

## COMMUNICATION AND MEDIA

A limited amount of press coverage was generated by HPA and FSA statements on the 17th September 2010 so on the 29th September, the HPA issued a statement proactively to the news media. This generated coverage by the Daily Telegraph, Scotsman and various trade and medical journals in the UK and abroad.

The campaign achieved some publicity in the national press but was widely covered by regional media, particularly in Greater Manchester where there had been a large local outbreak, in trade newspapers that are read by caterers and food wholesalers and, because of their interest in the Greater Manchester outbreak, by the Jewish press.

Subsequent media coverage of the link between bean sprouts and *Salmonella* infection included the Daily Mail, Telegraph, Scotsman, Herald, Manchester Evening News, Sun, various parts of the BBC and limited international interest (from the United States, France and Korea).

Following an episode of "Jamie Oliver's 30 Minute Meals" on Channel 4 in which the presenter Jamie Oliver was seen to serve and eat raw bean sprouts, contact was made the production company Fresh One Productions to advise of the risk. The production company mentioned their concern about the issue in a blog but the programme was subsequently rebroadcast unaltered.

At the time of writing, a TV production company has been in discussions with the HPA, FSA and other key partners to produce a programme for daytime television on the UK *S.* Bareilly outbreak, as part of a series called "Food Fighters".

## DISCUSSION AND CONCLUSIONS

The overall epidemiological and microbiological investigations implicated bean sprouts as the principal vehicle of *S.* Bareilly transmission, consistent with previous research showing that sprouted seeds can be a vehicle for transmission of *Salmonella* and other pathogens.[5] It is most likely that seeds contaminated with *S.* Bareilly of the outbreak strain were imported from China and that, despite disinfection procedures during the sprouting process at Producer 1, raw bean sprouts contaminated with low levels of *S.* Bareilly were distributed widely throughout the UK from this producer.

As the teriyaki salmon dish served at the wedding reception associated with the Greater Manchester outbreak was the only dish served which was known to have contained partly cooked bean sprouts, this is the most likely vehicle of infection for this outbreak. The epidemiological study into the Greater Manchester outbreak did not provide evidence of an association of bean sprouts with being a case but should not be misinterpreted as excluding it. A statistically significant association was found with three foods, of which one was roast chicken. Chicken was initially hypothesised as a possible food vehicle in the Greater Manchester outbreak and a statistically significant link was found, but no microbiological evidence of a link between chicken meat and *S*. Bareilly was demonstrated. A large number of food items were examined for an association with illness in this analysis and for each item there is a 5% chance of falsely demonstrating an apparent association when in fact no causal association exists. There are a number of other possible reasons why this study did not demonstrate an association of illness with bean sprout consumption. Cross-contamination between foods could have occurred (although it is unlikely when kosher rules are observed), diluting the association with an individual food item. In addition, although strenuous efforts were made to maximise the sample size, it was not possible to recruit three non-cases per case as initially envisaged, and furthermore, nearly 90% of the wedding guests reported consuming the teriyaki salmon dish. These factors reduced the power of the study to demonstrate a significant association of being a case with consumption of teriyaki salmon. The difficulties of data collection, with repeated revisions to the questionnaire due to initial incomplete information on menu items, and delays in data collection, may have led to bias in the collected data. A cautious interpretation of the epidemiological investigation of the Greater Manchester outbreak is to regard the findings as inconclusive.

The other epidemiological evidence (both descriptive and analytical) and the strong microbiological evidence of contamination of bean sprouts with *S.* Bareilly support the hypothesis of an association between consumption of bean sprouts and infection with *S.* Bareilly of the outbreak strain. Case control study designs are a useful study design where there is no clearly identifiable exposed group and can identify an association (for example the consumption of a particular food vehicle) with a relatively small sample size and in a shorter time than a cohort study. However, like any observational study they are prone to a number of biases. Possible biases include interviewer bias (differential accuracy in data collection due to interviewer awareness of case status) and recall bias (where cases are aware of a possible association between their illness and bean sprout consumption and more accurately recall recent bean sprout consumption than controls, leading to an artefactual association. Although there was a small amount of press interest in the possible link between *S.*

Bareilly and bean sprouts at the time of the case control study, this was mainly in the North West of England and in the trade press, and it is unlikely that many people in the general public were aware of a possible link between *Salmonella* and bean sprouts, so recall bias is unlikely to account for the findings. Another bias to consider in a case control study relates to the selection of controls. In our study, controls were selected by random digit dialling and this often gives a low response rate, meaning that the controls may not be representative of the general population. It is not possible to estimate what effect this could have had on our findings but it is again unlikely to be sufficient to account for the observed strong association between bean sprouts and *S.* Bareilly infection.

Although this investigation identified bean sprouts as a vehicle of infection, it was not able to conclude from this investigation that bean sprouts are the only food vehicle involved in *S.* Bareilly transmission, and other foods contaminated by *Salmonella* from bean sprouts could have contributed to this outbreak.

The bean sprouts implicated in this investigation were not ready to eat products (as is the case with most of those available commercially) but would be safe to eat if the instructions for correct preparation (washing and then cooking until piping hot) were followed. Not all labelling on bean sprouts makes the correct preparation of bean sprouts clear. Labelling should explicitly advise against eating bean sprouts without further preparation unless they are labelled as ready to eat.

Low dose intermittent contamination of bean sprouts may be too low or infrequent for detection by the methods currently used by commercial laboratories for quality control. The HPA recommends that a 100g sample be used for quality control testing and that the modified HPA NW FWE method be used for optimal detection.

It would have considerably facilitated the investigation of this outbreak if commercial laboratories detecting *Salmonella* in fresh produce as part of quality control testing were required to report to national surveillance systems and to submit positive samples for reference microbiology.

## ACKNOWLEDGEMENTS

Many thanks are due to staff at HPA NW (most of all to Debbie Anderson) for administrative and clerical support to the outbreak control team.

Thanks to colleagues throughout the HPA for sharing information from local investigations of *S.* Bareilly cases.

Thanks also to all colleagues not mentioned who assisted with the investigation.

## APPENDICES

### QUESTIONNAIRE – NORTH WEST ENGLAND WEDDING RECEPTION OUTBREAK

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### QUESTIONNAIRES – UK CASE CONTROL STUDY

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Bareilly InterviewScript.doc

### EXAMPLE PRESS RELEASES

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