# *Shigella flexneri* outbreak linked to a takeaway in Monmouthsire: Epidemiological findings

Date produced: 18th September 2023

Version: 1

Tarian incident: 16656

Report produced by Communicable Disease Surveillance Centre for the Outbreak Control Team

# Contents

1. Introduction ..... 4
1.1 Notes on causative organism ..... 4
1.2 Background to the incident ..... 4
1.3 Aims and objectives ..... 5
2. Methods ..... 6
2.1 Case definition(s) ..... 6
2.2 Data collection ..... 6
2.2.1 Line list ..... 6
2.2.2 Genomics Investigations ..... 6
2.3 Descriptive epidemiology ..... 6
2.4 Analytical study ..... 7
2.4.1 Hypothesis and study design ..... 7
2.4.2 Recruitment of cases and controls ..... 7
2.4.3 Questionnaire development ..... 7
2.4.4 Description of cases and controls ..... 8
2.4.5 Analytical epidemiology ..... 8
2.4.5 Additional analysis ..... 8
2.5 Information governance ..... 9
3. Results ..... 10
3.1 Descriptive epidemiology results ..... 10
Summary of cases linked to the outbreak ..... 10
Case-control study response rate ..... 12
Summary of cases and controls included in the study ..... 13
3.2 Analytical epidemiology results ..... 17
Univariable analysis ..... 17
Stratified analysis ..... 18
Multivariable analysis ..... 18
3.3 Additional analysis ..... 19
Dose-response effect of coleslaw consumption ..... 19
Dose-Severity analysis – hospitalisations and coleslaw ..... 19
Gender-bias amongst coleslaw orders ..... 20
4. Discussion ..... 21
4.1 Limitations ..... 22
5. Conclusions ..... 22
5.1 Recommendations ..... 22
6. References ..... 24
7. Appendix ..... 25

Appendix I: Identification of cases and controls for case-control study ..... 25

Appendix II: Enhanced surveillance questionnaire for case-control study ..... 26

# 1. Introduction

## 1.1 Notes on causative organism

Shigella is a gram negative bacteria responsible for the gastrointestinal illness, shigellosis [1]. The annual incidence of Shigella infection is estimated at 30 per 100,000 populations globally. Of the four species of Shigella, *S. sonnei* is most common in Europe, followed by *S. flexneri*. Both are characterised by 'bacillary dysentery', including profuse, sometimes bloody, diarrhoea, fever and vomiting [2]. *S. flexneri* is often more severe than *S. sonnei*, approximately 2% of people infected with *S. flexneri* go on to develop post-infectious arthritis (Reiter's syndrome) [3].

In 2022, a rise in *Shigella sp*. laboratory reports was recorded in Wales (data not published). This trend was matched by an increase in *S. flexneri* reported in England in 2021-2022, as reported by the UK Health Security Agency (UKHSA) [4]. However, it is worth noting that nearly 70% of *S. flexneri* cases reported to UKHSA in the first half of 2022 were in men who have sex with men (MSM) [4]. Where outbreaks of S. *flexneri* in the UK have been published, more often than not these outbreaks focus on transmission via sexual contact between MSM [5,6].

## 1.2 Background to the incident

On 17th February 2023, Public Health Wales (PHW) Health Protection Team (HPT) was contacted by Royal Gwent Hospital Microbiology, Aneurin Bevan University Health Board (ABUHB), following the identification of 9 new presumptive Shigella/enteroinvasive E.*coli* by PCR test on faecal samples. The cases, who had experienced gastrointestinal symptoms including diarrhoea and vomiting, were resident in the Abergavenny or Crickhowell areas of Monmouthshire and Powys local authorities. Four of nine faecal samples were requested by the same general practice (GP) surgery. At the time, four cases had been hospitalised as a result of their symptoms.

Over the weekend, cases were followed up, risk assessed and standardised exposure questionnaires were completed. From this initial investigation, all nine cases were found to have reported eating food from the same takeaway in Abergavenny (Takeaway A), on the 10th or 11th February 2023. An incident was opened on Tarian (ID: 16656) on 17th February 2023. Support was sought from Monmouthshire environmental health officers (EHOs), who visited the premises on the Friday evening to conduct an inspection.

An incident management team (IMT) was convened on 18th February 2023. Cases to date (n=14) were summarised, of which 10 had provided a sample that was PCR positive for shigella/enteroinvasive E.*coli* and a further 4 were symptomatic awaiting microbiological confirmation. Of those interviewed, 10 reported eating at Takeaway A, of which 9 ate from the premises on 10th February 2023. Symptom onsets were reported between 12th and 13th February 2023. It was noted that there had been substantial activity on social media regarding illness following recent consumption of food from Takeaway A, and it was possible that case numbers were higher than those currently notified.

The EHO's site visit identified a number of remediable issues around hygiene and cross-contamination risks, but the business remained open. The premises had been closed between 12th – 16th February 2023 for refurbishment. It had not been possible to collect any food samples for testing. Three staff members identified as working on 10th February 2023 had agreed to provide stool samples for microbiological testing.

The Consultant Microbiologist present at the IMT reported that two samples had undergone additional culture testing to identify the causative organism, *S. flexneri*. The working hypothesis was that an infected food handler working at Takeaway A had contaminated

food that was eaten by cases. As such, there was a risk of ongoing transmission as shigella can continue to shed for up to four weeks post-acute illness recovery [7].

By 23rd February 2023, 34 cases had been identified, of which 11 were culture positive for *S. flexneri*, a further 16 were PCR positive for shigella/enteroinvasive E.*coli* and 7 had GI symptoms compatible with the outbreak. 23 (68%) had eaten at Takeaway A on 10th February 2023. A further two staff had been identified as working on the dates in question.

Case numbers continued to rise over the following weeks. By 17th March 2023, 52 cases had been identified, of which 29 were culture positive for *S. flexneri* and 11 had been hospitalised. All five staff provided stool samples on two occasions but all samples were negative for shigella/enteroinvasive E.*coli* on PCR. The last case had a symptom onset date of 22nd February 2023 (Culture positive, secondary case). The outbreak control team (OCT) was closed on 17th March 2023, pending a summary briefing once all extended investigations had been concluded.

To further understand the exposures associated with GI illness, PHW Communicable Disease Surveillance Centre (CDSC) conducted a case-control study to investigate this outbreak.

## 1.3 Aims and objectives

The aim of this investigation was to determine the source of the *S. flexneri* outbreak, including any food vehicle, in order to inform the OCT and support management of this and future outbreaks of this nature/setting.

The objectives of this analysis were:

- To describe the outbreak in terms of person, place and time
- To report common exposures amongst cases
- To conduct a case-control study to identify exposures associated with being a probable or confirmed case

# 2. Methods

## 2.1 Case definition(s)

The following working case definitions were agreed by the OCT:

**Possible Case:**

A person with a GI illness compatible with bacillary dysentery, who is a resident of Abergavenny, Crickhowell or surrounding areas, with a symptom onset from 6th February 2023

**Probable Case:**

As above, plus epidemiological links to the incident including:

- A person who is a contact of a known case already linked to this incident

  *OR*

- Has eaten food purchased from Takeaway A from the 6th - 12th February 2023

*OR*

A Shigella PCR (ipaH) positive, culture negative/culture awaited, result from a local laboratory who is a resident of Abergavenny, Crickhowell or surrounding areas from 6th February 2023

**Confirmed Case:**

As above plus culture positive for *S. flexneri*

## 2.2 Data collection

### 2.2.1 Line list

A line list of cases (confirmed, probable and possible) was jointly maintained by PHW HPT and CDSC. The line list contained Tarian case ID (unique identifier), case demographic data (name, sex, age, address), date of symptom onset, hospitalisations, laboratory data (PCR test results, culture test results), case definition classification, date of takeaway order and a summary of menu items eaten (as reported at initial interview with HPT). The line list was later expanded to include genomics data.

### 2.2.2 Genomics Investigations

28 confirmed case samples were sent to UKHSA genomics reference laboratory, Colindale, for whole genome sequencing (WGS). The resultant single nucleotide polymorphism (SNP) addresses were returned to PHW on 7th March 2023. Information was added to the master line list for descriptive summary.

## 2.3 Descriptive epidemiology

Line list data were cleaned and analysed using Stata 14.2 and R. Cases linked to the outbreak and recorded on the master line list were described in terms of person, place and time, microbiological and genomic characteristics and hospitalisations. Epidemiological curves and age-sex pyramids were constructed.

## 2.4 Analytical study

### 2.4.1 Hypothesis and study design

The hypothesis ($H_1$) of this analytical study was that shigellosis is associated with the consumption of a food item from Takeaway A between 10th-11th February 2023.

The null hypothesis ($H_0$) was that shigellosis is not associated with consumption of food from Takeaway A, and is instead associated with a different exposure common to cases.

This hypothesis was tested using an unmatched case-control study design.

### 2.4.2 Recruitment of cases and controls

Cases were selected from the master line list. Cases with laboratory confirmed evidence of *Shigella sp* infection (i.e. probable and confirmed cases) who had an onset date from 11th February 2023 were included in case-control study. 34 cases (27 confirmed, 7 probable) met the definition for inclusion in the case-control study.

A control was defined as:

A person who has eaten food purchased from Takeaway A between 10th – 12th February 2023 but did not develop symptoms

*AND* has not tested positive for Shigella/Enteroinvasive E.*coli* on PCR.

Controls were selected by three methods:

i. Those identified by cases as individuals who had eaten from Takeaway A on 10th – 11th February 2023, either in the same order 'party' as cases or in different parties, but had not developed GI symptoms. 21 potential controls were identified this way.
ii. A list of customers who had ordered food from Takeaway A via an online application between 6th -11th February 2023 was obtained from Boost technologies on 13th March 2023. The list contained contact details of 42 customer orders, of which 4 were known confirmed or probable cases. Of the remaining 38, 2 people had ordered from Takeaway A on two separate occasions between 6th – 11th February. At this point, the epidemiological team were already confident that the outbreak was a point source exposure on either 10th or 11th February 2023. As such, this customer list was revised to exclude orders outside the dates of interest. This left 17 further potential controls for contact.
iii. A further 13 potential controls were identified by those who ordered via the app as people they had eaten with who had not developed GI symptoms.

Where control recruitment also led to the identification of symptomatic cases, these cases were appropriately included/excluded from the case-control study based on the availability of laboratory data.

Separate lists were kept to show cases and controls eligible for contact and when contact was made by a call handler. A flow diagram of case-control inclusion/exclusion is appended (Appendix I).

### 2.4.3 Questionnaire development

To address the hypothesis and support the identification of exposures associated with illness, the CDSC developed a questionnaire to collect information on exposure to Takeaway A, including food ordered and portion size, as well as symptom presentation and healthcare access. A menu of food items for order was obtained from Takeaway A's webpage, and ingredient lists for sauces and condiments were confirmed by EHO's during discussions with Takeaway A. To test the null hypothesis that the exposure was external to Takeaway A, the questionnaire also asked about visits to other food establishments

(restaurants/takeaways/cafes), grocery shopping, occupation and travel history. The same questionnaire was delivered to cases and controls.

A copy of the questionnaire is appended (Appendix II). An electronic copy of the questionnaire was developed as a Tarian Data Extension (TDE) to allow information to be filed directly within case records and to support data cleaning and analysis.

Questionnaires were delivered over the telephone by trained CDSC team members. Interviews were conducted between 1st – 20th March 2023. Responses were uploaded to the appropriate case/control TDE record.

### 2.4.4 Description of cases and controls

Questionnaires completed as part of the case-control study were downloaded from Tarian and cleaned in R and Stata to standardise data suitably for further analysis. As menu items were recorded in terms of portion size, binary (yes/no) variables were created for each menu item to allow for assessment of any exposure to an item.

Response rates of cases and controls eligible for the study were calculated. Respondent and non-respondent cases and controls were described in terms of age (mean, range) sex and postcode area of residence (data not available for controls). Chi-squared tests (t-tests for age) were calculated to show differences between case and control respondents vs. non-respondents.

Included cases and controls were described in terms of age, sex, postcode area of residence and date of order from Takeaway A (i.e. exposure date). Chi-squared tests (t-tests for age) were used to compare cases and controls and to inform later analytical calculations. Time of order from Takeaway A for cases and controls was also displayed graphically.

Cases were further described in terms of symptom presentation, incubation period and symptom duration (minimum, maximum, mean and interquartile range) and whether or not healthcare services were accessed (e.g. GP, NHS 111, Pharmacy, Hospital).

### 2.4.5 Analytical epidemiology

Univariable analysis was conducted on the exposures listed in the questionnaire (menu items, food eaten outside the home, food/grocery shopping, travel). Percentage of cases and controls exposed to each item were calculated (i.e. attack rate). Odds ratios and 95% confidence intervals (95%CI) with p-values were calculated for each item to identify statistically significant associations ($p<0.05$).

Items from the univariable analysis with a p-value<0.2 were investigated further by stratification to identify potential confounders and effect modifiers. The exposures tested in the stratified analysis were added into a multivariable logistic regression model in a forward stepwise approach to identify most likely vehicles of transmission. Likelihood ratio tests were conducted to assess the fit of models as new variables were added to the model.

### 2.4.5 Additional analysis

A dose-response analysis was conducted on variables included in the multivariable model to identify if greater exposure elevated odds of illness. Additionally, these variables were also included in a dose-severity analysis to ascertain whether greater exposure led to poorer outcomes.

A gender-response analysis was also conducted between variables of the multivariable model to ascertain whether gender was associated with increased odds of exposure to another variable in the model.

## 2.5 Information governance

All documents and files containing person identifiable information were handled and stored in compliance with the Data Protection Act (1998) and GDPR (2018) as well as by guidelines established by the local Caldicott guardian.

# 3. Results

## 3.1 Descriptive epidemiology results

### Summary of cases linked to the outbreak

As of 17th March 2023, 52 cases were linked to the outbreak (Table 1). 30 (58%) cases were female, and average case age was 41 years (range: 1-75 years, Interquartile range (IQR): 16-63 years)(Figure 1). 29 cases (56%) met the case definition for a confirmed case with a culture positive result for *S. flexneri*. 23 cases (44%) met the definition of a probable case, of which 8 (35%) had a PCR sample positive for *Shigella sp*. 11 cases (21%) were hospitalised as a result of their symptoms.

50 cases (96%) had eaten at the Takeaway A, the majority of which (n=40, 77%) ate food from the premises on the 10th February 2023. The two cases who did not eat at Takeaway A were thought to have been exposed to *Shigella sp* via direct contact with a case who had eaten from the restaurant (1 culture positive case, 1 probable PCR negative case).

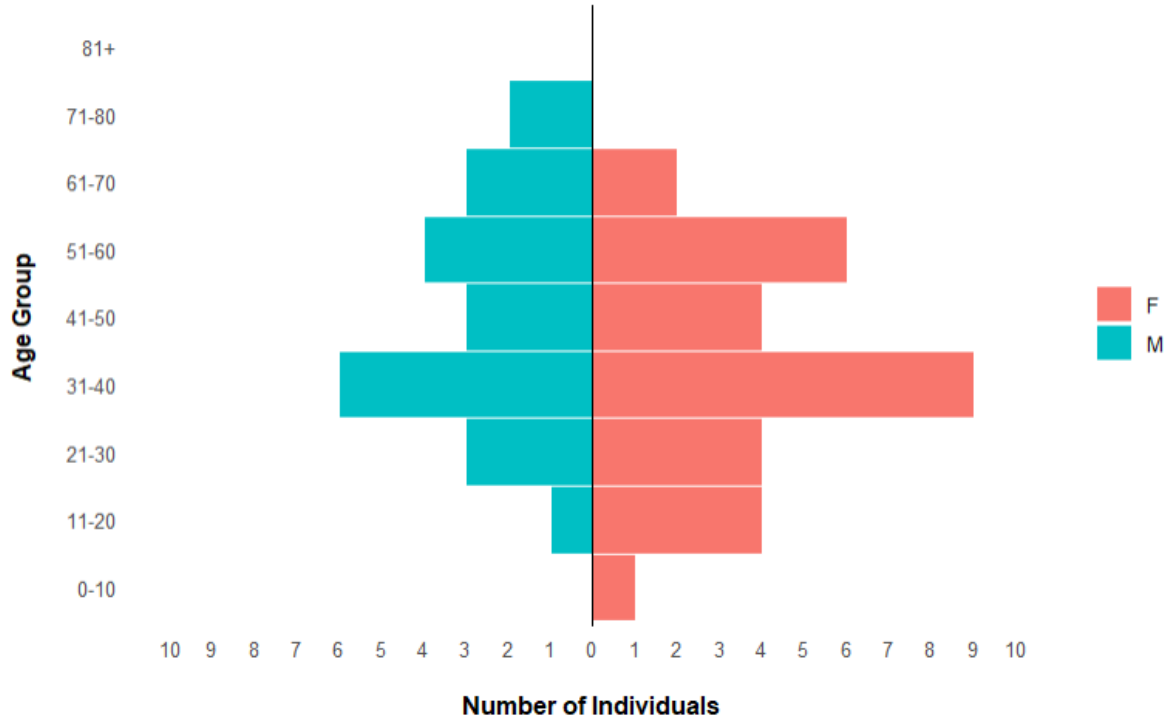
All except one case lived in South East Wales. The remaining case lived in South West England.

**Table 1:** Summary of cases linked to outbreak

| | n | % |
|---|---|---|
| **Total** | **52** | |
| Female | 30 | 58 |
| Age (mean, range) | | 41 (1-75) |
| Confirmed* | 29 | 56 |
| Probable | 23 | 44 |
| PCR positive | 8 | 35 |
| PCR negative** | 2 | 9 |
| No PCR sample | 13 | 57 |
| Hospitalised | 11 | 21 |
| Ate at Takeaway A?***: | 50 | 96 |
| 10th Feb 2023 | 40 | 77 |
| 11th Feb 2023 | 10 | 19 |
| Residential location | | |
| Abergavenny | 37 | 71 |
| Crickhowell | 10 | 19 |
| Ebbw Vale | 3 | 6 |
| Hereford | 1 | 2 |
| Raglan | 1 | 2 |

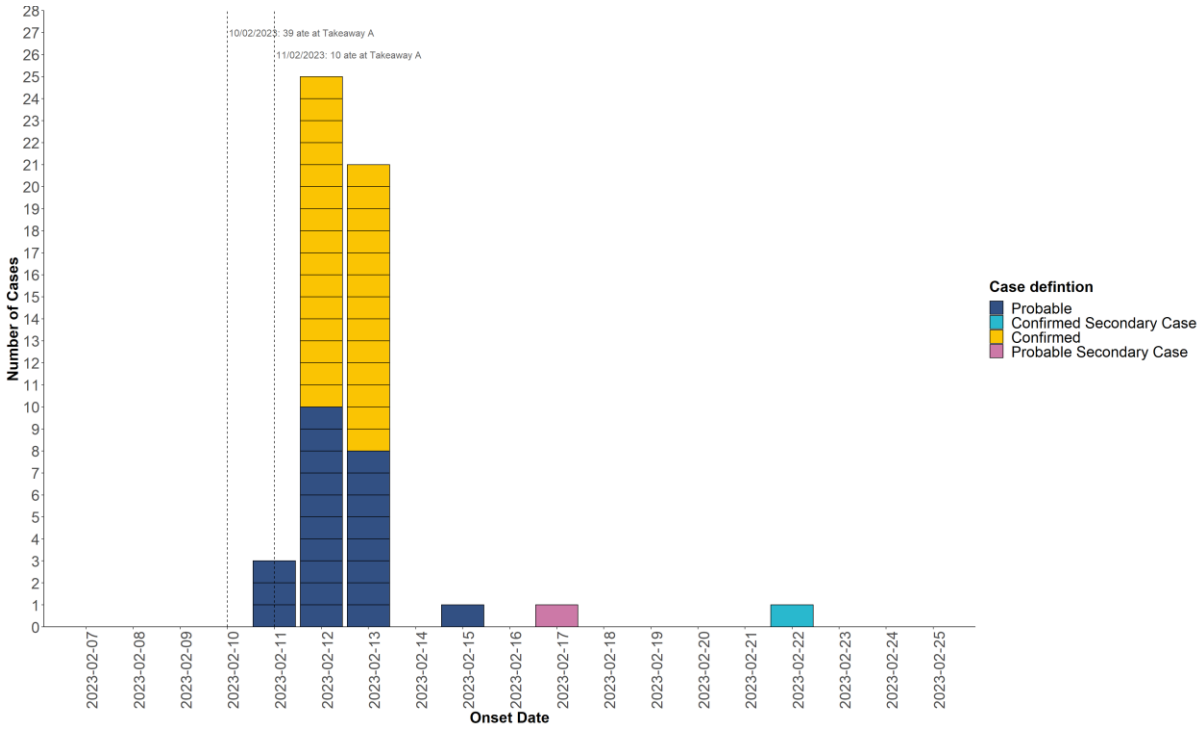
*1 confirmed case was a secondary transmission, had not eaten at Takeaway A
**1 probable PCR negative case was secondary transmission, had not eaten at Takeaway A
*** All primary cases had eaten at Takeaway A

**Figure 1:** Age-sex distribution of confirmed and probable cases linked to outbreak




The tight clustering of case onset, as shown on the epidemiological curve, would suggest a point source outbreak, with symptoms starting within 24-48 hours after exposure to Takeaway A (Figure 2). Cases infected through secondary transmission (i.e. symptomatic individuals who did not eat at Takeaway A, but are linked to known outbreak cases) are seen later on in the outbreak, after all confirmed or probable primary cases reported symptom onset.

**Figure 2:** Epidemiological curve of confirmed and probable cases linked the outbreak



All samples sent for sequencing (28 cases) were clustered within 5 single nucleotide polymorphisms (SNPs) (Table 2). This high level of relatedness for the samples of *S. flexneri* which underwent WGS, suggest a common exposure or event led to transmission.

**Table 2:** SNP address summary table

| SNP address | n | % |
|---|---|---|
| 4.145.455.1788.2389.2864.4235 | 14 | 27 |
| 4.145.455.1788.2389.2864.4247 | 1 | 2 |
| 4.145.455.1788.2389.2864.4248 | 1 | 2 |
| 4.145.455.1788.2389.2864.4249 | 4 | 8 |
| 4.145.455.1788.2389.2864.4250 | 1 | 2 |
| 4.145.455.1788.2389.2864.4252 | 1 | 2 |
| 4.145.455.1788.2389.2864.4253 | 1 | 2 |
| 4.145.455.1788.2389.2864.4259 | 1 | 2 |
| 4.145.455.1788.2389.2864.4261 | 1 | 2 |
| 4.145.455.1788.2389.2864.4262 | 1 | 2 |
| 4.145.455.1788.2389.2864.4267 | 1 | 2 |
| Same 5-SNP cluster* | 1 | 2 |
| No sample sent | 24 | 46 |

*Case resident outside Wales, but ate at Takeaway A on 10th February 2023. Full SNP not provided

## Case-control study response rate

After excluding likely secondary cases and probable primary cases with no/negative PCR result, 34 cases met the definition for inclusion in the case-control study. Of these, 31 (91%) completed the study questionnaire and were included in the final study (3 cases were not contactable). Of the 44 controls identified by cases (n=21, 48%) and from app data/parties (n=23, 52%), a total of 29 controls (64%) were successfully contacted and completed the study questionnaire (Table 3). There was no significant difference between the age, sex and residential location of respondent and non-respondent cases, and no significant difference between the age and sex of respondent and non-respondent controls (Table 4, 5).

**Table 3:** Response rate for cases and controls eligible for study

| | Cases | | | Controls | | |
|---|---|---|---|---|---|---|
| Age band | Reported | Analytical study | | Reported | Analytical study | |
| 10-19 | 2 | 2 | (100%) | 7 | 6 | (86%) |
| 20-29 | 5 | 5 | (100%) | 11 | 7 | (64%) |
| 30-39 | 8 | 8 | (100%) | 7 | 6 | (86%) |
| 40-49 | 4 | 3 | (75%) | 3 | 3 | (100%) |
| 50-59 | 8 | 8 | (100%) | 4 | 4 | (100%) |
| 60-69 | 5 | 3 | (60%) | 1 | 1 | (100%) |
| 70+ | 2 | 2 | (100%) | 0 | 0 | - |
| Unknown | 0 | 0 | - | 11 | 2 | (18%) |
| **Total** | **34** | **31** | **(91%)** | **44** | **29** | **(66%)** |

**Table 4:** Comparison of respondent and non-respondent cases

| | Cases | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total in sample | | Respondents | | Non-respondents | | *P*-value |
| | Mean | SD | Mean | SD | Mean | SD | |
| Age (years) | 44.1 | (17.0) | 42.9 | (17.0) | 56.3 | (14.6) | 0.197 |
| | n | % | n | % | n | % | |
| Gender | | | | | | | |
| Male | 12 | (35.3) | 10 | (32.3) | 2 | (66.7) | 0.234 |
| Female | 22 | (64.7) | 21 | (67.7) | 1 | (33.3) | |
| Postcode area | | | | | | | |
| NP15 | 1 | (2.9) | 1 | (3.2) | 0 | (0.0) | 0.814 |
| NP23 | 2 | (5.9) | 2 | (6.5) | 0 | (0.0) | |
| NP4 | 1 | (2.9) | 1 | (3.2) | 0 | (0.0) | |
| NP7 | 23 | (67.6) | 20 | (64.5) | 3 | (100.0) | |
| NP8 | 7 | (20.6) | 7 | (22.6) | 0 | (0.0) | |

**Table 5:** Comparison of respondent and non-respondent controls

| | Controls | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total in sample | | Respondents | | Non-respondents | | *P*-value |
| | Mean | SD | Mean | SD | Mean | SD | |
| Age (years) | 31.5 | (13.9) | 33 | (14.4) | 25 | (9.5) | 0.207 |
| | n | % | n | % | n | % | |
| Gender* | | | | | | | |
| Male | 22 | (50.0) | 18 | (62.1) | 4 | (26.7) | 0.81 |
| Female | 14 | (31.8) | 11 | (37.9) | 3 | (20.0) | |

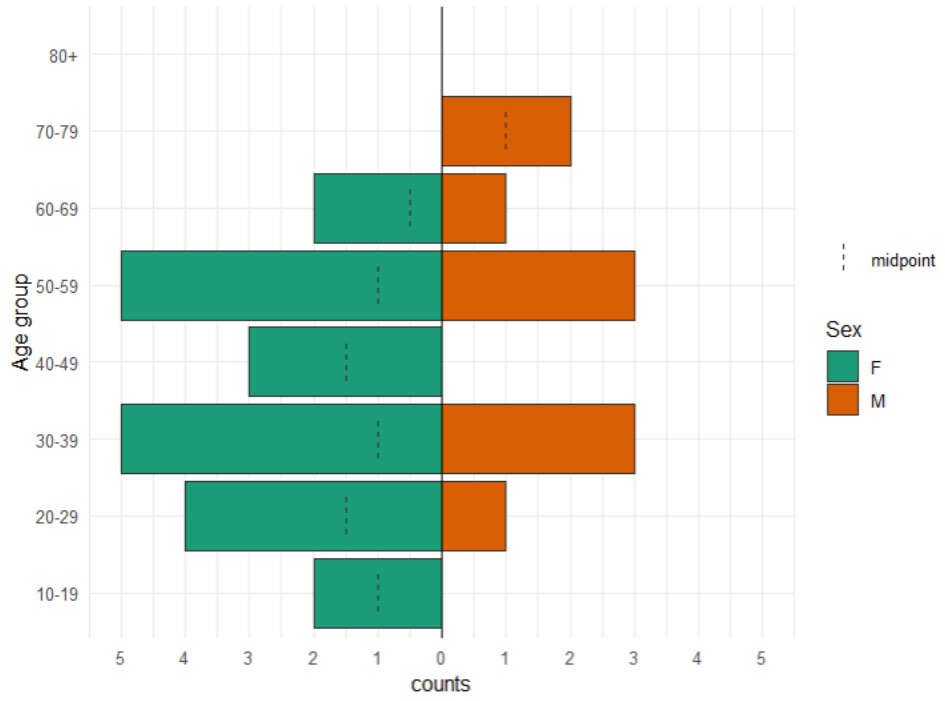
*Gender not available for 8 controls that did not respond

## Summary of cases and controls included in the study

The study included 31 cases and 29 controls. The average age of study participants was significantly older amongst cases (43 years, 11-75 years) compared to controls (33 years, 12-67 years) (p=0.024) Cases also included significantly fewer men than controls (Cases: 10, 32%; Controls: 18, 62%, p=0.021). Both factors were included in analytical analysis. There was no significant difference in location of residence or date of exposure to Takeaway A between cases and controls (Figure 3, Table 6).

**Figure 3:** Age-sex pyramids of (a) cases and (b) controls in case-control study

**a)**




**b)**

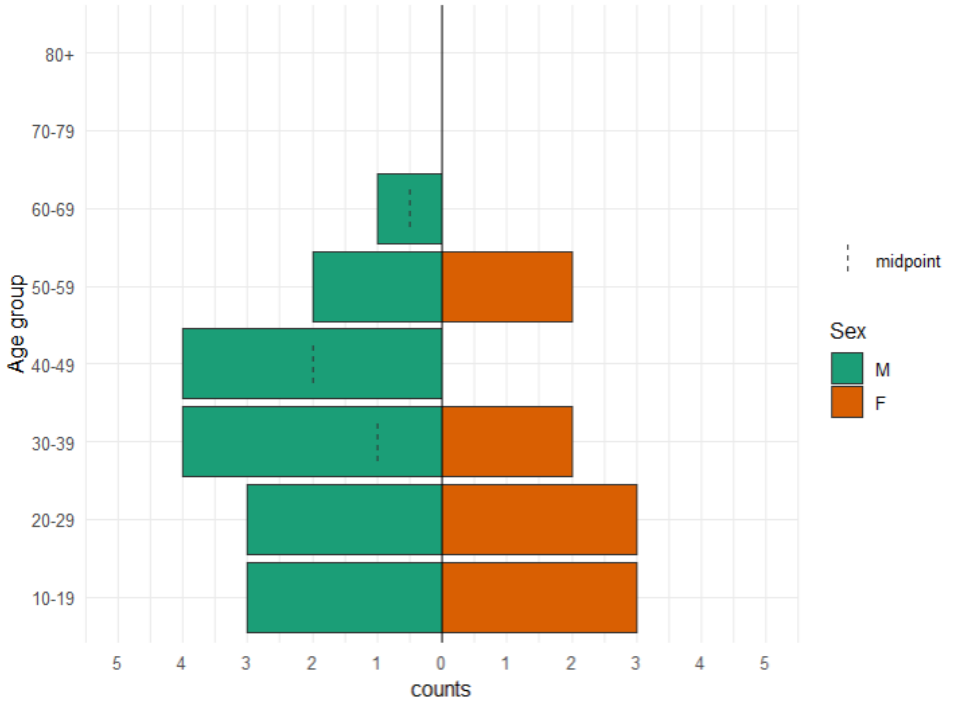


**Table 6:** Comparison of cases and controls included in the study

| | Total in sample | | Cases | | Controls | | *P* -value |
|---|---|---|---|---|---|---|---|
| Age/years (mean, range) | 39 (11-75) | | 43 (11-75) | | 33 (12-67) | | **0.024** |
| | n | % | n | % | n | % | |
| Male | 28 | (47%) | 10 | (32%) | 18 | (62%) | **0.021** |
| Postcode | | | | | | | |
| CF11 | 1 | (2%) | 0 | - | 1 | (3%) | 0.465 |
| NP15 | 2 | (3%) | 1 | (3%) | 1 | (3%) | |
| NP23 | 2 | (3%) | 2 | (6%) | 0 | - | |
| NP4 | 2 | (3%) | 1 | (3%) | 1 | (3%) | |
| NP7 | 42 | (70%) | 20 | (65%) | 22 | (76%) | |
| NP8 | 10 | (17%) | 7 | (23%) | 3 | (10%) | |
| Exposure Date | | | | | | | |
| 10th Feb 2023 | 49 | (82%) | 27 | (87%) | 22 | (76%) | 0.218 |
| 11th Feb 2023 | 10 | (17%) | 3 | (10%) | 7 | (24%) | |
| 12th Feb 2023 | 1 | (2%) | 1 | (3%) | 0 | - | |
| **Total** | **60** | | **31** | | **29** | | |

All cases interviewed reported symptoms related to *Shigella sp* infection. All cases reported diarrhoea, in 23 cases (74%) that included bloody diarrhoea (Table 7). The average incubation time from exposure to symptom onset was 2-3 days (IQR) (Table 8), and symptoms lasted on average 11 days (Table 9). 25/31 cases (81%) sought healthcare advice for their symptoms. This was most commonly through a GP (19, 61%). 9 cases (29%) required hospitalisation, with an average admission length of 4.5 days (range: 0-12 days) (Table 10).

**Table 7:** Symptoms reported by cases

| Symptoms | | |
|---|---|---|
| Any Symptom | 31 | (100%) |
| Abdominal pain | 31 | (100%) |
| Diarrhoea | 31 | (100%) |
| Stomach cramps | 26 | (84%) |
| Fever | 26 | (84%) |
| Shivering | 24 | (77%) |
| Bloody stools | 23 | (74%) |
| Nausea | 23 | (74%) |
| Vomiting | 21 | (68%) |
| Headache | 19 | (61%) |
| Muscle aches | 18 | (58%) |
| Rash | 0 | - |

**Table 8:** Incubation period from exposure to onset (days)

| Incubation period (days) | |
|---|---|
| Min | 0 |
| Mean | 2.4 |
| Max | 3 |
| IQR | 2-3 |

**Table 9:** Symptom duration (days)

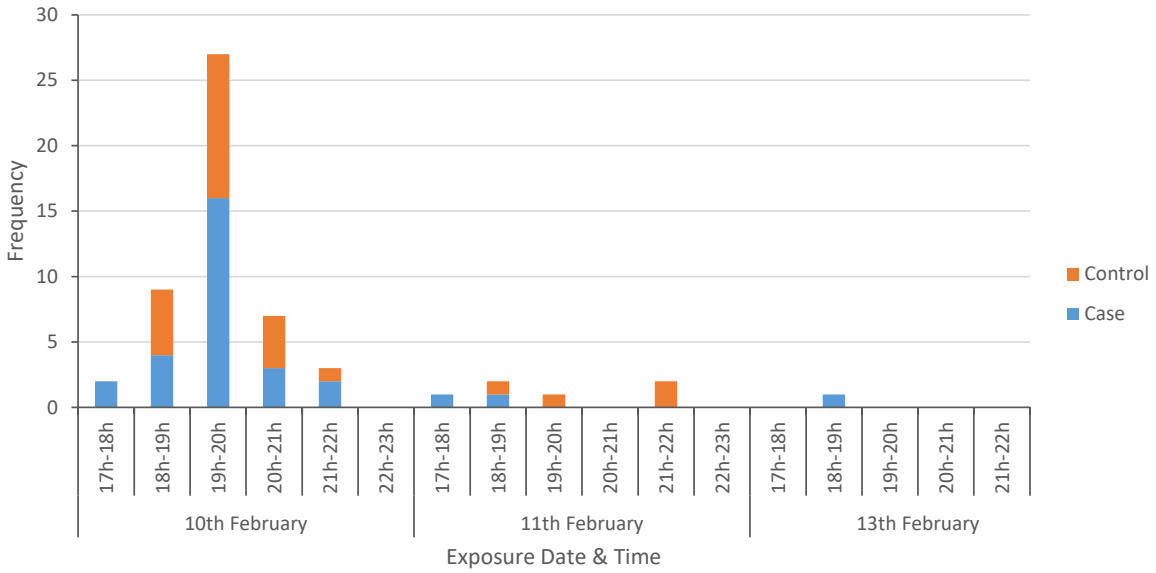
| Symptom duration (days) | |
|---|---|
| Min | 2 |
| Mean | 11.2 |
| Max | 20 |
| IQR | 7-15 |

**Table 10:** Healthcare advice-seeking behaviours by cases

| | | |
|---|---|---|
| GP | 19 | (61%) |
| A&E | 7 | (23%) |
| NHS Direct | 3 | (10%) |
| Pharmacy | 2 | (6%) |
| Hospital Admission | 9 | (29%) |
| Admission length (days) | | 4.5 (0-12) |

Of the 60 participants in the case-control study, 34 (57%) ordered from Takeaway A between 19:00 and 21:00 on 10th February 2023 (Figure 4).

**Figure 4:** Exposure (i.e. order) date and time of cases and controls



## 3.2 Analytical epidemiology results

### Univariable analysis

Percentage exposure, odds ratios (OR) with 95% confidence intervals (95%CI) and p-values were calculated for all items on the menu of Takeaway A, other food purchased and prepared outside the home (restaurants/cafes, etc.), grocery shopping locations, travel within the UK and abroad and key demographics. Table 11 shows the result of univariable analysis of exposures, where the calculated OR was greater than 1.0.

5/35 menu options showed a significant association ($p<0.05$) with being a case in the univariable analysis. In particular, coleslaw - which was eaten by 27 cases (87%) and 8 controls (28%) - had an OR of 17.7 (95%CI 4-87, $p<0.0001$). This was substantially greater association than other statistically significant menu items.

Univariable analysis also identified that being female was significantly associated with illness. The odds of being a case were 3.4 times greater in females compared to males (95%CI 1-11, $p<0.021$)

**Table 11:** Exposures in the study with an odds ratio greater than 1. Highlighted exposures had a p-value <0.05.

| | Cases | | Controls | | | | |
|---|---|---|---|---|---|---|---|
| **Exposures** | **n** | **%** | **n** | **%** | **OR** | **95% CI** | **p** |
| **Menu Item** | | | | | | | |
| Coleslaw | 27 / 31 | 87.1% | 8 / 29 | 27.6% | 17.7 | ( 4.09 - 87.22 ) | 0.000 |
| Garlic Mayonnaise | 27 / 31 | 87.1% | 17 / 29 | 58.6% | 4.8 | ( 1.16 - 23.04 ) | 0.013 |
| Doner Kebab | 8 / 31 | 25.8% | 2 / 29 | 6.9% | 4.7 | ( 0.80 - 48.55 ) | 0.050 |
| Cabbage | 18 / 31 | 58.1% | 7 / 29 | 24.1% | 4.4 | ( 1.27 - 15.55 ) | 0.008 |
| Lettuce | 26 / 31 | 83.9% | 16 / 29 | 55.2% | 4.2 | ( 1.12 - 17.71 ) | 0.015 |
| Cucumber | 24 / 31 | 77.4% | 14 / 29 | 48.3% | 3.7 | ( 1.07 - 13.17 ) | 0.019 |
| Onion | 20 / 31 | 64.5% | 12 / 29 | 41.4% | 2.6 | ( 0.81 - 8.33 ) | 0.073 |
| BBQ sauce | 4 / 31 | 12.9% | 2 / 29 | 6.9% | 2.0 | ( 0.26 - 23.59 ) | 0.438 |
| Chicken Shish Wrap | 2 / 31 | 6.5% | 1 / 29 | 3.4% | 1.9 | ( 0.09 - 117.92 ) | 0.594 |
| Tomatoes | 16 / 31 | 51.6% | 11 / 29 | 37.9% | 1.7 | ( 0.55 - 5.55 ) | 0.287 |
| Chicken Shish | 11 / 31 | 35.5% | 9 / 29 | 31.0% | 1.2 | ( 0.37 - 4.14 ) | 0.715 |
| **Food eaten outside home** | | | | | | | |
| British cuisine | 5 / 31 | 16.1% | 3 / 29 | 10.3% | 1.7 | ( 0.29 - 11.75 ) | 0.510 |
| **Food shopping** | | | | | | | |
| Iceland | 4 / 31 | 12.9% | 1 / 29 | 3.4% | 4.1 | ( 0.37 - 211.41 ) | 0.185 |
| Waitrose | 6 / 31 | 19.4% | 3 / 29 | 10.3% | 2.1 | ( 0.39 - 14.09 ) | 0.329 |
| Marks & Spencer | 2 / 31 | 6.5% | 1 / 29 | 3.4% | 1.9 | ( 0.09 - 117.92 ) | 0.594 |
| Tescos | 6 / 31 | 19.4% | 4 / 29 | 13.8% | 1.5 | ( 0.31 - 8.10 ) | 0.563 |
| **Other** | | | | | | | |
| Female | 21 / 31 | 67.7% | 11 / 29 | 37.9% | 3.4 | ( 1.05 - 11.41 ) | 0.021 |
| 20-49 years | 16 / 31 | 51.6% | 16 / 29 | 55.2% | REF | | |
| 50+ years | 13 / 31 | 41.9% | 7 / 29 | 24.1% | 1.9 | ( 0.59 - 5.87 ) | 0.292 |

## Stratified analysis

To assess for effect modifiers or confounders, exposures with calculated p-values<0.2 in the univariable analysis were stratified by exposure to coleslaw. The result of this stratified analysis are shown in Table 12.

The presence of a significant result to the test of homogeneity (i.e. p<0.05) for cabbage (p=0.02) and cucumber (p=0.01) may suggest the effect of coleslaw was being modified by these items. Likewise, the presence of a potential association between illness and garlic mayonnaise in the absence of coleslaw (lower 95%CI in unexposed strata= 1.17) might suggest effect modification. Interaction between these food items and coleslaw were considered when building the multivariable model.

**Table 12:** Stratification of key exposures, by exposure to coleslaw

| | Stratum-specific OR | | Test for | | | |
|---|---|---|---|---|---|---|
| Exposure | Exposed | Unexposed | Homogeneity | Crude OR | Adj OR | % change |
| Garlic Mayonnaise | 0.0 ( 0.00 - 3.19 ) | ( 1.17 - ) | | 4.8 ( 1.16 - 23.04 ) | 2.1 ( 0.40 - 11.11 ) | -55.9% |
| Cabbage | 0.8 ( 0.10 - 4.85 ) | 28.5 ( 1.22 - 1569.46 ) | 0.02 | 4.4 ( 1.27 - 15.55 ) | 2.0 ( 0.59 - 6.66 ) | -54.3% |
| Lettuce | 0.0 ( 0.00 - 3.19 ) | 4.9 ( 0.31 - 275.14 ) | | 4.2 ( 1.12 - 17.71 ) | 1.3 ( 0.24 - 6.52 ) | -70.1% |
| Cucumber | 0.5 ( 0.01 - 5.51 ) | 6.0 ( 0.37 - 336.88 ) | 0.01 | 3.7 ( 1.07 - 13.17 ) | 1.5 ( 0.37 - 6.34 ) | -58.1% |
| Onion | 0.6 ( 0.05 - 4.09 ) | 7.5 ( 0.45 - 419.18 ) | 0.09 | 2.6 ( 0.81 - 8.33 ) | 1.4 ( 0.40 - 5.05 ) | -44.9% |
| Doner Kebab | 1.1 ( 0.14 - 12.98 ) | ( 0.00 - ) | | 4.7 ( 0.80 - 48.55 ) | 1.8 ( 0.35 - 9.11 ) | -62.0% |
| Female | 6.0 ( 0.81 - 68.58 ) | 4.0 ( 0.25 - 227.11 ) | 0.79 | 3.4 ( 1.05 - 11.41 ) | 5.2 ( 1.23 - 21.85 ) | 50.6% |

## Multivariable analysis

The multivariable model identified two menu items as independent risk factors for infection: coleslaw (aOR 200, 95%CI 12-3220, p <0.001) and cabbage (aOR 71, 95%CI 3-1580, p=0.007). Previous stratification identified the potential for an interaction between these food items. As such, the model was adjusted to include this interaction. When both coleslaw and cabbage were consumed, the (adjusted) odds of being a case was substantially greater (aOR 886, 95%CI 26-30034, p<0.001). The model also included gender as an independent factor. Being female was associated with a higher odds of illness, when adjusting for all other variables (aOR 10, 95%CI 2-58, p=0.01) (Table 13).

No other menu items improved the fit of the model.

**Table 13:** Results of multivariable logistic regression model

| Exposure | aOR | 95% CI | p |
|---|---|---|---|
| Coleslaw only | 199.9 | ( 12.4 - 3219.9 ) | <0.001 |
| Cabbage only | 71.2 | ( 3.2 - 1579.6 ) | 0.007 |
| Coleslaw & cabbage | 886.2 | ( 26.1 - 30033.6 ) | <0.001 |
| Female | 9.9 | ( 1.7 - 57.7 ) | 0.011 |

*aOR: Adjusted Odds Ratio, CI confidence interval

## 3.3 Additional analysis

### Dose-response effect of coleslaw consumption

Cases were asked how much of each menu item they had ordered/eaten (none, small, large, extra large). Coleslaw portion sizes ordered by cases and controls were compared to identify any dose-response effect. The results of this calculation can be seen in table 14.

**Table 14:** Dose-response amongst cases and controls who ate coleslaw

| Coleslaw portion size | | Cases | Controls | OR | 95% CI |
|---|---|---|---|---|---|
| None | | 4 | 21 | 0.2 | ( 0.07 - 0.55 ) |
| Small | | 20 | 5 | 4.0 | ( 1.50 - 10.66 ) |
| Large/extra large | | 7 | 3 | 2.3 | ( 0.60 - 9.02 ) |
| Homogeneity | $chi^2$ | | 21.75 | | |
| | p-value | | 0 | | |
| Trend of odds | $chi^2$ | | 14.56 | | |
| | p-value | | 0.0001 | | |

The test for homogeneity would suggest that there is a significant difference between the odds of illness in people who ate only a small portion of coleslaw compared to those who ate no coleslaw ($chi^2$: 21.75, $p<0.0001$). Odds of illness were elevated in those who ordered large portions of coleslaw, but not significantly and not to the same extent as those who ate small portions. This is likely due to small numbers of cases and controls ordering large/extra large portions of coleslaw.

### Dose-Severity analysis – hospitalisations and coleslaw

74% of cases reported bloody diarrhoea. To assess whether there was an association between quantity of coleslaw consumed and severity of disease, we compared odds of coleslaw consumption of those who reported bloody diarrhoea with those who did not report bloody diarrhoea (cases or controls) (Table 15).

**Table 15:** Odds of coleslaw consumption in those who reported bloody diarrhoea

| | Bloody diarrhoea | | | No bloody diarrhoea | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exposed | Total | % | Exposed | Total | % | OR | 95% CI | p |
| Coleslaw (any) | 19 | 23 | 82.61 | 16 | 37 | 43.24 | 6.23 | ( 1.58 - 29.31 ) | 0.003 |
| Dose | | | | | | | | | |
| None | 4 | 23 | 17.39 | 21 | 37 | 56.76 | Ref | | |
| Small | 15 | 23 | 65.22 | 12 | 37 | 32.43 | 7.87 | ( 2.07 - 29.9 ) | 0.002 |
| Large/extra large | 4 | 23 | 17.39 | 6 | 37 | 16.22 | 3.5 | ( 0.66 - 18.34 ) | 0.138 |

A significant association was seen between those who ate coleslaw and those who reported bloody diarrhoea (OR 6.23, 95%CI 1.6-29, p=0.003). Odds of illness were elevated in those who ordered large portions of coleslaw, but not significantly (OR 3.5, 95%CI 0.6-18, p=0.14). A significant association was observed amongst those who ate small portions of coleslaw (OR 7.9, 95%CI 2-30, p=0.002). This is likely due to small numbers ordering large portions of coleslaw.

To further assess whether there was an association between quantity of coleslaw consumed and severity of disease, we compared odds of coleslaw consumption of those who were and were not hospitalised (cases or controls), regardless of symptom presentation (Table 16).

**Table 16:** Odds of coleslaw consumption in those who were hospitalised.

| | Cases (hospitalised) | | | Controls (not hospitalised) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exposed | Total | % | Exposed | Total | % | OR | 95% CI | p |
| Coleslaw (any) | 8 | 9 | 88.89 | 27 | 51 | 52.94 | 7.11 | ( 0.83 - 328.37 ) | 0.044 |
| Dose | | | | | | | | | |
| None | 1 | 9 | 11.11 | 24 | 51 | 47.06 | Ref | | |
| Small | 5 | 9 | 55.56 | 20 | 51 | 39.22 | 6 | ( 0.65 - 55.66 ) | 0.115 |
| Large/extra large | 3 | 9 | 33.33 | 7 | 51 | 13.73 | 10.3 | ( 0.92 - 155.06 ) | 0.059 |

The odds of exposure to coleslaw amongst hospitalised cases were 7 times greater than those of cases or controls who were not hospitalised. However, despite a significant p-value, this association should be interpreted with caution as the confidence intervals cross 1 (95%CI 0.83-328, p=0.04).

There is some evidence that the more coleslaw eaten, the odds of hospitalisation increases. However, this relationship is not significant and should be interpreted with caution.

## Gender-bias amongst coleslaw orders

To further investigate whether the relationship between being female and being a case was affected by a gender-response bias amongst coleslaw orders, we compared coleslaw orders amongst genders using a chi-squared test. The findings of this calculation were not significant ($Chi^2$: 0.49, p=0.48), suggesting that there is not significant difference between the number of men and women who ordered coleslaw (Table 17). This was further tested by comparing cabbage orders of men and women, which was also not significant ($Chi^2$: 0.76, p=0.38) (Table 18).

**Table 17:** Coleslaw orders by gender

| | Ate Coleslaw | | |
|---|---|---|---|
| Gender | No | Yes | Total |
| Male | 13 | 15 | **28** |
| Female | 12 | 20 | **32** |
| **Total** | **25** | **35** | **60** |
| Pearson $chi^2$ | 0.49 | | |
| p-value | 0.48 | | |

**Table 18:** Cabbage orders by gender

| | Ate Cabbage | | |
|---|---|---|---|
| Gender | No | Yes | Total |
| Male | 18 | 10 | **28** |
| Female | 17 | 15 | **32** |
| **Total** | **35** | **25** | **60** |
| Pearson $chi^2$ | 0.765 | | |
| p-value | 0.382 | | |

## 4. Discussion

In the UK, outbreaks of *S. flexneri* where transmission is linked to food are rare [8]. Here we describe a large outbreak, where the hypothesis of an association with the consumption of a food item from a takeaway between 10th-11th February 2023 is supported by a number of factors.

The onset of primary cases and the tight genomic clustering of samples that underwent WGS suggest a point source. Comparison to available genomic data on *S. flexneri,* for Wales and England, showed that this particular genomic profile has not been reported elsewhere in England or Wales – either within the time frame of the investigation, or since the establishment of routine WGS for Shigella isolates in the UK, in 2015. The closest sample was at the t:50 SNP level, i.e. genomically distinct in the context of an outbreak investigation, where Shigella isolates are considered to be linked to a common exposure, place or event, at the t10 level. The isolates in this investigation were related at the 5 SNP level, and a low amount of genetic diversity was identified; i.e. all these isolates are highly genomically related and indicative of a point source outbreak. No genomic similarities were identified between this cluster, and clusters of S. *flexneri* circulating in Wales, or S. *flexneri* clusters associated with travel to a specific region, or with the men who have sex with men (MSM) community.

A rise in *Shigella sp.* or symptoms linked to food poisoning was not reported outside the Abergavenny/Crickhowell area during this time. This would suggest that the outbreak was not associated with an item in the wider food supply chain and most likely was associated with contamination that occurred on the food premises.

The case-control study - which captured 91% of eligible, laboratory confirmed cases – identified that cases in this outbreak were characterised by particularly severe symptoms. Reporting of bloody diarrhoea was substantially higher than expected (75% reported vs. 10-50% expected [2]). Likewise, almost a third of cases included were hospitalised, substantially higher than the expected admissions rate of 3% [2]. Whilst virulence factors of the isolated organism were not investigated in this instance, it does show the potential burden of outbreaks of this nature on local health services, both primary and secondary care.

No potential vehicles outside Takeaway A were associated with illness. Of the available menu items, the multivariable logistic regression model showed that coleslaw was the most likely vehicle for infection. It is unclear how the coleslaw became contaminated. Site visits with EHO's at the beginning of the outbreak noted that the coleslaw was made fresh on-site, as opposed to buying in the product. As part of the outbreak investigation, all staff members and six family of staff were potted, but microbiological confirmation of Shigella infection was not identified in any of these samples.

Analysis of interactions between food items also identified cabbage as an interacting vehicle for infection. Where customers ate both coleslaw and cabbage, the odds of illness were much greater than eating either item on their own, though the effect of cabbage alone was much smaller than that of eating coleslaw without cabbage. As different cabbage types were used for the coleslaw and general salad cabbage, this may indicate cross-contamination. This could have occurred at any number of points (preparation, storage, service). Notes from the EHO's site visit identified "*a number of remediable issues around hygiene and cross-contamination risks*". It is possible these risks were in action at the time cases were exposed to the contaminated food.

There is no known biological reason why women would be more likely to become infected with *Shigella sp*. It is possible that the relationship in this case-control study is impacted by the inclusion criteria. In order to be included, cases needed to have a PCR sample

positive for *Shigella sp*. Generally, cases will have needed to access healthcare services (GP or hospital) in order to get a specimen pot. This relationship may be a reflection of access to healthcare, rather than a true association between gender and odds of infection, but this was not tested in this instance.

### 4.1 Limitations

All exposures and key dates were self-reported. As a bespoke questionnaire was developed, by the time the first cases and controls were interviewed on 1st March 2023, two weeks had elapsed since the initial exposure, and cases/controls identified via the app were not contacted until 4-5 weeks after exposure. It is possible that the time-delay and the self-reporting nature may have introduced bias. This effect was seen amongst portion size, with some participants unable to recall what size they ordered/size ordered did not match options available (e.g. Menu options: Small/large/extra large, case ordered 'regular').

Whilst the tests of association were appropriate, the sample size was relatively small. As such, the associations calculated here may not be a reflection of the true effect of the exposure-outcome relationship. As such, these calculations should be interpreted with caution.

## 5. Conclusions

The findings of the case-control study support the hypothesis that consumption of coleslaw from Takeaway A between 10th-11th February 2023 was associated with Shigellosis. Those who ate cabbage as well as coleslaw had a greater odds of illness. Whilst this investigation identified the most likely vehicle of infection, the original source remains undetected. Although uncommon in the UK, *S. flexneri* should be considered as a cause of foodborne outbreaks with a potentially high burden on primary and secondary healthcare services.

### 5.1 Recommendations

- Whilst no positive microbiological samples were obtained from food handlers, results from the case-control study suggest the contaminated coleslaw- which was made onsite - was the primary food item associated with illness. The findings of the EHO's site visit (not presented in this report) advised that there were concerns regarding hand hygiene and the potential for cross-contamination in food preparation or storage areas. **Environmental health colleagues and food standards regulators should continue to stress the importance of hand hygiene in catering industries, as a simple, cost-effective measure to curb infection transmission from potentially infectious (even if asymptomatic) persons to food items for sale.**
- Genomic sequencing of isolated organisms was important to both describe the outbreak and determine its potential origin. **Microbiology colleagues should ensure specimens linked to outbreaks are sent for genomic sequencing (where appropriate) at reference laboratories, to aid understanding of case clustering. In relation to GI data, CDSC should continue to strengthen relationships with UKHSA' GIFSOH (Gastrointestinal, Food Safety and One Health) and GBRU (Gastro Bacterial Reference Unit) colleagues to support access to genomics data from Welsh samples and to aid genomic surveillance of gastro-pathogens, including *S. flexneri*.**
- Identification of controls using data from food delivery applications increased the total number of controls by 143%, compared to just using controls identified by cases alone. As more catering establishments switch to using online applications to order food (in

addition to in-person/telephone orders), **Health Protection/CDSC must continue to adapt case and control finding to reflect how people may access exposures, including purchasing food. Identification and communication with app management systems to request data must be done quickly to avoid delay between exposure and interview. Guidance should be developed to support the selection of controls when utilizing this data.**

## 6. References

[1] Hale TL, Keusch GT. Shigella. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston: The University of Texas Medical Branch, Galveston; 1996.

[2] Hawker J, Begg N, Reintjes R, Ekdahl K, Edeghere O, van Steenbergen J. Communicable Disease Control and Health Protection Handbook. 4th ed. Hoboken, NJ: Wiley-Blackwell; 2019.

[3] CDC. Information for Healthcare Professionals | Shigella – Shigellosis [Internet]. 2023 [cited 2023 May 22]. Available from: https://www.cdc.gov/shigella/audience-medical-professionals.html

[4] Thorley K, Charles H, Mitchell H, Jenkins C, Godbole G, Sinka K. Sexually transmitted Shigella spp. in England – data up to quarter 2, 2022. September 2022 [Internet]. London; 2022 [cited 2023 May 19]. Available from: https://www.gov.uk/government/publications/non-travel-associated-shigella-infections/sexually-transmitted-shigella-spp-in-england-data-up-to-quarter-2-2022

[5] Richardson D, Devlin J, Fitzpatrick C, Pinto-Sander N. Sexually transmitted Shigella flexneri and Shigella sonnei in men who have sex with men. Sex Transm Infect [Internet]. 2021 May 1 [cited 2023 May 16];97(3):244–244. Available from: https://sti.bmj.com/content/97/3/244

[6] Gilbart VL, Simms I, Jenkins C, Furegato M, Gobin M, Oliver I, et al. Sex, drugs and smart phone applications: findings from semistructured interviews with men who have sex with men diagnosed with Shigella flexneri 3a in England and Wales. Sex Transm Infect [Internet]. 2015 Dec [cited 2023 May 16];91(8):598–602. Available from: https://sti.bmj.com/lookup/doi/10.1136/sextrans-2015-052014

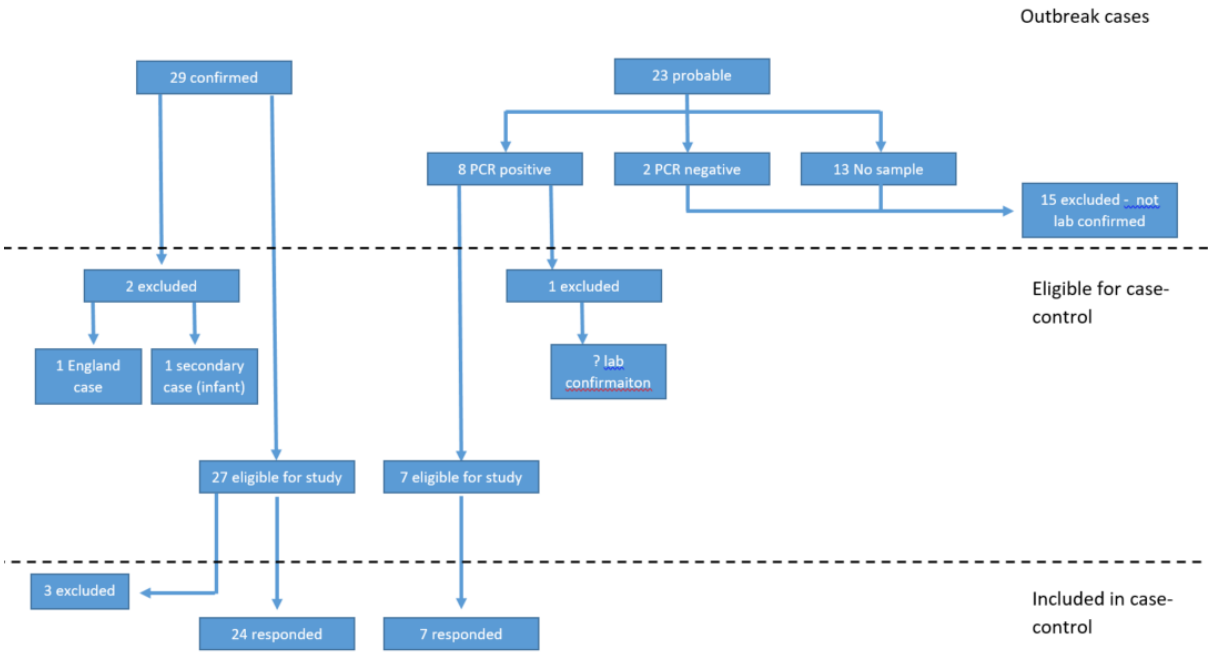
[7] European Centre for Disease Prevention and Control. Spread of multidrug-resistant Shigella in EU/EEA among gay, bisexual and other men who have sex with men [Internet]. 2023 [cited 2023 Aug 22]. Available from: https://www.ecdc.europa.eu/en/news-events/spread-multidrug-resistant-shigella-eueea-among-gay-bisexual-and-other-men-who-have-sex

[8] McLarty K, Paranthaman K, Jenkins C, Sedgwick J, Crawley-Boevey E. Lessons learned from the investigation and management of an outbreak of Shigella flexneri associated with a restaurant in London, 2019–2020. Public Health. 2022 Apr 1;205:130–2.

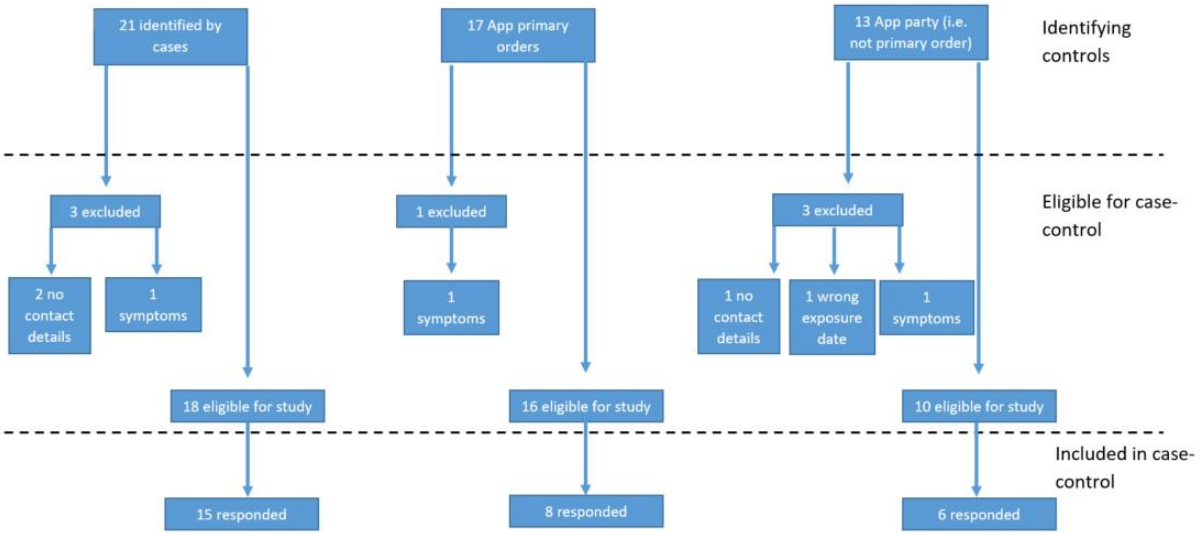
# 7. Appendix

## Appendix I: Identification of cases and controls for case-control study

a) Cases




b) Controls



## Appendix II: Enhanced surveillance questionnaire for case-control study

Shigella Incident 16656 Questionnaire_