Incident Management Team report: Outbreak of *E. coli* O157 PT21/28

July–September 2016

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Members of the public, patients and relatives of those affected by this outbreak

Contents

Executive Summary

This Incident Management Team (IMT) report presents the investigations and conclusions in relation to an outbreak of *E. coli* O157 PT21/28 in the summer of 2016. A total of 26 cases were identified in this outbreak, which occurred in two phases, firstly in July and then again in September.

The multi-agency IMT met 11 times between 22 July and 5 September to investigate and manage an outbreak of 20 laboratory confirmed cases of *E. coli* O157 PT21/28 infection with the same unique molecular profile (hereafter referred to as the "outbreak strain"), with onset dates between 2 July and 29 July. The IMT concluded that the source of the outbreak was consumption of an unpasteurised cows' milk cheese, Dunsyre Blue, and a voluntary recall of the suspected batches was undertaken by the food business on 29 July.

On 5 September the IMT stood down as more than a month had passed since the implementation of control measures on 29 July, no new cases had been identified and the immediate investigation and control of the incident was complete. Ongoing work with the food business was passed to the relevant competent authorities.

On 15 September the IMT reconvened following the identification of two new cases of *E. coli* O157 with the outbreak strain on 14 September. This second phase of the investigation involved six additional cases; five of which were linked to a cluster associated with a childcare setting in NHS Board A. Among the five childcare cluster cases, two were secondary cases who acquired their infection from close contact with the primary cases. NHS Board A convened a local IMT to investigate and manage this cluster. This local IMT fed into the national IMT. The national IMT met a further four times and as no further cases were identified, the national IMT stood down again on 12 October.

Based on the identification of new cases and results of microbiology testing from a number of cheeses produced by the food business, Food Standards Scotland issued a Food Alert for Action (FAFA) on 14 September for the recall of all cheese produced by the food business. The FAFA was also issued to the rest of the UK on 15 September. The FAFA was issued to inform and advise local authorities to identify food businesses which were likely or known to stock products subject to this FAFA and to take steps to ensure they were withdrawn from sale.

Overall, 26 cases of *E. coli* O157 were identified with the outbreak strain with onset dates from 2 July to 8 September. This comprised 24 primary cases and two secondary cases. Seventeen (65%) of the cases required admission to hospital and a three year old child, who was a primary case, died.

Extensive descriptive and analytical epidemiological and food chain investigations were undertaken, which provided strong evidence that Dunsyre Blue cheese was the vehicle of infection for the outbreak.

Of the 24 primary cases 15 (62.5%) are known to have consumed Dunsyre Blue cheese within the eight days before the onset of their symptoms.

In addition:

- two cases ate blue cheese purchased from a shop known to sell Dunsyre Blue but were unable to recall the name of the brand,
- one case attended a function at which Dunsyre Blue was served but did not recall eating it,
- one case ate blue cheese but there was no information available on the type,
- one case reported eating blue cheese but not Dunsyre Blue,
- one case's exposure information was extremely limited and it was not possible to determine cheese consumption history.

The three remaining primary cases were part of the childcare setting cluster. A direct link to Dunsyre Blue was not established for the childcare setting cluster but the organism could have been introduced into the childcare setting environment, by an unidentified asymptomatic or mildly symptomatic case.

When the childcare setting cases are excluded, 15/21 cases (71%) are known to have consumed Dunsyre Blue.

The descriptive epidemiological evidence indicating that Dunsyre Blue was the food vehicle responsible for the outbreak is supported by deficiencies in the procedures for the monitoring and control of pathogenic *E. coli* at the food business. The testing of Dunsyre Blue and other unpasteurised cheeses produced by the food business identified other shiga toxin producing *Escherichia coli* (STEC) and *stx* negative *E. coli* O157 which, although not the outbreak strain, demonstrated that potentially pathogenic *E. coli* were able to enter and survive the cheese production process at the food business. Positive results were obtained for cheese produced over a period of four months, indicating a systematic potential for STEC to enter the process and contaminate final products. Samples of raw cows' milk from the single dairy farm supplying the food business, taken a number of months after the production of the implicated cheese, identified two different strains of STEC, indicating the potential for milk used in the production of Dunsyre Blue to become contaminated. Furthermore, no evidence was provided to demonstrate how any STEC present in the raw milk supply would have been eliminated during the production process.

These findings are biologically plausible as Dunsyre Blue is an unpasteurised cows' milk cheese and cattle in Scotland are known to carry *E. coli* O157 PT21/28. Unpasteurised cheeses have previously been associated with other outbreaks of STEC infection.

Extensive investigations concluded that the source of the outbreak was the consumption of Dunsyre Blue. This conclusion was based on evidence from epidemiological and food chain investigations and supported by microbiological evidence and deficiencies identified in the procedures for the monitoring and control of STEC at the food business.

Throughout the investigation the paramount aim of the IMT was the protection of public health. To this end, products considered to pose a risk to the public were withdrawn from the market and the risks communicated to the public and professionals.

1 Background

1.1 Shiga toxin producing *Escherichia coli*

Shiga toxin producing *Escherichia coli* (STEC)[i] are a group of toxin-producing bacteria capable of causing gastrointestinal illness in humans. The incubation period for STEC infection is usually three to four days, seldom less than one day or more than eight days, but has been occasionally reported to be as long as 14 days[1]. The infectious dose required to cause illness is low, with fewer than 1,000 cells sufficient[2;3]. Clinical presentation ranges from asymptomatic infection to mild non-bloody diarrhoea, through bloody diarrhoea and haemorrhagic colitis to haemolytic uraemic syndrome (HUS), other presentations of thrombotic microangiopathy and, in a small number of cases, death. HUS develops in approximately 10-15% of *E. coli* O157 cases[4;5], with the highest rates in those under 15 years or over 65 years of age[6]. HUS mortality is reported to be between 3% and 5%, and death due to HUS is nearly always associated with severe extrarenal disease, including severe central nervous involvement[7].

1.2 Epidemiology of STEC infection in Scotland

In Scotland, the most common strain of STEC to cause illness is *E. coli* O157. The reported rates of *E. coli* O157 in Scotland rose substantially in the mid-1990s and remain consistently high compared to other countries within the UK and Europe. In 2015, a total of 183 reports of *E. coli* O157 were made to Health Protection Scotland (HPS), 170 from culture positive faecal samples (rate 3.2 per 100,000), 12 shiga toxin and rfb_{O157} gene positive by PCR (a bacterial DNA detection method) but culture negative samples and one on serology only (detection of *E. coli* O157 antibodies in blood)[8]. Among the culture positive isolates, PT21/28 was the most frequent phage type accounting for 34% of isolates. In the past 15 years, there has been no discernible trend in culture positive cases of *E. coli* O157, however there has, in recent years, been an increase in non-O157 STEC isolates with 75 such isolates reported in 2015. The observed increase in non-O157 isolates over the past few years is largely due to increased ascertainment resulting from a change in the referral pattern of faecal samples from diagnostic laboratories to the Scottish *E. coli* O157/VTEC Reference Laboratory (SERL)[8] which is able to test for non-O157 STEC organisms.

1.3 Sources of Infection

STEC can colonise the gastrointestinal tract of wild, farmed, and domesticated animals and be shed in their faeces. Cattle are considered the most important reservoir for STEC in humans; infection in cattle is non-pathogenic. Shedding of STEC by cattle is dynamic with individual farms having periods of apparent absence and periods of high prevalence[9]. Studies in Scotland have estimated a prevalence of *E. coli* O157 at farm level of approximately 20%[10], with modelling suggesting that whilst only 20% of farms are positive for *E. coli* O157 at any given time, approximately 80% may harbour infection at some point during the course of the year[11]. A number of factors have been postulated to influence farm level prevalence[11;12]. Within *E. coli* O157 positive herds there is heterogeneity in shedding, with a small number of high level or "supershedders", such that it has been estimated that about 80% of transmission arises from the 20% most infectious cattle[9;13].

[i] STEC is synonymous with VTEC (vero cytotoxin producing *E. coli*). Likewise *vtx* is synonymous with *stx* genes

Transmission to humans can occur as a result of direct contact with STEC-contaminated faecal material, from handling or petting animals[14;15] or by exposure to faecally contaminated soil or vegetation during recreational or occupational activities[16]. Exposure can also occur from consumption of water[17] or food which is contaminated. Fruit or vegetables can be contaminated if they come in contact with soil, animal faeces or manure which contains STEC. The use of water for irrigation of food crops and washing of fruit and vegetables has also been identified as a transmission route for STEC. A number of STEC outbreaks have been reported in the literature due to contaminated salads or vegetables, including slaw garnish[18],watercress[19], lettuce[20], sprouts[21-23], white radish[24] and handling raw leeks and potatoes[25].

Meat may be contaminated with STEC during the slaughter process with a number of meat related outbreaks being reported including the largest outbreak of *E. coli* O157 in Scotland[26], outbreaks due to beef burgers[2;27;28] and others due to cooked meats[29] .

Faecal contamination during the milking of cattle, sheep and goats can result in STEC contamination of raw milk[30]. There have been a number of STEC outbreaks associated with drinking raw milk[31-33]. Failure of pasteurisation or post-process contamination may also result in milk related STEC outbreaks[34]. Any contamination of the raw milk used to produce unpasteurised dairy products has the potential, unless sufficient additional control measures are in place, to result in the presence of STEC in the final ready to eat product. There have been a number of STEC outbreaks linked to the consumption of unpasteurised cheese[35;36] (more details on dairy related outbreaks in Table 11).

2 Outbreak Investigation

On the afternoon of 21 July 2016 the Scottish *E. coli* O157/VTEC Reference Laboratory (SERL) informed Health Protection Scotland (HPS) of eight confirmed cases of *E. coli* O157 PT21/28 with the same Multi Locus Variable-number tandem repeat Analysis (MLVA) profile. Cases were resident across five NHS Boards. There were an additional four cases of *E. coli* O157 PT21/28 for which the MLVA result was awaited. This initial alert led to the establishment of a Problem Assessment Group (PAG) that met on 22 July and subsequently became the National Incident Management Team.

The timeline for the key events in the outbreak are presented in Appendix 2.

2.1 Role and Responsibilities of the National Incident Management Team

As is standard practice for the investigation of national outbreaks of Infectious Intestinal Disease (IID), HPS convened a multi-agency Incident Management Team (IMT). The IMT was chaired by HPS and consisted of representatives from the Health Protection Teams (HPT) in the NHS Boards in which cases were resident, SERL, Food Standards Scotland (FSS) and relevant local authorities' Environmental Health Teams. Later, when cases were identified in England, membership was expanded to include Public Health England (PHE) and the Food Standards Agency (FSA). The Public Analyst Laboratories were also represented when food and environmental sampling was undertaken. Details of the agencies represented are provided in Appendix 3.

The investigation was undertaken in accordance with the Scottish Government guidance for the Management of Public Health Incidents[37].

It is the remit of the IMT to[37]

- Reduce to a minimum the number of cases of illness by promptly recognising the incident, defining how cases have been exposed to the implicated hazard, identifying and controlling the source of that exposure, and preventing secondary exposure;
- Minimise mortality and illness by ensuring optimum health care for those affected;
- Inform the patients, actually or potentially exposed groups, staff and clinical and management colleagues, the public, their representatives and the media of the health risks associated with the incident and how to minimise these risks; and
- Collect information which will be of use in better understanding the nature and origin of the incident and on how best to prevent and manage future incidents.

2.2 IMT meetings

The incident team initially met as a Problem Assessment Group on 22 July. The group met as an IMT on ten occasions between 26 July and 5 September, when the group initially stood down. On 15 September the IMT was reconvened and met on four occasions and stood down again on 12 October. Due to the fast moving nature of the outbreak, and to ensure the timely consideration of new information and action potentially required to protect

public health during the investigation, three *ad hoc* meetings of core members of the IMT (HPS, FSS, SERL and South Lanarkshire Council) were called by HPS at short notice. The *ad hoc* meeting on the evening of 14 September resulted in the reconvening of the National IMT on the morning of 15 September. Dates of all the IMT meetings are provided in Appendix 2.

2.3 IMT sub-group

Due to the complexity of discussions around the procedures in place at the food business, a sub-group of the IMT was established and chaired by FSS to progress the detailed and technical discussions to investigate processes at the food business. This sub-group comprised representatives from FSS, HPS and South Lanarkshire Council (SLC) and reported to the National IMT. When the IMT initially stood down on 5 September this sub-group also stood down, and ongoing work with the food business passed to SLC as the competent authority and FSS as per their usual food incident management procedures. When the National IMT was reconvened on 15 September, the sub-group of the IMT also reconvened and then stood down when the National IMT stood down on 12 October, following which SLC and FSS continued to work with the food business.

2.4 Incident Management Team in NHS Board A

During the course of the national investigation, a cluster of cases of *E. coli* O157 PT21/28 with the outbreak MLVA profile was identified associated with a childcare setting in NHS Board A. NHS Board A HPT convened a local IMT to manage this cluster, including the screening of all children and staff at the childcare setting and a number of household contacts as per the national guidance[1] and local investigations into the source of infection. This local IMT was chaired by a Consultant in Public Health Medicine (CPHM) from NHS Board A and included representatives from HPS. NHS Board A provided updates of local investigations to the National IMT. This functioned as a standalone IMT rather than a sub-group to the National IMT.

3 Case definitions

The case definitions evolved as the outbreak progressed to take account of cases identified by PHE on the basis of Whole Genome Sequencing (WGS) rather than Multi Locus Variable-number Tandem Repeat Analysis (MLVA). Below are the final case definitions.

Confirmed case:

A case of *E. coli* O157:H7 PT21/28 with the outbreak MLVA profile,

or

a single locus variant of the outbreak MLVA profile and an epidemiological link to a confirmed case,

or

with a whole genome sequence profile within the same 5 SNP cluster.

Probable case:

A case of *E. coli* O157:H7 PT21/28 for which MLVA or WGS is awaited, with an epidemiological link to a confirmed outbreak case,

or

A case of *E. coli* O157:H7 for which phage typing and MLVA/WGS is awaited, with an epidemiological link to a confirmed outbreak case,

or

A case of *E. coli* O157:H7 PT21/28 with a MLVA single locus variant from the outbreak MLVA profile, for which WGS is awaited.

Possible case:

A case of *E. coli* O157:H7 PT21/28 for which MLVA is awaited (Scotland only).

Secondary case:

A confirmed or probable case with onset two or more days after another confirmed or probable case that is a household or other close contact, if the exposure likely occurred outside the place of residence.

4 Descriptive Epidemiological Investigation – Methods

4.1 Case finding

Case finding was through reports of *E. coli* O157:H7 PT21/28 and the subsequent MLVA profile from SERL. Public Health England (PHE) discontinued routine MLVA typing of STEC isolates replacing this with WGS in 2015. Isolates were exchanged between SERL and the PHE Gastrointestinal Bacteria Reference Unit (GBRU) to allow comparison by WGS with *E. coli* O157:H7 PT21/28 cases in the rest of the UK and the Republic of Ireland (ROI) and the rapid identification of outbreak cases. Wales, Northern Ireland and the ROI routinely send STEC isolates to GBRU for WGS therefore providing a mechanism to capture any cases resident in these areas.

Close contacts of confirmed cases were screened where indicated in line with guidance for the management of STEC in Scotland[1] to identify any secondary cases.

4.2 Questionnaires and case interviews

As part of the routine response to STEC infections in Scotland, NHS Board Health Protection Teams (HPTs) (or Environmental Health Officers (EHO) on their behalf) interview all reported cases of STEC infection. These interviews are conducted on the same day they are reported (or as soon as possible thereafter) using a standard national enhanced surveillance form[38]. These forms are routinely sent to HPS to be entered onto the national database for the enhanced surveillance of STEC infection in Scotland. A similar process operates in other parts of the UK. As per usual practice, HPS obtained copies of the completed surveillance forms for cases associated with this outbreak. To help identify any common links, initial cases were also re-interviewed by the HPTs as soon as possible after the identification of the outbreak using a longer and more in-depth trawling questionnaire developed by PHE for use in outbreak investigations (Appendix 4). The trawling questionnaire asks about a wide range of exposures in the seven days prior to onset of symptoms in the case, including travel, events or functions attended, recreational and outdoor exposures, contact with animals, as well as a very detailed food history for a wide range of foods eaten both within the home and outside including salads, vegetables, fruits, meats, poultry, dairy products, sandwiches and burgers, eggs, cakes and biscuits, desserts and puddings, chocolate, snack food, sauces, nuts and seeds, and herbs and spices (Appendix 4). Based on the results of the initial trawling questionnaires, a more focused questionnaire was developed by HPS which collected detailed information in relation to the meals consumed outside the home and the foods identified from the surveillance forms and initial trawling questionnaires as being of particular interest and biologically plausible. These foods included beef products, cheese, sandwiches and burgers, salad vegetables, herbs and fruits. Subsequent cases were interviewed with the more focused trawling questionnaire. As required, the local HPT re-contacted the cases to seek further clarification or request additional information.

Where necessary, due to difficulties in recall of the exact foods eaten or the component parts of dishes eaten at hotels/restaurants, EHOs contacted the premises to obtain details of the constituent parts of dishes, foods ordered by the case during their stay or the menu for particular functions/ events that cases had attended. This enabled the confident identification of the exact foods eaten.

4.3 Interviews with close contacts of cases in childcare setting cluster

As part of the investigation by NHS Board A into the cluster of cases with the outbreak strain associated with a childcare setting, in addition to the interviews for confirmed cases, interviews were conducted by Board A HPT, using the focused questionnaire with the parents/caregivers of cases and the childcare leaders. These questionnaires focused on foods consumed in the 14 days prior to the onset of the first case within the cluster.

4.4 Summarising the descriptive epidemiological evidence

Throughout the investigation HPS collated information from the surveillance forms, trawling questionnaires and focused questionnaires to identify common exposures among cases. In considering the information, HPS took into consideration the likely commonality/rarity of each exposure in the general population and biological plausibility. Common exposures were investigated further by seeking information on brands/locations purchased and through EHOs identifying brands/suppliers of foods served in hotels/restaurants. For example, whilst a number of cases reported eating blue cheese from a cheese board in a hotel/restaurant they were often unaware of the brands of cheese on the cheese board, therefore EHOs visited the relevant premises to clarify.

This information was summarised and presented to the IMT throughout the outbreak to inform risk assessment, identify areas for further investigation and guide risk management.

4.5 General population food consumption information

As blue cheese consumption was mentioned by a large proportion of the cases, the IMT obtained information on the frequency of blue cheese consumption in the general population.

Information on the consumption of blue cheese in the general population was provided by Food Standards Agency from the National Diet and Nutrition Survey (NDNS) report for the period 2008/09-2011/12[39].

5 Descriptive Epidemiological investigation – Results

5.1 Number of confirmed cases

A total of 26 confirmed cases were identified, five of which were associated with the childcare cluster in NHS Board A including two cases who were close contacts of confirmed cases and considered to be secondary cases. Therefore there were:

- 21 primary cases not linked to the childcare cluster,
- 3 primary cases linked to the childcare cluster,
- 2 secondary cases linked to the childcare cluster.

PHE informed HPS of an additional case of *E. coli* O157 diagnosed on serology who had stayed at the same hotel as one of the confirmed outbreak cases. This individual stayed at the hotel during the first week in July (arriving four days after the confirmed case had left) and had also consumed Dunsyre Blue during their stay. However as diagnosis was made on serology, no molecular typing information could be obtained and therefore the individual did not fulfil the case definition for a confirmed or probable case and was not included in further investigations nor counted in the final case numbers.

5.2 Demographics of confirmed cases

Twenty-one of the 26 confirmed cases were resident in Scotland (spread across seven NHS Boards), four were resident in England and one in the Republic of Ireland (ROI).

Three of the four cases resident in England and the case resident in the ROI visited Scotland during their incubation period. The fourth case resident in England did not travel to Scotland during their incubation period.

Eighteen (69%) of the 26 confirmed cases were female and eight (31%) male (Figure 1).

The mean age of cases was 38.9 years and median age 35 years. Excluding the five cases associated with the childcare cluster, the mean age was 47.1 years and median 55 years.

Figure 1 Age band and sex of confirmed cases in the outbreak of *E. coli* O157 PT21/28 (n=26)

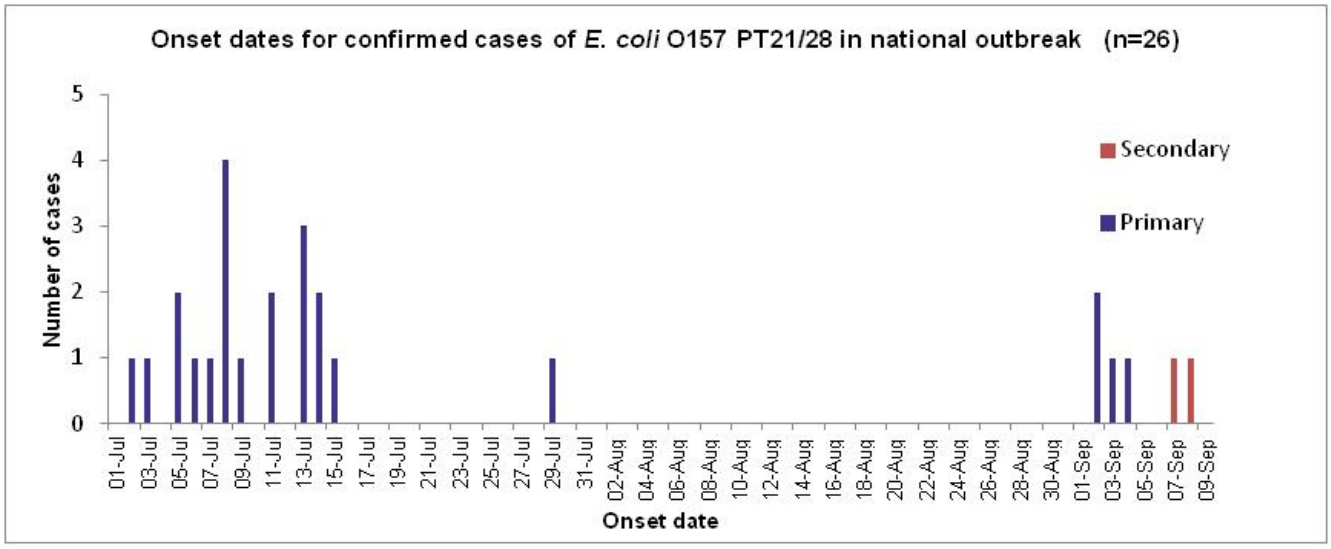
5.3 Epidemic curve

Onset dates ranged from 2 July to 8 September.

For the original 20 cases investigated before the IMT was initially stood down on 5 September, onset dates ranged from 2 July to 29 July, with 19/20 occurring in the 14 days between the 2 and 15 July (Figure 2).

The national IMT was reconvened on 15 September in response to additional cases. In total six additional cases were identified, one unconnected to the childcare cluster and five (3 primary and 2 secondary) associated with the childcare cluster. Dates of onset for the six additional cases ranged from 2 to 8 September.

Figure 2: Onset dates for confirmed cases of *E. coli* O157 PT21/28 (n=26)



5.4 Clinical presentation

Information on the presence or absence of bloody diarrhoea was available for 23 cases, 21 (91%) of whom reported bloody diarrhoea.

Of the 26 confirmed cases, 17 (65%) required admission to hospital.

Two cases (8%) developed HUS, one of whom, a three year old child died on 2 September 2016.

5.5 Case exposures

Information on both food and non-food related exposures were investigated throughout the investigation. Other than the five cases associated with the childcare cluster, and two cases who had consumed food from the same hotel, there were no common links between the remaining 19 cases with respect to places visited or events attended, nor in animal or environmental exposures. Consumption of some food products other than blue cheese was reported by more than one primary case, although these were a mixture of brands/types of products indicative of no common source. No vehicle or source was reported that accounted for as high a number of primary cases as blue cheese (Dunsyre Blue or unspecified). These

exposures are described below. Details of some of the other food products consumed by cases is presented in Table 5.

5.6 Blue cheese and Dunsyre Blue consumption

Throughout the investigation the IMT considered a number of biologically plausible vehicles of infection. A vehicle of interest that emerged early in the investigation was blue cheese and ultimately one particular type of blue cheese – Dunsyre Blue. As detailed below the descriptive epidemiological evidence linking Dunsyre Blue to the outbreak strengthened as the investigation progressed and more information became available leading the multi-agency IMT to conclude Dunsyre Blue was the most likely source of the outbreak.

The way in which this conclusion was reached is detailed below:

On 22 July 2016, the initial PAG reviewed the available exposure information for the eight confirmed cases identified. There was no obvious commonality, but seven cases were known to have a history of either staying at a hotel within Scotland or eating at commercial premises within Scotland and five had reported eating blue or soft cheese. At this stage there was limited information on types/brands.

By 26 July 2016, at the first IMT, a total of 13 confirmed cases had been identified, nine of whom reported consumption of blue cheese. For three of the nine who reported blue cheese consumption the cheese specified was one produced by Errington Cheese Limited (ECL). By comparison, when the HPS National STEC surveillance database was checked, only two of the other 75 cases of STEC reported up to that point in 2016, and for whom exposure information was available, reported eating blue cheese. Whilst acknowledging that this was not a direct comparison of datasets, the IMT agreed the number of cases in this outbreak who reported eating blue cheese was unexpectedly high and warranted further investigation. These further investigations included visits by EHOs to the hotels/restaurants where cases had eaten to gain more information into all types of cheese as well as salads, herbs and garnishes used by the businesses and continued follow up with cases.

By the IMT held on 28 July 2016, there were a total of 14 confirmed cases. Information on whether they had consumed blue cheese was available for 12 cases; seven had consumed Dunsyre Blue, two had possibly consumed Dunsyre Blue and three did not report eating Dunsyre Blue. The evidence from the trawling questionnaires had not identified any other specific vehicle in common to the cases. Supply chain information available at the IMT meeting provided by one of the main suppliers of Dunsyre Blue to the restaurants in which cases had reported eating, identified two particular batches of Dunsyre Blue, C22 and D14, as common to the restaurants where cases were known to have consumed the cheese. The IMT concluded there was sufficient evidence to recommend a product recall for the two batches of Dunsyre Blue C22 and D14 (see control measures) to prevent further cases and protect public health. This voluntary recall took place on 29 July. On 4 August 2016, this main supplier subsequently stated to their local authority that they were now unable to provide information on exact batches they had supplied to particular premises. The IMT considered this update at the meeting of 4 August and whether a wider withdrawal of Dunsyre Blue was required. The IMT concluded that such action was not indicated at that point in time as there was no evidence of new cases with exposure dates after the recall. Furthermore, the batches on sale at the time the cases were exposed would now be past their best before date and unlikely to be in circulation.

As the investigation continued, more cases were identified and information continued to become available for existing cases. On 12 October, when the IMT stood down at the end of the investigation, 15 of the 24 primary cases (62.5%) were known to have consumed Dunsyre Blue, another two cases had eaten blue cheese from a shop selling Dunsyre Blue but were unable to recall the type of cheese, and one case had attended a function at which Dunsyre Blue was served but did not recall eating it. Of the remaining six primary cases, one case had eaten blue cheese but due to limited information available from the case, the IMT was unable to determine the type of blue cheese or where it had been purchased from, one case reported eating blue cheese but not Dunsyre Blue and for one case there was very limited exposure information available such that it was not possible to determine if the case had consumed blue cheese (Table 1). The three primary cases associated with the childcare setting cluster are discussed below.

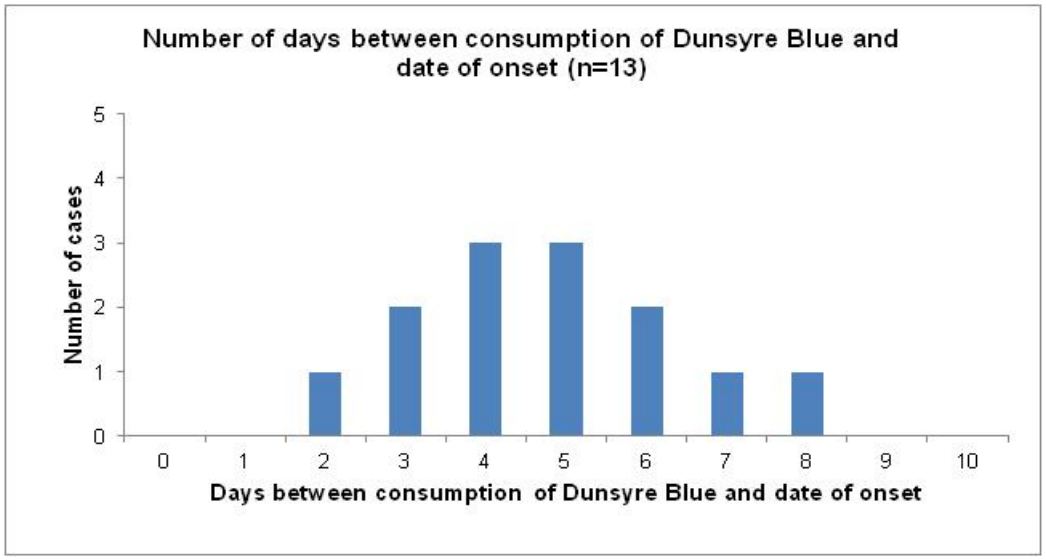
Table 1: Consumption of Dunsyre Blue cheese by primary cases – final information

Potential exposure to Dunsyre Blue/ blue cheese	Number of primary cases	Percentage of primary cases (n=24)	Percentage of non-child care setting cluster primary cases (n=21)
Consumed Dunsyre Blue	15	62.5	71.4
Consumed blue cheese from a shop known to sell Dunsyre Blue but unable to recall type of cheese purchased	2	8.3	9.5
Attended a function at which Dunsyre Blue was served but does not report consuming it	1	4.2	4.8
Ate blue cheese – no information available on place of purchase or brand	1	4.2	4.8
Ate blue cheese but not Dunsyre blue	1	4.2	4.8
Limited exposure information	1	4.2	4.8
Childcare setting cluster *	3	12.5	N/A
Total	24		

* See below for hypothesis for exposure for the childcare setting cluster.

Fifteen cases are known to have consumed Dunsyre Blue prior to the onset of illness, for 13 of these cases a date of consumption is known, for the remaining two cases there are multiple dates on which the cheese may have been consumed. For the 13 cases with a single known date of exposure both the mean and median time between consumption and onset of symptoms was 5 days (range 2-8 days) (Figure 3), which falls within the recognised incubation period for STEC infection[1].

Figure 3: Number of days between consumption of Dunsyre Blue and date of onset (n=13)



All fifteen cases known to have consumed Dunsyre Blue did so in hotels or restaurants. Details of the dishes in which the cheese was consumed are contained in Table 2.

Table 2: For the 15 cases known to have consumed Dunsyre Blue details of dish in which the cheese was consumed

Consumption of Dunsyre Blue	Number of cases	Percentage of cases known to have eaten Dunsyre Blue (n=15)
Cheese consumed in hotel/restaurant	15	100%
Details of how the Dunsyre Blue was consumed in the hotel/restaurant		
Dunsyre Blue on cheese board in hotel/restaurant	9	60%
Dunsyre Blue in hotel/restaurant menu items but not part of cheese Board	4	27%
Dunsyre Blue eaten at work at hotel/restaurant	2	13%

5.6.1 Other cheeses on the cheese boards

Fifteen cases consumed Dunsyre Blue within a hotel/restaurant. For nine of these cases this was as part of cheese from a cheese Board (Table 2). As these cheese boards often contained multiple types of cheese, investigations were undertaken to determine if there was any other cheese common to these cheese boards apart from Dunsyre Blue (Table 3).

This showed that there were no other cheeses common to all nine cheese boards that cases reported eating Dunsyre Blue from.

It is unsurprising that other cheeses were reported to have been consumed given the number of cases eating from cheese boards. However, four cases who consumed Dunsyre Blue at hotels/restaurants did so as part of other dishes and not from a cheese board.

Table 3: Cheese on cheese boards reported by the nine cases who consumed Dunsyre Blue from a hotel/restaurant cheese board

Type of cheese on cheese board	Number of cases
Dunsyre Blue	9
Other cheese B	6
Other cheese C	4
Other types of cheese which appeared on only one cheese board	12 different types of cheese

* multiple cheeses were present on some of the cheese boards alongside the Dunsyre Blue.

5.7 Consumption of blue cheese in the general population

Estimates of blue cheese consumption in the general population aged 19 years and over based on a four day food diary of approximately 2,000 consumers was provided by FSA from the National Diet and Nutrition Survey data.

Table 4: Estimate of consumption of blue cheese among National Diet and Nutrition Survey participants based on 4 day food diary (excluding recipes) by UK consumers during 2008/9-2011/12

Age group	Number of consumers reporting blue cheese consumption in the previous four days	Percent consumers (%)
Adults 19 yrs & older	41	2.2
Female adults 19yrs & older	15	1.2
Males adults 19yrs & older	26	3.2

Note: that consumption or exposure estimates made with a small number of consumers reporting consumption may not be statistically reliable. As a guide, estimates based on less than 60 consumers reporting consumption or exposure should be treated with extreme caution.

Whilst the estimates of blue cheese consumption from the National Diet and Nutrition Survey data (based on four day food diaries) are based on small numbers, they highlight that blue cheese consumption is not a common food exposure as it was only reported by 2.2% of the approximately 2,000 consumers surveyed in the previous four days (if this was scaled up, it would approximate to 3.8% in the previous seven days). This low rate of blue cheese consumption is in contrast to the cases within this outbreak, furthermore the National Diet and Nutrition Survey data relates to all types of blue cheese, while in this outbreak the blue cheese was one particular artisan variety (Table 1).

Among the 21 primary cases not linked to the childcare setting, 19 (90%) reported the consumption of blue cheese (15 Dunsyre Blue, two blue cheese from shop known to sell Dunsyre Blue, one blue cheese but place of purchase is unknown and one reported eating blue cheese of a different type).

The artisan nature of Dunsyre Blue means it is only sourced from wholesalers and some specialist retailers and is not sold through supermarkets hence it is unlikely to be a commonly consumed product.

5.8 Investigation of childcare cluster in NHS Board A

NHS Board A undertook extensive investigations into the cluster of five cases associated with a childcare setting. This included in-depth exposure histories for the cases and the food histories for the parents/caregivers of the cases and childcare setting leaders covering the 14 days prior to onset of the first case.

Neither the cases, nor the adults who were interviewed reported eating Dunsyre Blue during the time period in question and the source of the infection into the childcare setting was not established. The most likely hypothesis considered by the National IMT was that the bacteria were introduced into the venue in which the childcare was held by an unidentified infected individual with subsequent spread to the childcare group through environmental contamination e.g. faecal contamination of the toilet facilities.

NHS Board A in line with national guidance[1] undertook screening of all children and staff attending the childcare setting. This screening did not identify anyone as an asymptomatic carrier of the outbreak strain, suggesting that introduction to the childcare environment was by an individual who had ceased to excrete the pathogen, or was not directly connected with the group. The venue in which the childcare is held is used by other people/organisations and not solely the childcare group, and so it is possible that there could have been an infected individual who used the venue during the relevant time period, but who was not identified during the outbreak investigation. It is known that infection with *E. coli* O157 presents with a range of severity from asymptomatic to fatal infection, and therefore someone who was either asymptomatic or mildly symptomatic may well have contaminated the environment without themselves being identified.

One confirmed case not connected to the childcare cluster in NHS Board A had an onset date in early September and had eaten Dunsyre Blue on 26 August. This suggests that there was still some contaminated cheese available for consumption at the end of August and therefore there may have been other unidentified cases occurring during that time period.

6 Analytical Epidemiological investigation – Methods

6.1 Case case analytical study

Analytical epidemiological investigations are undertaken to test the hypothesis that a particular exposure (in this instance consumption of a specific food) suggested by the descriptive epidemiology is the most likely vehicle of cases' infection. There are a number of analytical study designs which can be undertaken depending on the nature and size of the outbreak including case control or cohort studies. The ability to undertake such studies depends on the availability of new cases (upon whom the hypothesis has not been generated) who can be recruited in sufficient numbers for study's findings to reach statistical significance. Following discussions the IMT decided not to conduct a case control study. This was based on the small number of new cases identified after the link with blue cheese was established on which to test the hypothesis and the fact that the descriptive evidence had been strong enough to warrant control measures, including informing the public of the suspected vehicle. The public's knowledge of the suspicion could invalidate the results of the study by influencing the responses of cases and controls.

Another design of analytical study is a case case study, in which cases from previous outbreaks are used instead of controls. The case case analysis is a proxy for a case control study. In a case control study the controls would be selected from the same population as the cases and differentiated from them only by their disease status. This is not the case for the case case study and the results must be interpreted bearing in mind potential biases associated with the selected cases from previous outbreaks. For example, you may fail to demonstrate an association with consumption of a particular food vehicle if previous outbreaks had the same cause.

A case case study was conducted to compare food exposures among the 24 primary cases to those in previous outbreaks. This study used 23 cases from previous outbreaks of STEC and *Salmonella* infection between 2008 and 2016. In none of these previous outbreaks was cheese the suspected vehicle of transmission, thus providing a comparison population who should have an exposure to cheese similar to the general population.

We used 38 questions in the study selected from the trawling questionnaires used in previous outbreaks. These covered a range of cheese exposures and other biologically plausible food vehicles, for example burgers and salads as well as some unlikely exposures such as chocolate which were expected to be the same in the two groups. The principal analysis was the estimation of the odds ratio of consuming cheese if you are a case in this or previous outbreaks. Fisher's exact test was used to test if the odds ratio was different from the null hypothesis value of one, indicating no association, and 95% confidence intervals for the odds ratio calculated. Although animal exposures are a known source of STEC infection, the information from cases did not identify any commonality in animal exposure and therefore this was not included in the analysis.

As there were 38 questions in the analysis and as there was no predefined hypothesis associated with any of the food items in the questionnaire the Benjamini and Hochberg multiple comparisons method was used[40]. This adjusts the p value of the individual tests so that the overall significance level associated with all 38 tests is 5%. This adjustment is necessary to reduce the possibility of a false positive result – reporting an association between case status and exposure when, in fact, there is no association.

When performing one significance test there is a 5% chance of rejecting the null hypothesis of no association between case status and exposure when there is no association. When performing two tests on different exposure variables this probability increases to 9.7%, assuming independence and with 38 tests the chance of reporting at least one significant association increases to 85.5%, assuming independence of tests. Thus without the adjustment for multiple comparisons there is a high likelihood of reporting a false positive result. The individual p value for each significance test are reduced to a much lower level to ensure that over the 38 tests the chance of reporting one false positive result is 5% assuming there are no associations over all 38 exposures.

All statistical analysis was carried out using R version 3.2.2.

The analysis was conducted twice during the initial stages of the investigation before all the cases were identified and then again at the end of the outbreak when information was available for all 24 primary cases. On this occasion three separate analyses were conducted:

1. All 24 primary cases from the current outbreak compared to all 23 cases in the previous outbreaks,
2. The 21 primary cases who were not part of the childcare cluster,
3. All 20 primary cases of 16 years or older.

Analysis 2 and 3 above are largely the same as the childcare cluster cases were less than 16 years old. However analysis 3 is justified as all the cases from the previous outbreaks were adults and this is a more valid comparison, by comparing the adult cases in this outbreak with the adult cases from previous outbreaks.

6.2 Bayesian modelling

The second analytical epidemiology methodology employed was Bayesian modelling. This technique estimates the odds ratio of being a case when exposed to blue cheese compared to being a case when not exposed and is used to quantify if the proportion of outbreak cases reporting an exposure is higher than expected compared to a control population. This modelling is used in this report where there are no population controls and exposure to cheese in the controls is represented by a prior probability distribution. Information about the distribution of the exposure within the control population may be unknown in which case the model would be represented by a uniform distribution within the control population between 0 and 1, or the model may be informed by prior information on the prevalence of exposure in the control population. A uniform distribution for the proportion of the control population exposed means that values such as 0.05, 0.1, 0.2 and 0.4 of the population exposed are equally likely and we have no prior information on which values are more likely than others. Such prior information as exists for the consumption of blue cheese suggests that this is not a food product which is eaten by a large proportion of the population and more informative prior distributions are represented by a beta distribution which still varies over the whole range from 0 to 1 but has a peak towards the lower end of the distribution at 0.05 or 0.10, corresponding to 5% and 10% of the control population exposed.

Our analysis used both a non-informative distribution where distribution of eating blue cheese within the control population was represented by a uniform distribution to range from 0 to 100% and two informative models based upon information obtained from other sources.

The first of these was based on estimates from some restaurants who estimated either from menu order records or experience that about 5 to 10% of diners order the cheese board, this information was obtained during the EHO visits to premises cases had eaten at. As this is based on those dining out at these restaurants for the general population the percentage of eating blue cheese is likely to be lower and the model used an estimate centred upon 1% of the population.

The second was based on the National Diet and Nutrition Survey (Table 4) and used an estimate of 3.5%.

The Bayesian modelling was conducted twice during the early stages of the investigation before all the cases were identified and at the end once information was available for all 26 cases.

A more technical explanation of the Bayesian modelling methodology is contained in Appendix 5.

7 Analytical Epidemiological investigation – Results

7.1 Case Case analytical study

The case case study using the 24 primary cases in the current outbreak identified four exposures which were statistically significantly associated with being a case in the current compared to previous outbreaks in order of significance (Table 5):

i. Eating blue cheese away from home
ii. Eating out at hotels
iii. Eating out at British restaurants
iv. Eating hard white cheese away from home

Table 5: Results of case case study

The p value is the unadjusted p value.

Sig indicates if the difference was significant once the Benjamini and Hochberg multiple comparisons method was applied[40]. This adjusts the p value of the individual tests so that the overall significance level associated with all 38 tests is 5%

Question	Current outbreak		Previous outbreak cases		Odds Ratio	Lower CI	Upper CI	P value	Sig**
	Yes	No	Yes	No					
Blue cheese eaten away from home	17	7	0	21	Inf	8.69	Inf	<0.0001	Y
Eating out at hotels	14	10	0	22	Inf	5.55	Inf	<0.0001	Y
Eating out British Restaurants	18	6	4	18	12.55	2.75	73.40	0.0001	Y
Hard white cheese eaten away from home	9	12	0	21	Inf	2.74	Inf	0.001	Y
Other soft cheese eaten away from home	6	15	0	21	Inf	1.37	Inf	0.021	N
Burgers away from home	7	13	2	19	4.92	0.77	55.82	0.067	N
Parsley eaten away from home	3	12	0	20	Inf	0.58	Inf	0.070	N
Strawberries away from home	4	17	0	20	Inf	0.67	Inf	0.107	N
Parsley eaten at home	0	14	4	18	0.00	0.00	2.30	0.141	N
Blue cheese at home	4	17	1	22	5.00	0.44	265.85	0.176	N
Hot chicken eaten away from home	10	10	5	15	2.92	0.66	14.50	0.191	N
Cheese spread eaten at home	2	19	6	17	0.31	0.03	2.02	0.245	N
Goats cheese away from home	0	21	1	7	0.00	0.00	14.86	0.276	N
Snack foods	6	2	20	2	0.31	0.02	5.20	0.284	N
Strawberries eaten at home	12	10	8	14	2.06	0.54	8.35	0.364	N
Handled potatoes	10	9	6	2	0.38	0.03	2.92	0.405	N

Other types of biscuits	7	2	12	9	2.55	0.36	30.90	0.419	N
Any eating out	24	0	22	1	Inf	0.03	Inf	0.489	N
Goats cheese eaten at home	1	20	1	8	0.41	0.00	35.29	0.517	N
Tomatoes eaten away from home	10	12	7	14	1.65	0.41	6.90	0.536	N
Processed cheese eaten at home	2	19	2	7	0.38	0.02	6.22	0.563	N
Burgers eaten at home	1	16	3	20	0.43	0.01	5.89	0.624	N
Handled carrots	7	12	4	4	0.60	0.08	4.32	0.675	N
Handled onions	9	10	5	3	0.55	0.07	3.84	0.678	N
Wrapped chocolate	4	5	12	9	0.61	0.09	3.78	0.694	N
Steak at home	4	13	4	19	1.45	0.23	9.34	0.702	N
Mixed salad leaves eaten at home	7	14	8	11	0.69	0.16	2.99	0.745	N
Mixed salad leaves away from home	9	13	6	13	1.49	0.35	6.71	0.746	N
Hot chicken eaten at home	14	6	14	8	1.32	0.31	5.99	0.750	N
Lettuce at eaten at home	8	13	10	12	0.74	0.18	2.93	0.760	N
Tomatoes eaten at home	10	11	13	10	0.71	0.18	2.68	0.763	N
Steak away from home	2	19	3	18	0.64	0.05	6.27	1	N
Cheese spread away from home	1	20	0	21	Inf	0.03	Inf	1	N
Other soft cheese at home	3	18	3	20	1.11	0.13	9.37	1	N
Hard white cheese at home	14	8	14	9	1.12	0.29	4.46	1	N
Sandwiches away from home	14	8	13	9	1.04	0.26	4.25	1	N
Lettuce away from home	8	13	8	12	0.92	0.22	3.87	1	N
Processed cheese away from home	0	21	0	8	NA	NA	NA	0.05	N

* not all questions were answered by all cases, partly due not all cases having exactly the same questionnaire administered, especially for the cases from previous outbreak investigations compared to the current investigation and compared to each of these previous outbreak.

** Variables ordered by level of significance

Table 5 presents the results when the case case analysis was run at the end of the study with the 24 primary cases. However, the analysis was run twice during the investigation before all the cases were identified and gave similar results.

If the childcare cluster cases are excluded or only adults included then in addition to the above four exposures “eating other soft cheese away from home” is also associated with being a case in the current outbreak. The fact that “eating hard cheese away from home”

and “eating other soft cheese away from home” both feature in the analysis is likely to be the result of confounding i.e. cases who ate Dunsyre Blue were more likely to eat other cheese from cheese boards as well. As discussed previously no other cheese was identified that accounted for the same high proportion of cases as Dunsyre Blue (Table 3).

From the previous outbreaks 0 out of 21 (2 were not asked this question), cases reported eating blue cheese away from home compared to 17 out of the 24 primary cases in this current outbreak. Because there is a zero the odds ratio is infinity but the lower 95% limit is still 8.69, meaning that eating blue cheese outside the home was at least 8 times more likely among the cases in this outbreak than in other outbreaks. When the childcare cluster cases are excluded the numbers change to 17 out of 21. This refers to blue cheese eaten away from home, in addition to these cases there are a small number of cases who also ate blue cheese at home but due to small numbers this was not statistically significant.

The case case study was only able to investigate cheese consumption down to the level of general type of cheese i.e. blue cheese, hard white and not specific types/brands as this level of information was not sought for the cases from the earlier outbreaks. The case case study identified a significant association with eating blue cheese, even without taking into account the additional evidence that it was one specific artisan type of blue cheese that was identified among cases in the current outbreak.

7.2 Bayesian modelling

The final Bayesian modelling conducted at the end of the investigation was based upon 26 cases among whom 17 ate blue cheese away from home.

Using this methodology an odds ratio close to 1 would indicate the level of exposure among the outbreak cases was not statistically significantly different from the control population, and the larger the estimate of the odds ratio, the greater the strength of the difference between the cases in the outbreak and the control population in eating blue cheese away from home.

When the Bayesian modelling is conducted using the information from the previous outbreak cases, as used in the case case study where 0 out of the 21 cases had consumed blue cheese away from home, the median odds ratio is 59 (95% credible interval 8, 1721).

When the estimate for blue cheese consumption away from home is centred on 1%, with a range of 0-4% the odds ratio is 270 (95% Crl 39, 7729). When the estimate is kept at 1% but the range extended from 0 to 10% the odds ratio is 196 (95% Crl 14, 93814).

When the estimate for blue cheese is based on the National Diet and Nutrition Survey at 3.5%, with a range of 0 to 8%, the odds ratio was 78 (95% Crl 16, 264). When the estimate is kept at 3.5% but the range extended from 0 to 16%, the odds ratio was 50 (95% Crl 8, 940).

The wide credible interval (the Bayesian equivalent of the confidence interval) show the uncertainty in these estimates, however all the odds ratios and the lower limit of the credible intervals are well above 1, with odds ratios as high as 78 when using the estimate based on the National Diet and Nutrition Survey indicating that blue cheese consumption away from home was statistically significantly more common among cases in this outbreak than any of the estimates used for the control population. All versions of the analysis provided very high estimates for the odds ratios. It would be necessary to assume that between 30-40% of the

population usually eat blue cheese away from home before the lower limit of the 95% credible interval approaches 1 and the exposure among the outbreak cases is no longer different from what would be expected.

A more technical explanation of the Bayesian modelling results is contained in Appendix 5.

8 Clinical Microbiological Investigation – Methods

8.1 Referral of samples to SERL

Faecal samples from symptomatic individuals are submitted to local diagnostic laboratories for culture, and presumptive isolates of *E. coli* O157 are then forwarded to SERL for confirmation and typing. In addition, faeces testing negative at the local diagnostic laboratory but from individuals with symptoms suggestive of an STEC infection, or from symptomatic contacts of known cases, are also forwarded to the SERL for more sensitive testing in line with current Scottish guidance[1].

SERL uses real-time PCR to detect shiga toxin genes (*stx1* and *stx2*), including all variants, and a gene specific for *E. coli* O157 (rfb_{O157}) in each submitted sample. Samples which are positive by PCR are reported to the sending laboratory and then cultured to confirm STEC infection. If real-time PCR detects the presence of an *E. coli* O157 organism, then Immunomagnetic Separation (IMS) is carried out to aid in the isolation of an organism.

8.2 Phage Typing

All *E. coli* O157 isolates are sub-typed using phage typing[41] and Multi Locus Variable number tandem repeat Analysis (MLVA)[42]. Phage typing tests the susceptibility of each confirmed *E. coli* O157 isolate to a standard panel of sixteen different bacteriophages. The output is a phage infection profile based on the lysis pattern produced by each phage. This profile is then compared with the international phage typing scheme and a phage type is assigned.

8.3 Multi Locus Variable number tandem repeat Analysis

MLVA is a typing method used to determine relatedness of *E. coli* O157 strains isolated from different patients. This method detects the number of repeat DNA sequences at eight different sites of the *E. coli* O157 genome. The output is a string of eight numbers - the MLVA profile - each number representing the number of repeats at each of the eight sites. SERL commenced routine MLVA typing in December 2012, and has a database of approximately 1,200 clinical *E. coli* O157 MLVA entries, against which each new MLVA profile is compared. If strains of *E. coli* O157 from different patients share the same MLVA profile (or share the same number of repeats at seven of the eight sites – this is called a single locus variant), this demonstrates the strains are closely related. When an MLVA match occurs, the strains are forwarded to PHE for whole genome sequencing (WGS).

In order to compare *E. coli* O157 isolates in Scotland with isolates from cases in England and Wales, SERL sends Scottish isolates to PHE for WGS.

8.4 Whole Genome Sequencing

For WGS, DNA was extracted by PHE from cultures of STEC O157 for sequencing on the Illumina HiSeq 2500 instrument as described previously in the work of Jenkins *et al* [19]. High quality Illumina reads were mapped to the STEC O157 reference genome Sakai (Genbank accession BA000007). Single Nucleotide Polymorphisms (SNPs) were identified and core genome positions that had a high quality SNP in at least one isolate were extracted and used to derive the maximum likelihood phylogeny of the isolates.

Genomes were compared by a Bioinformatician at PHE to the sequences held in the PHE STEC O157 WGS database. This database comprises genomes from over 2,000 cultures of STEC O157 submitted to Gastrointestinal Bacteria Reference Unit (GBRU) between 1982 and 2016. The majority of isolates were from human cases in the UK reporting domestically

acquired infection, although cases associated with foreign travel and isolates from domestic cattle and from food samples were also included. Isolates of STEC O157 with fewer than five SNPs differences within their core genome were considered closely related and likely to have an epidemiological link[43].

At PHE the SNP address is used to provide an isolate level nomenclature that can be used to group isolates at different levels of genomic similarity. Isolates with identical SNP addresses have no changes in their core genome. To generate the SNP address, hierarchical single linkage clustering was performed on the pairwise SNP difference between all isolates at various distance thresholds (Δ250, Δ100, Δ50, Δ25, Δ10, Δ5, Δ0). The result of the clustering is a SNP address that can be used to describe the population structure based on clonal groups. Although isolates greater than 5 SNPs apart are unlikely to be part of the same temporally linked outbreak, deeper phylogenetic relationships within the 10 or 25 SNP clusters may provide epidemiologically useful information or associations.

9 Clinical Microbiological Investigation – Results

9.1 MLVA profiles

SERL confirmed 21 cases of *E. coli* O157 infection resident in Scotland shared the same phage type (PT21/28), shiga toxin gene profile (*stx1* negative, *stx2* positive) and the same MLVA profile (7, 4, 6, 14, 7, 8, 8, 12) or a single locus variant thereof. This particular MLVA profile and variant were unique to the SERL MLVA database which meant this strain had not been observed in Scotland since the introduction of the MLVA method in December 2012. In addition, SERL received isolate DNA from a patient residing in the ROI but with Scottish exposures and isolates from two cases residing in England. The MLVA profile from these three cases also matched the outbreak MLVA profile. The remaining two cases were resident in England and confirmed as part of the outbreak through WGS.

9.2 WGS Analysis

Isolates from the 21 outbreak cases resident in Scotland were sent by SERL to PHE for WGS. This revealed that these isolates differed by fewer than 5 SNPs. A further five isolates differing by fewer than 5 SNPs following WGS were identified by PHE as being part of the outbreak. This included the ROI isolate and four isolates from patients resident in England. Sixteen of the 26 isolates have the identical SNP address 4.4.4.2160.3025.3143 indicating identical genomes. The remaining outbreak isolates differ by single SNPs from this profile. Therefore the isolates from all 26 cases were confirmed by WGS to differ by fewer than 5 SNPs.

Research has shown that core genome sequences of *E. coli* O157 from temporally linked cases that share a common epidemiological exposure are the same, or fall within 5 SNPs of each other[43], and therefore all 26 cases within this 5 SNP cluster belonged to this outbreak.

WGS analysis also confirmed that the Scottish outbreak strains were *E. coli* O157:H7 and possessed *stx2a/2c* and *eae* genes.

Figure 4: Phylogenetic tree for the Whole Genome Sequencing for the outbreak isolates. Maximum likelihood phylogeny of 26 *E. coli* O157:H7 genomes rooted against the closest outlier strain (H153840762).

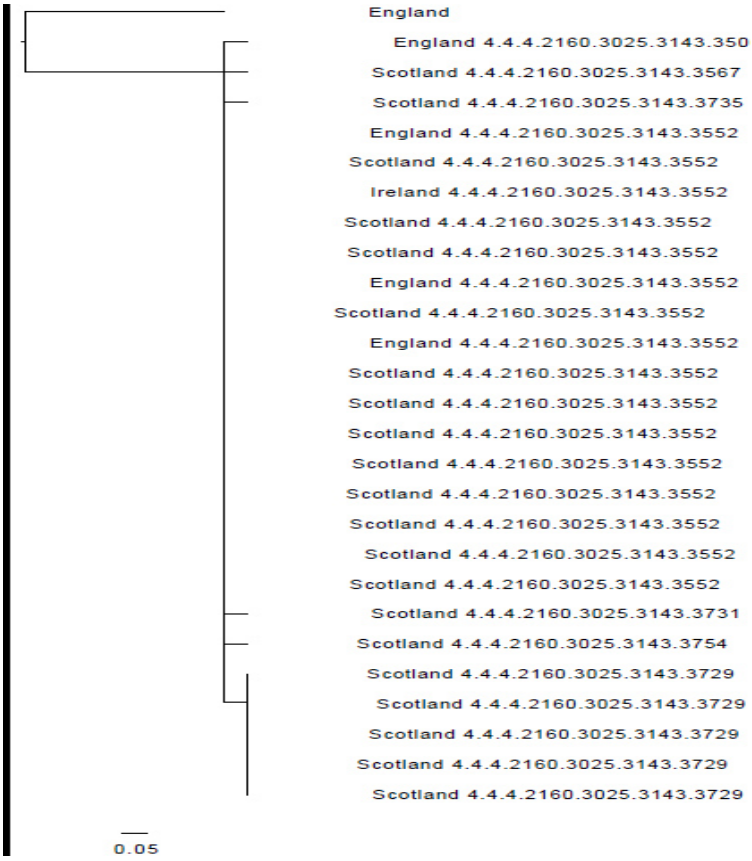


Figure 4 shows the phylogenetic tree for the WGS results for the 26 isolates, the smallest branch on the tree is 1 SNP, showing that all cases fall within the same 5 SNP cluster. The genetic similarity between the isolates is consistent with the cases being exposed to the same source.

10 Food Chain Investigation – Methods

Throughout the investigation, HPS provided information on the premises that cases had eaten at and the foods consumed to FSS to facilitate food chain investigations. FSS coordinated food chain investigations carried out by FSS and local authority Environmental Health Teams.

10.1 Environmental Health Officer visits to premises

On 26 July FSS informed local authorities of premises where cases had consumed or purchased particular products from. FSS asked local authorities to visit premises and provide full details of all types of cheese, salads and garnishes used/sold by the business as well as information about their suppliers. Local authorities were provided with details of the meal(s) consumed by cases and asked to secure information on the following:

- Name of establishment,
- Type of establishment,
- Address of establishment,
- Type of product (including products that contained either cheese, salad and/or garnishes),
- Brand names,
- Batch codes,
- Durability dates,
- Supplier (including address and approval number if applicable).

Local authorities were also asked to identify if there was more than one supplier for any specific product and, if so, asked to provide details.

Throughout the investigation FSS communicated with FSA, who were involved as there were premises in England where cases had consumed cheese, and information was obtained on the type and supply chain of the cheese consumed.

10.2 Supply chains

One major supplier (Supplier A) and some smaller suppliers of Dunsyre Blue were identified via local authority visits to a number of hotels/restaurants and shops. FSS asked local authorities in which there were relevant suppliers to obtain information from suppliers on the batches of cheese delivered to relevant premises and dates of delivery.

11 Food Chain Investigation – Results

11.1 Supply chain for Dunsyre Blue

Figure 5 shows the distribution chain for Dunsyre Blue to the premises where cases reported consuming or purchasing the product. All 15 cases known to have consumed Dunsyre Blue did so from premises supplied by Supplier A, this was also established to be the main supplier of Dunsyre Blue in Scotland.

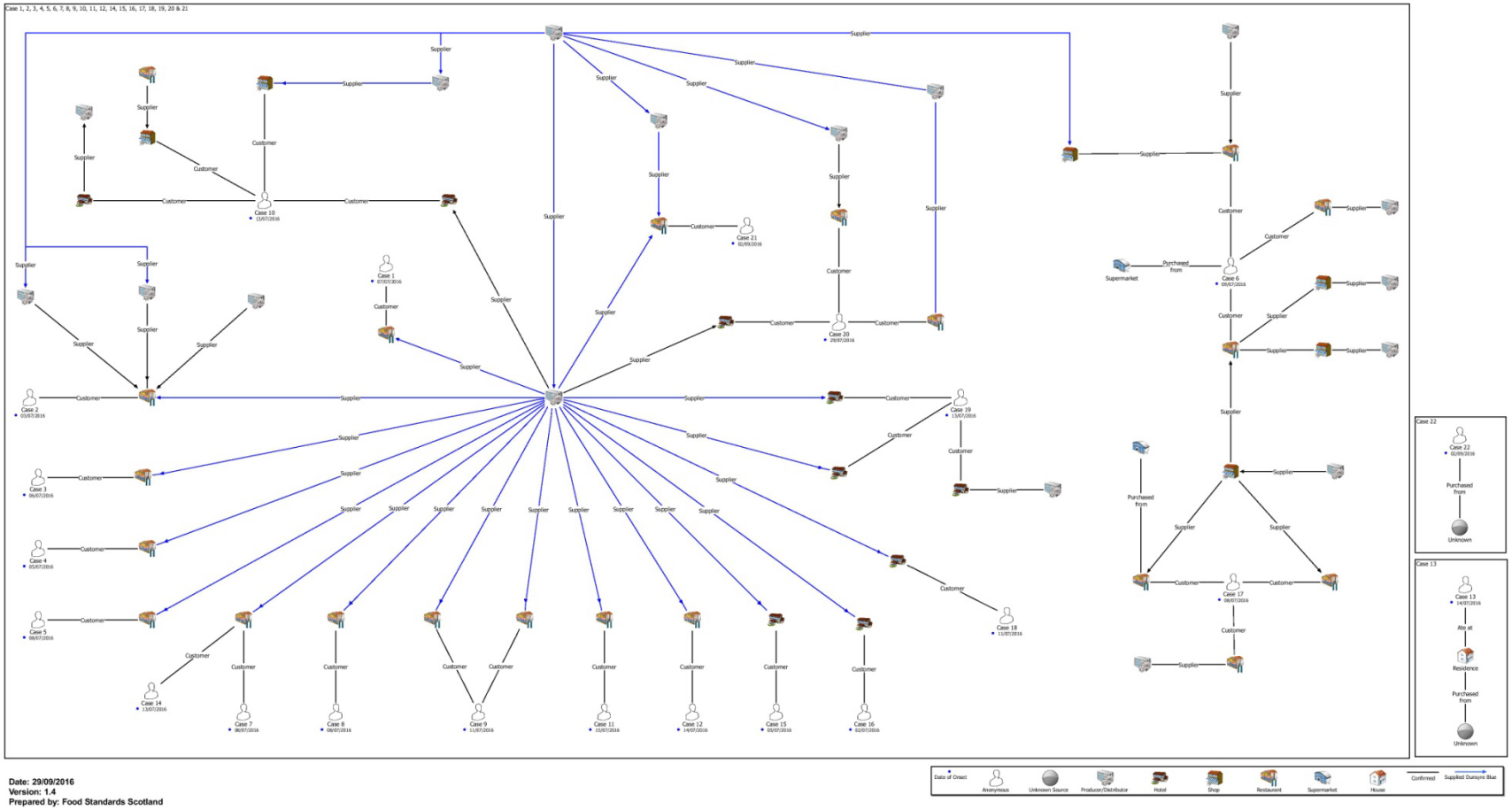
The competent local authority visited Supplier A on 28 July. Information was provided indicating that the relevant premises had received batches of C22 and/or D14 of Dunsyre Blue prior to the dates on which the cases visited the premises. The IMT considered this information at the meeting of 28 July, which informed the decision to recommend a recall of these two specific batches.

Supplier A informed their local authority on 4 August that although they had received batches C22 and D14 from ECL they were no longer certain which batches they had supplied to which premises. Subsequent analysis by FSS of the delivery notes from Supplier A showed that Dunsyre Blue was supplied between 16 June and 04 July (a 19 day period) to premises where 15 cases had consumed food, providing evidence that the source of infection for the first 20 cases was one or a small number of contaminated batches of Dunsyre Blue.

The information collected by local authorities during their visits to premises where cases had eaten did not identify any commonality among the consumption/supply of salads or garnishes that accounted for the same high proportion of cases as Dunsyre Blue.

Most Dunsyre Blue is sold within Scotland, with a smaller amount distributed to the rest of the UK. For batches C22 and D14, 67% and 71% respectively of the supply direct from Errington Cheese Ltd was to Scottish businesses.

Figure 5 Diagram of distribution chain for Dunsyre Blue to the premises where cases reported consuming or purchasing the product.



Date: 29/09/2016
Version: 1.4
Prepared by: Food Standards Scotland

12 Food Business Operator Investigation

12.1 Cheese produced by Errington Cheese Ltd

Errington Cheese Ltd (ECL) is a manufacturer of unpasteurised cows' and ewes' milk cheese based in South Lanarkshire (Table 6), for which South Lanarkshire Council (SLC) is the competent authority (Food Hygiene (Scotland) Regulations 2006). The last scheduled food hygiene inspection was carried out in October 2015.

Table 6: Types of cows' and ewes' milk cheese produced by ECL

Cheese	Milk	Description
Dunsyre Blue	Unpasteurised cow	Blue cheese
Maisie's Kebbuck	Unpasteurised cow	Semi-hard white cheese
Lanark White	Unpasteurised ewe	Semi-hard white cheese
Lanark Blue	Unpasteurised ewe	Blue cheese
Corra Linn	Unpasteurised ewe	Hard white cheese
Sir Lancelot	Unpasteurised ewe	Lactic cheese

Each batch of each type of cheese is alphanumerically coded, where the letter denotes the month of production and the number corresponds to the day of production e.g. C14 was made on 14 March. Eighty-eight batches of Dunsyre Blue were produced between 01/03/16 and 25/08/16. A batch is about 160-200kg of cheese; a batch consists of one day's production of cheese, made from typically 2000 litres of milk.

ECL receive one delivery of cows' milk a day from a single dairy farm transported by a tanker company. The ewes' milk is from their own flock of sheep milked on their own farm.

FSS contacted SLC on 26 July to advise that cheese produced by ECL had a tentative link to an outbreak of *E. coli* O157, and asked SLC to obtain information on distribution by ECL of Dunsyre Blue and Lanark Blue cheeses from the start of June 2016, this information was provided to FSS on 27 July.

12.2 Food legislation applicable to cheese production

The Food Safety Act 1990 remains the overarching piece of legislation governing food safety in Scotland, however much of the detailed food law applicable to ECL at the time of this outbreak is derived from EC Regulations:

- **Regulation EC 178/2002** lays down the general principles and requirements of food law in the EU. These regulations include food safety requirements, contained within (Article 14). Article 14 places a duty on food business operators (FBOs) to ensure food placed on the market is not 'unsafe', i.e. injurious to health, or unfit for human consumption. It lays down the provisions for FBOs to withdraw and recall 'unsafe' foods from the market (Article 19). Regulation EC 178/2002 is enforced by The General Food Regulations 2004, which make it an offence not to comply with Articles 14 and 19 in Regulation EC 178/2002.

- **Regulation EC 852/2004** is the main EU regulation applying to all food businesses on the hygiene of foodstuffs. It contains **Article 5**, which requires FBOs to put in

place, implement and maintain a permanent procedure or procedures based on Hazard Analysis and Critical Control Point (HACCP) principles. This includes requirements for FBOs to identify any hazards associated with their production system and to establish controls for ensuring these hazards are prevented, eliminated or reduced to acceptable levels. It further requires that FBOs establish procedures to verify that these controls are working effectively and that it keeps documents and records to demonstrate the effective application of these measures.

- **Annex II to Regulation EC 852/2004** contains the general hygiene requirements for all food business operators. Article 4, Annex II, Chapter IX – provisions applicable to foodstuffs, states that:
  "*A food business operator is not to accept raw materials or ingredients, other than live animals, or any other material in processing products, if they are known to be, or might reasonably be expected to be, contaminated with parasites, pathogenic microorganisms or toxic, decomposed or foreign substances to such an extent that, even after the food business operator had hygienically applied normal sorting and/or preparatory or processing procedures, the final product would be unfit for human consumption*."

- **Regulation EC 853/2004** lays down specific additional hygiene rules for food of animal origin. It requires premises to be approved, which ECL was and continues to be so. It also contains Annex III, Section IX of which applies to raw milk and dairy products. ECL were found to be compliant with this Regulation, both prior to and following the outbreak.

- **Regulation EC 2073/2005** sets out the microbiological criteria to be adhered to in foods produced in the EU. No standard for raw milk cheese [a standard was added for sprouted seeds in 2013] exists within this regulation in relation to STEC or *E. coli* O157, however recital 14 provides information on their stance of VTEC organisms. It states that "*The SCVPH (Scientific Committee on Veterinary Measures relating to Public Health) issued an opinion on verotoxigenic E. coli (VTEC) in foodstuffs on 21 and 22 January 2003. In its opinion it concluded that applying an end-product microbiological standard for VTEC O157 is unlikely to deliver meaningful reductions in the associated risk for the consumers. However, microbiological guidelines aimed at reducing the faecal contamination along the food chain can contribute to a reduction in public health risks, including VTEC. The SCVPH identified the following food categories where VTEC represents a hazard to public health: raw or undercooked beef and possibly meat from other ruminants, minced meat and fermented beef and products thereof, raw milk and raw milk products, fresh produce, in particular sprouted seeds, and unpasteurised fruit and vegetable juices*."

- **The Food Hygiene (Scotland) Regulations 2006**, as amended, create the offences in terms of not complying with Regulations EC 852/2004, 853/2004 and 2073/2005.

12.3 Industry Best Practice Guidance – The Specialist Cheesemakers Assured Code of Practice

In addition to the aforementioned legislative requirements, the UK industry guide applicable to the cheese industry is the "Specialist Cheesemakers Assured Code of Practice", The Specialist Cheesemakers Association (SCA) Edition 1, 2015. This document describes itself as "not intended to be an authoritative guide to cheesemaking – its prime aim is to provide guidance on good hygiene practices". It would therefore be considered "best practice" for cheesemakers to follow this Code.

12.4 Application of food safety controls at ECL

ECL had identified a number of pathogens, including *E. coli* O157, as potential hazards and had established controls, which they considered would be effective in reducing the risk of cheese being contaminated with pathogens to an acceptable level. It should be noted that an acceptable level for STEC in a ready to eat food would be absence, due to the low infective dose required for these organisms to cause illness. The risk of *E. coli* O157 was considered by ECL to be low throughout the primary production phase and processing phase of the operation.

At the time of the outbreak ECL had identified two critical control points (CCPs) in respect to their Food Safety Management System based on HACCP principles. The first CCP related to their milk supply with a critical limit of 10°C having been set for the acceptance of raw milk delivered to the premises. The second CCP related to "cheesemaking acidity" with a critical limit for blue cheese being an acidity of not less than 0.5% at 5th turn/last turn of the day, and a pH of less than 4.9 at the morning turn.

The significant hazards which these two CCPs were identified to control, i.e. prevent, eliminate or reduce to acceptable levels were, in relation to the milk supply, "formation of toxins from the growth of *Staphylococcus aureus* from incoming or prolonged storage at warm temperatures" and, in relation to cheesemaking acidity, the "growth of pathogenic organisms (*Staphylococcus aureus, Salmonella, Listeria monocytogenes*) and development of toxin due to low level of lactic acid and starter populations in relation to target makes." Whilst these controls will also contribute to a reduction in STEC/*E. coli* O157, the scientific literature, and the SCA Assured Code of Practice recognises that STEC/*E. coli* O157 is able to survive certain cheesemaking processes. However, ECL had not undertaken any technical assessment or sampling to enable them to demonstrate the extent to which STEC or *E. coli* O157 that may have been introduced via the raw milk supply, would have been capable of surviving and growing throughout the production and maturation process for their cheese.

As such operational pre-requisite programmes (oPRPs) to prevent the introduction of faecal contamination into the raw milk supply represented the primary control for STEC in ECL cheeses. In order to assess the effectiveness of these oPRPs, it is necessary to verify measureable or observable action criteria and identify corrective actions to control the hazard. Controls in relation to ECL's raw milk supply consisted of the observance of good animal health and husbandry, together with the application of good agricultural and hygiene practices to minimize opportunities for raw milk to be contaminated with pathogens. However, scientific evidence for the impact of animal husbandry on STEC shedding by cattle is inconclusive, and it is therefore important that appropriate verification is undertaken to

assess the effectiveness of hygiene practices in preventing contamination of the raw milk supply.

Whilst ECL had a specification in place for their supplier of raw cows' milk (a single dairy farm), including a requirement that the milk was free from *E. coli* O157, this was not being verified with regard to the STEC hazard. The verification of hygiene standards relating to the raw milk supply involved supplier audits, the reviewing of somatic cell count levels for both cows' and ewes' milk, and microbiological sampling for indicator organisms. Raw milk was being sampled weekly for aerobic colony counts and *Enterobacteriaceae* levels, and monthly for *Staphylococcus aureus* and *Listeria.* No specific testing had been undertaken to verify the absence of *E. coli* O157 in ECL's raw milk supply in accordance with their specification. The SCA Assured Code of Practice recommends that a test schedule be implemented in respect to STEC in raw milk, and although it does not specify the frequency of this testing, it recommends a risk based approach. Cheesemakers should establish their sampling frequency based on a number of factors; principally their relationship with the milk producer, the type of cheese produced, the size of business and any requirements imposed by customers. The Code of Practice proposes that frequency of testing could be anything from weekly to six monthly, but should be reviewed periodically and amended according to results. It suggests that for many specialist cheesemakers quarterly testing of pathogens in raw milk might seem to be appropriate.

In addition to the sampling regime for their raw milk, ECL also carried out microbiological testing at the curd stage of cheese production including weekly sampling for both generic *E. coli* and *Listeria,* monthly sampling for *Salmonella* and twice monthly sampling for *Staphylococcus aureus.*

The testing regimes applied by ECL for cheeses produced leading up to the outbreak did not follow the recommendations of the SCA Assured Code of Practice for generic *E. coli* testing, as well as routine checks for *E. coli* O157 in both raw milk and cheese. Despite this, ECL were accredited to the Safe and Local Supplier Approval (SALSA) and the SCA Standard for the 'manufacture, maturation and packing of soft blue and hard pressed cheese made from raw cows' and ewes' milk' at the time of the outbreak and were found on audits to be compliant with the contents of it.

It is unclear whether adoption of the SCA testing recommendation would have enabled ECL to routinely identify *E. coli* O157 contamination of affected batches of milk, however the absence of any STEC testing regime prevented ECL from being able to demonstrate that their food safety management system was capable of controlling this hazard. This incident has highlighted that there is a need to strengthen existing guidance on STEC risks in raw milk production, particularly with regard to appropriate validation and verification of controls.

Sampling undertaken by SLC following the outbreak has indicated that the existing controls, which ECL had in place were not sufficient or failed on some occasions to prevent STEC being present in final product, at or immediately prior to the point of sale (see food microbiology results section for positive results). The actual controls that had failed to prevent contamination were not identified, although the original contamination is most likely to have occurred during the milking of animals which were shedding the pathogen. It is also necessary to consider the possibility that cross contamination in the processing environment could have led to the contamination of cheese types from different milk sources, which may

have attributed to sample results which identified the same strains of *E. coli* in both cows' and ewes' milk cheese.

Following the outbreak ECL reviewed their Food Safety Management system. The revised document will now include more detailed information on the verification being carried out by ECL during on-farm audits, and the sampling plan was amended to include testing for *E. coli* O157 and STEC. Finally, every batch of raw milk will be sampled for the presence of *E. coli* O157 as a means of validating controls applied at the milking stage. The microbiological testing regimes undertaken by ECL leading up to and subsequent to the outbreak are presented in Table 7 and Table 8.

Table 7: Microbiological testing undertaken by ECL at the time of the outbreak

Microbiological test	Raw milk	Cheese curd
Aerobic Colony counts	Weekly	
Enterobacteriaceae	Weekly	
Staphylococcus aureus	Monthly	2 x Monthly
***Listeria* species**	Monthly	Weekly
Salmonella		Monthly
***E. coli* – generic**		Weekly
***E. coli* O157**		
STEC		

Sampling plan states that "where results are unsatisfactory all cheese will be tested on a positive release basis until results are satisfactory for at least a 3 month period"

Table 8: New microbiological testing revised by ECL since the outbreak

Microbiological test	Raw milk	Cheese curd	Finished Product
Aerobic Colony counts	Weekly/monthly		
Enterobacteriaceae			
Staphylococcus aureus	Weekly/monthly	Weekly/monthly	
***Listeria* species**	Weekly/monthly	Weekly/monthly	Quarterly
Salmonella	Weekly/monthly	Weekly/monthly	Quarterly
***E. coli* – generic**	Weekly/monthly	Weekly/monthly	
***E. coli* O157**	Every batch	Weekly/monthly	
STEC	Quarterly		Quarterly

Sampling plan states that testing will be undertaken "weekly, if results are satisfactory over a 1 month period, revert to monthly"

12.5 Summary of deficiencies identified in ECL's food safety management system with regard to the risk of STEC

- No specific testing of raw milk or cheese to assess for the presence of *E. coli* O157 or STEC
- Identification of the risk of *E. coli* O157 as low in the absence of any specific testing regime to verify this
- No evidence and/or validation to demonstrate the extent to which controls applied during the cheese production and maturation process would have been capable of controlling the introduction, survival and or proliferation of the *E. coli* O157 or STEC hazard in the products or the processing environment.

12.6 Visits to ECL by SLC and sampling undertaken

SLC took five informal samples of Dunsyre Blue on 29 July in response to the epidemiological link to Dunsyre Blue identified by the IMT on 28 July.

At the initial stages the investigation focused on gathering information requested by the IMT and reviewing the information in relation to the food safety management arrangements used by ECL in relation to controls for *E. coli* O157. A site visit was carried out on 12 August and went through the cheese making process and SLC considered if any additional controls could be employed by ECL.

SLC determined to take samples of cheese to obtain information relating to water activity and pH at the point of dispatch to inform SLC's discussions with ECL on their procedures based on HACCP principles. On 23 August SLC took two informal samples of Dunsyre Blue that were ready to be placed on the market for microbiological quality and physicochemical testing. A younger cheese (batch F15) and a more mature cheese (batch E31) were taken, with a view of comparing the physico-chemical characteristics. On 25 August SLC were advised of a presumptive positive for non-O157 STEC for batch F15 (for details of all samples results see Food Microbiology section), at this point the work of SLC moved to checking for potential contamination of other batches.

On 26 August formal samples were taken of Dunsyre Blue that was ready to be placed on the market. SLC took samples from batches E12, E24, F2 and F13 at that time.

On 29 August, SLC took nine formal samples of cheese from batch F15 of Dunsyre Blue and 30 formal samples from other available batches of Dunsyre Blue.

On 31 August, SLC took four formal samples of batch E24 of Dunsyre Blue. In addition, formal samples were also taken of Masie's Kebbuck (unpasteurised cows' milk cheese) and of Corra Linn, Lanark Blue, Sir Lancelot and Lanark White (all unpasteurised ewes' milk cheeses).

SLC took samples of raw milk and swab samples from the dairy farm that supplied the cows' milk to ECL for their cheese on 29 September and this was followed up with further sampling from 17-20 October.

On 7 October SLC took environmental swabs from the ECL premises.

12.7 Microbiology results provided by ECL to SLC

As part of the investigation SLC requested from ECL the microbiological sampling results available for samples since March 2016.

The tanker company provided SLC with available results of both Bactoscan and somatic cell counts for the cows' milk received in March and April (when C and D batches would have been produced, although none of the results available related to the milk used in batches C22 or D14). All available results for Bactoscan and somatic cell counts were satisfactory and well within standard values. Bactoscan results indicate the level of bacterial contamination from external sources, e.g. milking equipment which has not been adequately cleaned or poor udder or teat preparation, and can indicate a high level of environmental pathogens. Somatic cell counts are the main indicators of milk quality. They represent cells shed in response to infection e.g. when a cow/ewe is suffering from mastitis.

At the time of the outbreak ECL undertook weekly testing of the raw cows' milk for hygiene indicator organisms (colony counts <100,000 cfu/ml and *Enterobacteriaceae* <10,000 cfu/ml) with monthly checks for *Staphylococcus aureus* and *Listeria* spp (Table 7).

ECL provided SLC with results of raw cows' milk sampling undertaken between 2 March 2016 and 19 July 2016 which showed that levels of *Enterobacteriaceae* ranged from <10 to 1260 cfu/ml. All these results are below the criteria set in ECL's sampling plan. Results were available for the cows' milk used to produce batch C22 of Dunsyre Blue. The aerobic colony count was 1,300 cfu/ml, *Enterobacteriaceae* 1,100 cfu/ml and *Listeria* species were not detected; these results were satisfactory. As per ECL's sampling plan these samples were not tested for *E. coli* or STEC. No sample results were available for the raw milk used for batch D14.

Results for aerobic colony counts ranged from 570 to 28,000 cfu/ml which meets ECL's own and EU criteria (<100,000 cfu/ml). However the SCA Assured Code of Practice recommends a stricter criterion for a plate count at 30°C of <10,000 cfu/ml; 6 of the 18 samples were ≥ 10,000 cfu/ml thus exceeding the SCA guidance.

ECL provided SLC with results of testing of pooled samples (usually 3 or 4) of Dunsyre Blue they undertook between 2 March and 12 July. *E. coli* results for the 20 pooled samples ranged from <10 to 640 cfu/g, which do not exceed ECL's set criteria or that of the SCA guidance (<10,000 cfu/g). Results for *Listeria* spp., *Salmonella* and *S. aureus* were all satisfactory. No testing had been undertaken for *E. coli* O157 or STEC.

12.8 Private Water Supplies

Private water supplies have previously been implicated in outbreaks of STEC infection. ECL are supplied by a private water supply from a spring that serves the food business, farm and three further residential properties. SLC confirmed that the supply is sampled annually and the most recent sample was in April 2016. The sample was satisfactory, additionally all results from the supply have been satisfactory since 2011. The supply has a UV treatment system, which was installed in 2008. The source storage tank is fenced off to prevent access by livestock. Visits by SLC to ECL confirmed that no water from the private water supply is added at any stage of the cheese production process.

12.9 Tanker Company

ECL receives cows' milk from a single dairy farm. The milk is transported by a tanker company. The relevant local authority visited the tanker company, reviewed relevant documentation and undertook extensive sampling from four of the tankers. The swabs from the tankers were tested for generic *E. coli*, *E. coli* O157, *Listeria monocytogenes, Enterobacteriaceae* and total viable counts. All results were satisfactory. The local authority inspecting the tanker company did not identify any areas of concern.

13 Food Microbiological Investigation – Methods

13.1 Products sampled

Throughout the investigation a number of samples of Dunsyre Blue and other cheese produced by ECL were sampled. These samples can be divided into a number of groups:

- Where possible EHOs visiting the hotels/restaurants at which cases had eaten took samples of Dunsyre Blue on the premise at the time of the visit. As these visits occurred a number of weeks after the case consumed the implicated cheese, it is unlikely that the cheese on the premise was the same batch as that served to the case.
- Some of the cheese returned to local authorities as part of the voluntary recall of batches C22 and D14 and the Food Alert for Action. In addition, cheese was sampled by local authorities while visiting premises that stocked Dunsyre Blue. From the voluntary recall only batch D14 was available for testing.
- As discussed above SLC undertook extensive sampling of cheese produced by ECL, milk samples and environmental swabs.
- The relevant local authority took environmental samples from the tanker company that transports the cows' milk from the dairy farm to ECL.

13.2 Examination of cheese, milk and environmental samples at City of Edinburgh Council Scientific Services

During the investigation 74 cheese samples, 21 milk samples and 25 environmental swabs were submitted by SLC to City of Edinburgh Council Scientific Services (ESS) who are their appointed Food Examiner under the Food Safety (Sampling & Qualifications) (Scotland) Regulations 2013[44]. ESS is a FSA authorised EU Food and Feed Official Control Laboratory (OCL)[45]. An additional three cheese samples were submitted by other local authorities as part of the investigation, including two cheese samples of batch D14 from Orkney Islands Council following the recall.

A range of standard United Kingdom Accreditation Service (UKAS) accredited culture tests were applied to examine the samples including enumeration tests such as generic *E. coli* per gram, *Enterobacteriaceae* per gram and detection of *E. coli* O157 per 25 gram using immuno-magnetic separation (IMS). Confirmation of *E. coli* O157 was by serology using latex agglutination kit and biochemical tests (API 20E) before submission to SERL for further confirmation. DNA extracted from samples was also examined by real time PCR using a UKAS flexible scope accredited procedure based on an ISO[ii] method ISO/TS 13136:2016[46] for virulence gene markers such as *stx1*, *stx2* and *eae*.

Initially modified Tryptone Soya Broth (mTSB) which had added antibiotic supplements to suppress competing bacteria was used as the enrichment broth of choice since this is optimised for recovery of *E. coli* O157:H7. With guidance from SERL, Buffered Peptone Water (BPW) was used as an enrichment broth to improve recovery of stressed *E. coli* O157 and *E. coli* non-O157 strains. The use of either mTSB or BPW enrichment broths is part of the standard accredited method at ESS. Research has shown the application of BPW for the resuscitation of non-O157 STEC[47]; this published work was discussed by EU member states

[ii] ISO (the International Organisation for Standards) is a worldwide federation of national standards bodies

E. coli STEC/VTEC reference laboratories at the annual EU reference laboratory workshop in Rome (2016) and is likely to be included in an update to the STEC PCR reference method ISO 13136[48]

To further assist recovery of live STEC organisms from the BPW enrichment broth for some samples “acid shock” was used to suppress competing bacteria. The use of acid shock was advocated by the Norwegian National STEC/VTEC laboratory[49] and is likely to be included in an update to the STEC PCR standard reference method ISO 13136.

The results of the examination were assessed against the Health Protection Agency (HPA) Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market (November 2009)[50], Annex II Chapter IX of Regulation (EC) 852/2004 on the hygiene of foodstuffs or the Specialist Cheesemakers Association Guide (2015) as appropriate. Samples which were presumptive positive for either *E. coli* O157 by culture or shiga toxin gene (*stx*) positive by real time PCR at ESS were submitted to SERL who confirmed ESS findings as described below. Formal certificates for legal and enforcement purposes were issued to SLC under Food Safety (Sampling & Qualifications) (Scotland) Regulations 2013[44]

In addition to the 74 samples submitted to ESS, during the course of the investigation cheese samples submitted by other local authorities to Glasgow, Aberdeen and Dundee Scientific Services, underwent similar testing for *E. coli* O157. As not all Public Analyst laboratories are able to undertake testing for the *stx* genes, samples were referred to either ESS or Tayside Scientific Services for *stx* testing.

13.3 Examination of cheese, milk and environmental samples at SERL

SERL received 88 samples from ESS in order to confirm PCR results and aid in the isolation of shiga toxin - producing organisms. Real-time PCR was used to detect the presence of a number of key genes (as described in Clinical Microbiology Investigation – Methods section) and isolation was achieved by carrying out individual PCR tests on a number of different colonies from culture plates or by IMS (for isolation of *E. coli* O157). Once an organism was isolated, its identity as *E. coli* was confirmed using biochemical tests.

Sixteen *E. coli* isolates (from nine different cheese samples) containing either or both virulence genes (*stx1, stx2)* and/or the gene specific for *E. coli* (rfb_{O157}) were forwarded to PHE for WGS.

14 Food Microbiological Investigation – Results

14.1 Microbiology results

The presence of an STEC is considered to be confirmed when one or more *stx* genes are detected in a cultured *E. coli* strain. Detection of *stx* gene(s) alone is considered a "presumptive positive" until an *E. coli* is isolated and cultured[iii].

The Specialised Cheesemakers Association target for *Enterobacteriaceae* in soft and semi soft cheese is less than 10,000 cfu/g (colony forming units per gram of cheese). The HPA guidelines for assessing the Microbiological Safety of Ready-to-Eat Foods placed on the market considers *Enterobacteriaceae* unsatisfactory if levels are greater than 10,000 cfu/g.

The Specialised cheesemakers Association target for generic *E. coli* (all types not just STEC) in soft and semi soft cheese is <10,000 cfu/g. The HPA generic *E. coli* criteria do not apply to raw milk cheese, but the sample is considered unsatisfactory and potentially injurious to health and/or unfit for human consumption if *E. coli* O157 or other STEC are detected.

[iii] As defined in the draft *UK Working Policy on Detection of STEC in Food by Official Controls And Food Business Operator Sampling and Testing*. Previously out to consultation at http://www.foodstandards.gov.scot/consultation-uk-working-policy-detection-stec-food-official-controls-and-food-business-operator

Table 9: Results of Food Microbiology testing from Edinburgh Scientific Services, SERL and PHE

Batch No	Local Authority	Sampled by LA on	LA Sample Description	*E. coli* O157 by IMS culture	eae (EPEC) qPCR of broth	*stx1* qPCR of broth	*stx2* qPCR of broth	Enterobacteriaceae by culture	*E. coli* (generic) by culture	Coagulase +ve *Staphylococci*	Total Viable Count 30°C by culture	Comments on results. Colony identification by WGS at PHE
				per 25 gram	per 25 gram	per 25 gram	per 25 gram	cfu/gram	cfu/gram	cfu/gram	cfu/gram	
E26	SLC	29/07/2016	Dunsyre Blue	ND	ND	ND	ND	NT	<10	NT	NT	
E12	SLC	29/07/2016	Dunsyre Blue	ND	ND	ND	ND	NT	<10	NT	NT	
E30	SLC	29/07/2016	Dunsyre Blue	ND	Detected	ND	ND	NT	<10	NT	NT	*eae* presumptive in broth, not confirmed by culture
E17	SLC	29/07/2016	Dunsyre Blue	ND	ND	ND	ND	NT	<10	NT	NT	
E25	SLC	29/07/2016	Dunsyre Blue	ND	Detected	ND	ND	NT	<10	NT	NT	*eae* presumptive in broth, not confirmed by culture
D14	OIC	01/08/2016	Dunsyre Blue	ND	NT	ND	ND	NT	<10	NT	NT	
D14	OIC	01/08/2016	Dunsyre Blue	ND	NT	ND	ND	NT	<10	NT	NT	
E31	SLC	23/08/2016	Dunsyre Blue	ND	Detected	ND	ND	80	10	<10	NT	*eae* presumptive in broth, not confirmed by culture
F15	SLC	23/08/2016	Dunsyre Blue	ND	Detected	ND	Detected	430	10	<10	NT	Colony identified as *E.coli* O unidentifiable :H20 *stx2d* ST 1308 eae negative. *eae* presumptive in broth only
F13	SLC	26/08/2016	Dunsyre Blue	ND	NT	ND	ND	NT	NT	NT	NT	
E12	SLC	26/08/2016	Dunsyre Blue	ND	NT	ND	ND	NT	NT	NT	NT	
F2	SLC	26/08/2016	Dunsyre Blue	ND	NT	ND	ND	NT	NT	NT	NT	
E24	SLC	26/08/2016	Dunsyre Blue	ND	NT	ND	Detected	NT	NT	NT	NT	*stx2* presumptive in broth not confirmed by culture
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	60,000	<10	NT	NT	*stx2* presumptive not confirmed by culture. Unsatisfactory Enterobacteriaceae
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	800	<10	NT	NT	*stx2* presumptive in broth not confirmed by culture
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	350,000	<10	NT	NT	Colony identified as *E.coli* O unidentifiable :H20 *stx2d* ST 1308 *eae* negative. Unsatisfactory Enterobacteriaceae
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	24,000	10	NT	NT	*stx2* presumptive in broth not confirmed by culture. Unsatisfactory Enterobacteriaceae
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	13,000	<10	NT	NT	*stx2* presumptive in broth not confirmed by culture. Unsatisfactory Enterobacteriaceae

F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	Detected	Detected	90	<10	NT	NT	*stx1* and *stx2* presumptive in broth not confirmed by culture
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	310	20	NT	NT	*stx2* presumptive in broth not confirmed by culture
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	2,900	10	NT	NT	*stx2* presumptive in broth not confirmed by culture
F15	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	60,000	100	NT	NT	*stx2* presumptive in broth not confirmed by culture. Unsatisfactory Enterobacteriaceae
F6	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	30	<10	NT	NT	
F7	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	170,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
F8	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	330,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
F9	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	1,800,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
F13	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	<10	<10	NT	NT	
F14	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	330	60	NT	NT	
G21	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	54,000	40	NT	NT	Unsatisfactory Enterobacteriaceae
G25	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	18,800,000	620	NT	NT	Unsatisfactory Enterobacteriaceae
G26	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	6,000,000	340	NT	NT	Unsatisfactory Enterobacteriaceae
G27	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	1,020	10	NT	NT	
G28	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	11,200,000	50	NT	NT	Unsatisfactory Enterobacteriaceae
H2	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	810	<10	NT	NT	
H3	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	5,400,000	50	NT	NT	Unsatisfactory Enterobacteriaceae
H4	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	280,000	10	NT	NT	Unsatisfactory Enterobacteriaceae
H9	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	120,000	10	NT	NT	Unsatisfactory Enterobacteriaceae
H10	SLC	29/08/2016	Dunsyre blue	ND	NT	ND	ND	3,400	10	NT	NT	
F21	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	99,000	120	NT	NT	Unsatisfactory Enterobacteriaceae
F22	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	59,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
F20	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	900	20	NT	NT	
F27	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	150,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
F29	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	130,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae

F30	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	1,310,000	200	NT	NT	Unsatisfactory Enterobacteriaceae
F28	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	7,300,000	13,600	NT	NT	Unsatisfactory Enterobacteriaceae and generic *E.coli* (SCA)
G5	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	78,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
G6	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	610,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
G11	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	7,200,000	<10	NT	NT	Unsatisfactory Enterobacteriaceae
G12	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	Detected	13,200	<10	NT	NT	*stx2* presumptive in broth not confirmed by culture. Unsatisfactory Enterobacteriaceae
G13	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	12,000,000	20	NT	NT	Unsatisfactory Enterobacteriaceae
G19	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	1,020,000	100	NT	NT	Unsatisfactory Enterobacteriaceae
G20	SLC	29/08/2016	Dunsyre Blue	ND	NT	ND	ND	2,600	100	NT	NT	
E24	SLC	31/08/2016	Dunsyre Blue	ND	Detected	ND	ND	500	20	NT	NT	*eae* presumptive in broth, not confirmed by culture
E24	SLC	31/08/2016	Dunsyre Blue	ND	ND	ND	ND	480	<10	NT	NT	
E24	SLC	31/08/2016	Dunsyre Blue	ND	Detected	ND	ND	130	<10	NT	NT	*eae* presumptive in broth, not confirmed by culture
E24	SLC	31/08/2016	Dunsyre Blue	ND	ND	ND	Detected	350	<10	NT	NT	stx_2 presumptive in broth not confirmed by culture
E25	SLC	31/08/2016	Corra Linn,	ND	NT	ND	ND	10	<10	NT	NT	
E26	SLC	31/08/2016	Lanark Blue,	ND	NT	ND	ND	620	<10	NT	NT	
E24	SLC	31/08/2016	Lanark Blue	ND	Detected	Detected	Detected	<10	<10	NT	NT	Colony identified as *E.coli* O unidentifiable :H20 *stx2d* ST 1308 *eae* negative. *eae* and *stx1* presumptive in broth only
F14	SLC	31/08/2016	Lanark Blue	ND	NT	ND	ND	500,000	30	NT	NT	Unsatisfactory Enterobacteriaceae
G19	SLC	31/08/2016	Maisie's Kebbuck	ND	NT	ND	ND	1,500	<10	NT	NT	
G14	SLC	31/08/2016	Lanark White	Detected	NT	ND	ND	30	20	NT	NT	Colony identified as *E coli* O157 H42, *stx* -ve *eae* -ve ST7077
H13	SLC	31/08/2016	Lancelot	ND	NT	ND	ND	20	20	NT	NT	
C22	SLC	31/08/2016	Corra Linn,	ND	NT	ND	ND	<10	<10	NT	NT	
F15	SLC	31/08/2016	Corra Linn	ND	ND	ND	ND	20	<10	NT	NT	
I9	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	130	< 10	NT	NT	
I7	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	310	10	NT	NT	
H5	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	10	< 10	NT	NT	

H3	SLC	13/09/2016	Lanark White	Detected	NT	ND	ND	< 10	< 10	NT	NT	Colony identified as *E. coli* O157 H42, *stx* -ve *eae* -ve ST7077
G27	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	< 10	< 10	NT	NT	
H24	SLC	13/09/2016	Lanark White	Detected	NT	ND	Detected	30	< 10	NT	NT	Colony identified as *E coli* O157 H42, *stx* -ve *eae* -ve ST7077 *stx2* presumptive in broth only
H31	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	70	< 10	NT	NT	
I2	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	20	< 10	NT	NT	
G12	SLC	13/09/2016	Dunsyre Blue	ND	NT	ND	ND	2,300	10	NT	NT	
Not Known	SLC	13/09/2016	Lanark White	ND	NT	ND	ND	10	< 10	NT	NT	
G14	SLC	13/09/2016	Lanark White	Detected	NT	ND	ND	50	< 10	NT	NT	Colony identified as *E. coli* O157 H42, *stx* -ve *eae* negative ST7077
N/A	HC	19/09/2016	Dunsyre Blue sample Hotel A	ND	NT	ND	ND	120	<10	<10	NT	
N/A	SLC	29/09/2016	Tank Outlet	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Milk - raw bulk tank	ND	NT	NT	Detected	NT	NT	NT	NT	Colony identified as *E. coli* O15 H16 *stx2g*, STand/or LT genes *eae* negative ST325
N/A	SLC	29/09/2016	Filter housing	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Liner Tube - Cluster 4	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Cluster Head - Cluster 4	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Jetter - Custer 4	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Trough Water	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	29/09/2016	Filter Sock	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Piercing Machine Swab	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab white cheese vat surface – internal	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Steel table top green room - where moulds located	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Steel table top Green room - holding cheese moulds	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab large vat blue cheese room	ND	NT	NT	ND	NT	NT	NT	NT	

N/A	SLC	07/10/2016	Swab wooden shelf white cheese maturing room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab stainless steel floor drain blue cheese room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab middle vat blue cheese room/ stainless steel	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab table top white cheese room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab mixer tap blue cheese cleaning room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab small vat blue cheese room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab hose outlet blue cheese room	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Curster swab 5th right	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab milk filter housing – internal	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab internal bulk tank - Ewe's	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Bulk tank outlet- Ewe's	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab bulk tank internal - cows milk	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	07/10/2016	Swab bulk tank outlet - Cow's milk	ND	NT	NT	ND	NT	NT	NT	NT	
N/A	SLC	17/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$< 1.00x10^3$	
N/A	SLC	17/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.10x10^4$	
N/A	SLC	17/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$2.90x10^4$	
N/A	SLC	17/10/2016	Raw milk	ND	NT	NT	ND	NT	10	NT	$3.00x10^4$	
N/A	SLC	17/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$3.20x10^5$	
N/A	SLC	18/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$5.00x10^3$	
N/A	SLC	18/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.00x10^4$	
N/A	SLC	18/10/2016	Raw milk	ND	NT	NT	ND	NT	10	NT	$3.00x10^3$	
N/A	SLC	18/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$< 1.00x10^3$	
N/A	SLC	18/10/2016	Raw milk	ND	NT	NT	ND	NT	10	NT	$1.50x10^4$	

N/A	SLC	19/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.00x10^3$	
N/A	SLC	19/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.00x10^3$	
N/A	SLC	19/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$3.00x10^3$	
N/A	SLC	19/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$4.00x10^3$	
N/A	SLC	19/10/2016	Raw milk	ND	NT	NT	Detected	NT	20	NT	$2.00x10^3$	Colony identified as *E coli* O150:H2 *stx2* and *stx1a* ST P3233
N/A	SLC	20/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.00x10^3$	
N/A	SLC	20/10/2016	Raw milk	ND	NT	NT	ND	NT	10	NT	$3.00x10^3$	
N/A	SLC	20/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$5.00x10^3$	
N/A	SLC	20/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$3.00x10^3$	
N/A	SLC	20/10/2016	Raw milk	ND	NT	NT	ND	NT	< 10	NT	$1.00x10^3$	

Samples of cheese, milk and swabs were initially tested at ESS. Isolated colonies or DNA presumptive *E. coli* O157 or STEC were sent to SERL for verification. Colony identification was by WGS at PHE. Due to the high number of *stx* presumptive broths not all samples were prioritised for extensive attempts to isolate an STEC colony to confirm its presence. No attempt was made to isolate an *E. coli* with *eae* only since the outbreak strain was a *stx* positive organism.

Presumptive STEC and/or unsatisfactory *Enterobacteriaceae*/generic *E. coli*

Unsatisfactory: Potentially injurious to health and/or unfit for human consumption

SLC	South Lanarkshire Council	NT	Not Tested	cfu	colony forming unit
OIC	Orkney Islands Council	ND	Not Detected	+ve	Positive
HC	Highland Council	<	less than		

Microbiological Assessment Criteria - Sample Unsatisfactory If

	Enterobacteriaceae cfu/g	*E. coli* cfu/g	*E. coli* O157 & STEC in 25g
SCA soft and semi soft cheese	> 10,000	> 10,000	Detected
SCA hard cheese	> 100	> 100	Detected
HPA ready to eat food guidance	> 10,000	Does not apply to raw milk cheese	Detected

Table 10 Results for *E. coli* O157 for cheese tested at Glasgow, Tayside or Aberdeen Scientific Services

Local Authority	Establishment	Batch Information	Sample Information	Date Reported	Result *E. coli* O157 / 25g
Perth & Kinross	Hotel B	Not known	Dunsyre Blue Cheese 500g	04/08/2016	Negative
Glasgow	Restaurant A	Not known	Lanark Blue	01/08/2016	Negative
Glasgow	Restaurant A	Not known	Dunsyre Blue cheese Dressing	01/08/2016	Negative
Glasgow	Restaurant A	Not known	Dunsyre Blue cheese	01/08/2016	Negative
Glasgow	Restaurant A	Not known	Lanark Blue Butter (Dunsyre Blue Cheese used)	01/08/2016	Negative
Glasgow	Restaurant B	Not known	Dunsyre Blue	01/08/2016	Negative
Glasgow	Hotel C	Not known	Dunsyre Blue	01/08/2016	Negative
Fife	Supplier B	Not known	Dunsyre Blue	08/08/2016	Negative
East Ayrshire Council	Supplier A	D14	Dunsyre blue	08/08/2016	Negative
East Ayrshire Council	Supplier A	D14	Dunsyre Blue	08/08/2016	Negative
Dumfries and Galloway	Hotel D	E18 / 15	Dunsyre Blue	03/08/2016	Negative
Dumfries and Galloway	Hotel D	N/A (Portion)	Dunsyre Blue	03/08/2016	Negative
Dumfries and Galloway	Hotel E	E18 / 15	Dunsyre Blue	03/08/2016	Negative
Dumfries and Galloway	Hotel E	N/A (Portion)	Dunsyre Blue	03/08/2016	Negative
Glasgow	Supplier C	F1 (12 samples)	Dunsyre Blue	15/10/2016	Negative
East Dunbartonshire	Outlet A	E10 or E12 (2 samples)	Dunsyre Blue	01/09/2016	Negative
East Renfrewshire	Supplier D	Not Known	Lanark Blue	12/09/2016	Negative
North Lanarkshire	Hotel F	C26	Lanark Blue	19/09/2016	Negative

These samples were also found to be negative for STEC (samples from Glasgow Scientific services tested for STEC at ESS, and samples from Tayside and Aberdeen Scientific Services tested for STEC at Tayside Scientific Services).

Twelve samples of Dunsyre Blue, one of Lanark Blue and two dressings/butters made by a restaurant using Dunsyre Blue were tested in Glasgow, Tayside or Aberdeen Scientific Services. These samples were all negative for *E. coli* O157. As these samples had been taken from hotels/restaurants/suppliers information on batch number was not always available.

14.2 Results for Dunsyre Blue

The initial information available from Supplier A identified batches C22 and D14 as those delivered to the premises where the cases had consumed Dunsyre Blue. No samples of C22 were available for testing. All four samples of D14 (two submitted by Orkney Islands Council and two by East Ayrshire Council) tested were negative for STEC.

Batch F15: A total of ten samples were taken from batch F15 all tested positive for the *stx2* gene by PCR. Two of these samples were cultured and sequenced by WGS and identified as *E. coli* serotype: O unidentifiable:H20, sequence type (ST): 1308 and *stx* subtype stx*2d*.

Batch E24: Detection of the *stx2* gene by PCR in two samples, laboratories unable to isolate an organism for confirmation and typing.

Batch G12: Detection of the *stx*2 gene by PCR in one sample, laboratories unable to isolate an organism for confirmation and typing.

***Enterobacteriaceae* results:** A total of 58 samples of Dunsyre Blue were tested at ESS on the basis that the cheese was presented for sale and ready to eat, 27 (46.6%) of these had *Enterobacteriaceae* higher than the SCA target for soft/semi soft cheese of <10,000 cfu/g, these positive results were obtained across a large number of batches (F7, F8, F9, F15, F21, F22, F27, F28, F29, F30, G5, G6, G11, G12, G13, G19, G21, G25, G26, G28, H3, H4 and H9). The highest value of 18,800,000 cfu/g was identified from a batch of G25, 1880 times greater than the HPA Guidelines and SCA target.

Generic *E. coli* results: The SCA target for generic *E. coli* in soft/semi soft cheese is <10,000 cfu/g. This was exceeded in a sample from batch F28, with a count of 13,600 cfu/g.

14.3 Positive results for ewes' milk cheese samples

Lanark White Batch G14. Sorbitol fermenting shiga toxin negative *E. coli* O157 isolated and identified as *E. coli* O157:H42 ST 7077, *stx* negative. The same result was obtained from a second sample from Batch G14 (same organism as in Batches H3 and H24).

Lanark White Batch H3: Sorbitol fermenting shiga toxin negative *E. coli* O157 isolated and identified as *E. coli* O157:H42 ST 7077, *stx* negative (same organism as in Batches G14 and H24).

Lanark White Batch H24: Sorbitol fermenting shiga toxin negative *E. coli* O157 isolated and identified as *E. coli* O157:H42 ST 7077, *stx* negative (same organism as in Batches G14 and H3).

Lanark Blue Batch E24: Detection of the *stx2* gene by PCR in a non-O157 *E. coli* i.e. an STEC, organism isolated and by WGS identified as *E. coli* O unidentifiable:H20 ST 1308 *stx2d* (same as identified from batch F15 of Dunsyre Blue, these are a ewes' milk and a cows' milk cheese produced three weeks apart).

***Enterobacteriaceae* results:** The *Enterobacteriaceae* result for batch F14 of Lanark Blue of 500,000 cfu/g exceeded the HPA Guidelines and SCA target of <10,000 cfu/g.

Limited sampling during the investigation was undertaken of the other cheese produced by ECL. Samples were taken from one batch of Maisie's Kebbuck; three batches of Corra Linn

and one batch of Sir Lancelot. No *E. coli* O157 or *stx* genes were detected in these five samples.

14.4 Cows' Milk results

A raw cows' milk sample taken on 19 October isolated a *stx1* and *stx2* positive non-O157 *E. coli* i.e. an STEC and was identified by WGS as *E. coli* O150:H2 ST P3233, *stx* subtype *stx1a/ stx2a*

A raw cows' milk sample taken on 29 October isolated a *stx2* positive non-O157 *E. coli* i.e. an STEC, and was identified by WGS as *E. coli* O15:H16 ST 325, *stx* subtype *stx2g.*

14.5 Environmental swab results

The environmental swabs taken at ECL by SLC all tested negative for STEC. These swabs were taken some months after the implicated batches were produced and at a time when cheese production had ceased and after the company would have had an opportunity to conduct a deep clean of the premises.

14.6 Further sampling undertaken after the IMT stood down

SLC undertook additional sampling after the IMT stood down. A range of potentially pathogenic *stx* negative *E. coli* O157 and *stx* positive *E. coli* non O157 were detected in seven batches of Corra Linn cheese (details in Appendix 6).

15 Biological plausibility

The IMT considered Dunsyre Blue cheese a biologically plausible vehicle for an outbreak of *E. coli* O157.

15.1 Previous STEC outbreaks associated with dairy products

The major route of STEC contamination of milk is faecal. STEC excreted in faeces soils the teats of animals, and the milk is subsequently contaminated during the milking process. STEC could also potentially persist if milking equipment and pipelines are not adequately cleaned or are not well designed or maintained[51]. There is some controversial evidence of intra-mammary sources of STEC (pre/sub-clinical mastitis)[51].

STEC contamination of milk has been associated with a number of milk and dairy product outbreaks of STEC. Consumption of contaminated soft and semi-soft cheese has been implicated in outbreaks, especially those made from unpasteurised cows' and goats' milk *E. coli* O157 was linked to the majority of outbreaks, but O110, O103, O26 and O119 have also been implicated [51].

Table 11: STEC outbreaks associated with unpasteurised cheese (2000-2015)

Year	Cheese	Serotype	Number of cases	Country	Reference
2002-03	Gouda	O157	13	Canada	[52]
2004	Goats cheese	O157	3	France	[35]
2005	Raw milk cheese	O26	16	France	[53]
2008-09	Raw milk cheese	O157	16	Canada	[54]
2010	Gouda	O157	41	USA	[36]
2013	Raw milk Gouda	O157	29	Canada	[55]

Based on table of Farrokh *et al*[51]

There have been several documented outbreaks of *E. coli* O157 in Scotland associated with cheese made from unpasteurised milk, with three described between 1994 and 1999[30;56;57].

Whilst there are a few reports of STEC outbreaks associated with pasteurised milk[34;58] and cheese, these were probably due to faulty pasteurisation and/or post processing contamination[51].

There are studies on the prevalence of STEC in cheese made from raw milk and the few studies there are have shown variation in results from 0 to 19.9% of samples[59-64], however direct comparison of the studies isn't possible due to differences in methodologies. For example the US Food & Drug Administration (FDA) study of 1,606 samples[60] utilises an enrichment broth containing antibiotic supplements[65] which may be inhibitory to some STEC or stressed *E. coli* O157.

- USA – STEC was not detected in raw milk hard and semi-hard cheese samples (n=29) at retail[62],
- USA – The FDA did not detect *E. coli* O157:H7 in any of 1,606 samples. STEC was detected in 11 of the 1,606 samples, the FDA considered one of them to be pathogenic, an isolate of *E. coli* O111:H8 in a hard, raw goats' milk cheese[60],

- Scotland - *E. coli* O157 not detected in raw milk cheese tested 1997-1999 purchased from retail premises (n=739) (albeit methods not as sensitive as those currently used)[61],
- England – *E. coli* O157 not detected in samples tested for raw milk cheese (n=545) sampled 2010-2011, not tested for STEC. *Listeria monocytogenes* was detected in 1.8% of samples[59],
- Switzerland – STEC detected in 5.4% of raw milk hard and semi-hard cheese collected from producers (2006-2008) (n=1,422)[63],
- France – STEC detected in 19.9% of raw milk hard cheese samples at retail (n=272)[64].

STEC is not the only pathogen of concern with respect to unpasteurised cheese with outbreaks reported of *Salmonella*[66-68], brucellosis[69], *Streptococcus equi*[70], and staphylococcal food poisoning[71].

15.2 Legislation relating to raw milk/milk products in Scotland

The sale of raw milk and raw cream intended for direct human consumption is currently prohibited in Scotland by virtue of Regulation 32 and Schedule 6 of The Food Hygiene (Scotland) Regulations 2006, in line with Article 10 (8) of Regulation (EC) No. 853/2004 allowing Member States to adopt national rules in this area.

There are currently no restrictions on the sale of raw milk cheeses in Scotland, subject to such products having been produced in accordance with EU food hygiene regulations (Regulation (EC) No. 852/2004 and Regulation (EC) No. 853/2004).

16 Summary of evidence

- The descriptive epidemiology showed 15/24 primary cases (15/21 excluding the childcare cluster) consumed Dunsyre Blue in the 8 days prior to onset of symptoms and, as described in Table 1, other cases may well have done so but that level of information was not available: two cases ate blue cheese purchased from a shop known to sell Dunsyre Blue but were unable to recall the brand, one attended a function at which Dunsyre Blue was served but does not recall eating it. One ate blue cheese but there was no information on the type, one reported eating blue cheese but not Dunsyre Blue, and for one case there was extremely limited exposure information available making it impossible to determine cheese consumption history. The likely route of transmission for the five cases in the childcare cluster as discussed previously was via contamination of the venue environment and secondary spread.

- The consumption of blue cheese among cases was considerably higher (19/21; 90%, primary cases not linked to the childcare cluster reported consumption of blue cheese) than the 3.8% (in the past 7 days) reported in the general population (Table 4).

- Bayesian modelling demonstrated that the high proportion of cases consuming blue cheese is highly unlikely to have occurred by chance, with an odds ratio of 78 (95% CrI 16, 264) based on a prior knowledge of consumption of blue cheese of 3.5%. It would be necessary to assume that about 30-40% of non-cases consumed blue cheese before the lower limit of the 95% credible interval approaches 1.

- The case case analytical study showed a statistically significantly higher proportion of primary cases in this outbreak consumed blue cheese away from home than did cases in previous outbreaks (Table 5). Both the analytical studies looked at exposures to blue cheese and did so independently of the evidence from the descriptive epidemiology, which was to the level of one specific artisan type of blue cheese – Dunsyre Blue. The findings from both analytical studies are consistent with the hypothesis that the outbreak was caused by Dunsyre Blue.

- The time period between consuming Dunsyre Blue and the onset of symptoms in cases was within the incubation period for *E. coli* O157 infection (Figure 3).

- No other biologically plausible food or other exposure was identified that linked such a high proportion of cases.

- The outbreak cases were linked by a unique MLVA profile not previously seen in Scotland and not seen since the last date of onset (8 September) suggesting a common source.

- All outbreak cases were within the same 5 SNP cluster by WGS, the genetic similarity between the clinical isolates is consistent with a single source.

- *E. coli* O157 of phage type 21/28 are not normally seen in imported cases of infection and rarely in other countries, indicating that the causative food vehicle was in all likelihood produced in the UK rather than an imported product.

- Food chain investigations identified a 19 day period during which most of the premises at which cases consumed Dunsyre Blue received deliveries, suggesting contamination of one or a limited number of batches with the outbreak strain.

- Whilst the microbiological sampling of cheese produced by ECL did not identify the outbreak strain, it did identify other *E. coli* strains which were considered by the IMT to have the potential to cause human illness. STEC organisms and *stx* negative *E. coli* O157 were identified in samples of different types of cheese produced by ECL over a number of months – demonstrating the ability of potentially pathogenic strains to enter and survive the cheese production process and be present in the final ready to eat product.

- STEC was isolated from samples of milk taken at the dairy that supplies ECL with cows' milk for the production of Dunsyre Blue (Table 9).

- ECL's controls for *E. coli* O157 related to the hygienic production of raw milk. ECL operated a sampling programme for raw milk which did not include generic *E. coli, E. coli* O157 or STEC. The SCA Assured code of practice recommends that *E. coli* and *E. coli* O157 is included in routine testing programmes for raw milk, with quarterly testing considered appropriate for many specialist cheesemakers.

- The results of microbiological testing of raw milk and cheese samples taken by SLC during investigations indicated that the food safety management system applied by ECL was not effective in preventing contamination with potentially pathogenic *E. coli* and STEC in the final product.

- That most cases occurred in or were exposed in Scotland and were otherwise unrelated suggests a food vehicle mainly distributed within Scotland with more limited distribution to other parts of the UK. This fits with the distribution profile of Dunsyre Blue.

- The only known case with an exposure outside Scotland had consumed Dunsyre Blue at a hotel in England supplied with this product. Most Dunsyre Blue is sold within Scotland, with a smaller amount distributed to the rest of the UK.

- Unpasteurised cheese is a biologically plausible vehicle and has previously been associated with STEC outbreaks (Table 11) and previous studies have demonstrated carriage of *E. coli* O157 PT21/28 among cattle in Scotland.

17 Control Measures

17.1 Recall for batches C22 and D14

The IMT meeting of 28 July considered the epidemiological information obtained from cases and information obtained by EHOs visiting a number of premises where cases had eaten and information on batches of cheese supplied by Supplier A, which at that stage in the investigation identified batches C22 and D14 as the common batches delivered to the premises where the cases had eaten. Additionally the investigation had not identified any other biologically plausible vehicle in common between the cases. It was therefore the unanimous view of the IMT to request a recall of batches C22 and D14 of Dunsyre Blue to mitigate risk to consumers and protect public health.

On 29 July, ECL advised SLC and FSS that they had instigated a voluntary recall of batches C22 and D14 on the evening of 28 July. In line with standard procedure, FSS issued a Product Recall Information Notice (PRIN 47/2016) to provide advice to the public and local authorities about this recall. This was accompanied by media statements from both FSS and HPS (on behalf of the IMT) to ensure the public were aware of the advice not to consume these two batches.

FSS sent a Rapid Alert System for Food and Feed (RASFF) to the authorities in Singapore as the implicated batches may have been sent to businesses in Singapore.

On 4 August Supplier A advised their local authority that they could no longer be confident about information on batch numbers provided to individual premises. The IMT considered this update and whether a wider withdrawal of Dunsyre Blue was required. The IMT concluded that such action was not indicated at that point in time as there was no evidence of new cases with exposure dates after the recall. Furthermore, the batches on sale at the time the cases were exposed would now be past their best before date and unlikely to be in circulation.

17.2 Batch F15 not to be placed on the market

On 25 August the IMT was advised by ESS and SERL of provisional results of sampling of batch F15 of Dunsyre Blue which had been sampled by SLC as part of the ongoing food safety investigation at ECL. This had identified the presence of a *stx*2 gene in the sample (a presumptive positive for STEC). SLC contacted ECL to inform them of this and determined that batch F15 was not on the market and assurances were provided by ECL to SLC that this batch would not be placed on market. Subsequently on the 29 August, a non-O157 strain of STEC which contained the *stx2* gene was confirmed in this sample. Due to the food safety and public health concerns this raised, on 29 August, an additional nine formal samples were taken at ECL from three cheese in the same batch (F15), which all tested positive for the presence of the *stx2* gene. Colonies were isolated from two of the samples and subsequently sent for WGS by PHE later (13 September) identified as serotype: O unidentifiable: H20, sequence type: 1308 and *stx* subtype *stx*2d.

17.3 Recall of Dunsyre Blue batch E24

On 30 August the presence of the *stx2* gene (presumptive positive for STEC) was identified in a sample from a batch of Dunsyre Blue (E24). ECL agreed to withdraw this batch from wholesale. On 4 September FSS and SLC were advised of a further positive for *stx2* in another sample of E24. On 8 September ECL agreed to a voluntary recall of batch E24 of

Dunsyre Blue, which had been placed on the market, following presumptive positive results for STEC in this batch. In line with standard procedure FSS issued a Product Recall Information Notice (PRIN 47/2016 Update 2) to provide advice to the public and local authorities about this recall on 8 September.

17.4 Recall of Lanark White batch G14

The IMT stood down 5 September, with ongoing food safety investigations coordinated by SLC and FSS. On 9 September ESS confirmed the presence of shiga toxin negative *E. coli* O157 from a batch of Lanark White G14, a ewes' milk cheese. SERL confirmed that *stx* negative *E. coli* O157 organisms had previously been found to cause cases of severe illness in humans. The Food Examiner at ESS declared this product "*Unsatisfactory: Potentially injurious to health and / or unfit for human consumption"* ECL declined to undertake a voluntary recall of Lanark White G14. Therefore FSS initiated a withdrawal of batch G14 of Lanark White under the terms of Article 14 (8) of Regulation (EC) No 178/2002. The Food Alert for Action (FAFA) for Lanark White batch G14 was issued by FSS on 10 September.

17.5 Recall of all batches of cheese produced by ECL

On the evening of 14 September, HPS re-convened a meeting of core members of the IMT (HPS, FSS, SLC, and SERL) to consider recent developments including the confirmation that afternoon by SERL of two new cases with the outbreak MLVA profile, one of whom was known to have consumed Dunsyre Blue prior to onset. At this stage cheese exposure details were not available for the second new case. At that meeting it was also discussed that a sample taken from G12 batch of Dunsyre Blue had tested presumptive positive for STEC (*stx2* positive) (this batch had not been placed on the market), and that another sample from a further batch of Lanark White, batch H24, had tested presumptive positive for STEC. The group considered:

- The occurrence of two additional cases, one of which was known to have consumed Dunsyre Blue nearly a month after the recall of batches C22 and D14.
- That there was no specific evidence that one of the initial cases with an onset date in July (case was identified after the initial recall) had eaten either of the two batches implicated at the start of the outbreak (C22 and D14).
- Positive results were now being obtained for non-O157 STEC and shiga toxin negative *E. coli* O157 from a number of batches of not only Dunsyre Blue but also Lanark White, with the potential to cause illness.
- The ongoing concerns about the HACCP in place at ECL when these products would have been produced meant there was no assurance as to the safety of these products.

All attendees at the meeting agreed that action was required to mitigate any further risk to the public through the recall of all batches of cheese produced by ECL, whether this was done voluntarily by the company or by a FAFA.

SLC contacted ECL on the evening of 14 September advising them of the latest developments and asking the company to voluntarily recall all batches of their cheese on a precautionary basis. The company did not respond in the requested time frame set by FSS and consequently FSS issued a Food Alert for Action (FAFA) covering all known cheeses made by ECL on the evening of 14 September. It was issued in terms of article 14(8) of Regulation (EC) No 178/2002, using FSS's powers contained in Regulation 3(2) of the

General Food Regulations 2004, and the local authorities were requested to contact food businesses and take steps to ensure the cheese was withdrawn using the provisions contained in Regulation 6(b) of the 2004 Regulations, if necessary using their seizure powers under Section 9 of the Food Safety Act 1990 and Regulations 23 and 27 of the Food Hygiene (Scotland) Regulations 2006.

All local authorities were advised of the FAFA on the evening of 14 September and a supporting statement was placed on the FSS website the same night.

On 15 September FSS issued an update to the FAFA of 14 September as FSS became aware of further ECL cheese, Sir Lancelot cheese, on the market.

The full IMT was re-convened on the morning of 15 September.

On 9 November FSS issued a further update to the FAFA, this clarified the wording on the FAFA to remind local authorities that they were requested to identify food businesses which are likely or known to stock products subject to this FAFA and to take steps to ensure they are withdrawn from sale. Local authorities should ensure that this withdrawal is effective and that the products to which it applies are not placed on the market, if necessary using local powers available to them under the Food Safety Act 1990, the General Food Regulations 2004, and the Food Hygiene (Scotland) Regulations 2006.

Subsequent to the recall of all batches on 14 September, additional sampling results became available from both cheese and milk samples (see food microbiology results section and Appendix 6). The identification of multiple strains of *stx* positive *E. coli* and *stx* negative *E. coli* O157 in cheese produced by ECL demonstrated that adequate control measures were not in place to prevent STEC being present in the final ready to eat product and supported the decision taken to recall all batches to protect public health.

17.6 Improvements made by the cheese manufacturer (ECL)

ECL had not been following the testing regimes recommended in the SCA Assured Code of Practice for verifying the effectiveness of their food safety management system. They had not been routinely sampling their raw milk or cheese for *E. coli* O157 (or other STEC). Although Regulation (EC) No. 2073/2005 (as amended) has no specific criteria for *E. coli* in cheese made from raw milk it is recommended in the SCA Assured Code of Practice that the raw milk supply and cheese be routinely tested for indicator *E. coli* and investigation undertaken if a change in trend is detected. It is also recommended that a risk assessment is performed to assess the need for periodic monitoring for *E. coli* O157. ECL did not commence testing for *E. coli* O157 until they were advised in July of the epidemiological link to one of their cheeses.

ECL carried out a review of their food safety management arrangements in light of the outbreak and the Enforcement Letter issued by FSS on 7 October 2016. SLC advised ECL that their revised food safety management arrangements were satisfactory in January 2017. ECL proposes to validate their raw milk hygiene controls by testing every batch of raw milk for *E. coli* O157.

The revised food safety management arrangements, including ECL's sampling plan, meets the requirements of the SCA Assured Code of Practice.

18 Communications

18.1 Professionals

A HPS Health Protection Alert was issued on 22 July to NHS Board Health Protection Teams, Microbiologists, Scottish Government and Food Standards Scotland for onward cascade to local authority Lead Food Officers, advising of the outbreak and ongoing investigation. An updated Health Protection Alert was issued on 29 July. Throughout the investigation updates were shared with professional colleagues.

HPS liaised throughout the investigation with colleagues in PHE to support the identification and investigation of cases resident outside Scotland.

When a case resident in ROI was identified, HPS liaised with the Health Protection Surveillance Centre, Dublin.

FSS liaised with colleagues in FSA and the food safety authorities in other countries as appropriate through the Rapid Alert System for Food and Feed (RASFF).

18.2 Errington Cheese Limited

Communication with ECL was mainly by SLC as the competent authority.

18.3 Public

Media communication was led by HPS on behalf of the IMT. During the investigation HPS issued six proactive press statements. Between 22 July and 31 October HPS also coordinated the response to a total of 79 media enquires.

Additional media enquires in relation to food safety and the withdrawal of cheese were received and responded to by FSS. During the investigation between 22 July and 31 October FSS issued 9 press statements and responded to 45 media enquires.

Product Recall Information Notices and Food Alerts for Action were posted on the FSS website: www.foodstandards.gov.scot

19 Discussion

The descriptive and analytical epidemiological and food chain evidence provided strong evidence of Dunsyre Blue cheese being the causative vehicle for the outbreak. This conclusion was strongly supported by the microbiological findings and deficiencies in the HACCP processes.

The fact that not all primary cases could be directly linked to Dunsyre Blue was not unexpected and similar to other food related outbreaks[26;72]. In addition, a number of cases which could not be directly linked to Dunsyre Blue, did consume blue cheese of an unspecified brand and one case provided only very limited exposure information. There are a number of reasons why not all of the primary cases could be directly linked back to Dunsyre Blue cheese including:

- Cases may have been poor historians and be unable to recall all foods consumed especially as some interviews were conducted a number of weeks after they would have been exposed.
- Some people purchased blue cheese from food outlets without knowing exactly which brand they were buying.
- There could well have been cross contamination from Dunsyre Blue to another food consumed by a case.
- Cases defined as primary could in fact have been secondary cases from an unidentified primary case who was exposed to Dunsyre Blue or another secondary case.
- Pieces of blue cheese may be in dishes such as salads without the knowledge of consumer.

Two cases were classified as secondary cases, this is in keeping with the documented occurrence of person to person transmission of STEC [4], and is consistent with other foodborne outbreak investigations[26;73]. As discussed previously, the IMT considered the cluster in the childcare setting to be due to the introduction of contamination into the venue environment possibly by an unidentified symptomatic or asymptomatic individual.

The outbreak was likely due to the contamination of one or a small number of batches of Dunsyre Blue. Each batch of cheese is approximately 160- 200kg in size, meaning that the number of exposed individuals was greater than the number of cases identified in the outbreak, this is not unexpected. The number of cases reported in this outbreak is in keeping with that reported in other cheese related outbreaks (Table 11). Whilst *E. coli* O157 is known to have a low infectious dose[2;3], clinical presentation varies from asymptomatic to fatal infection, therefore not all those infected will have developed symptoms or developed symptoms severe enough to seek medical attention and subsequent laboratory identification. It follows that the 26 cases identified during the outbreak investigation is likely to be an underestimate of the true number of cases. Indeed HPS was advised of a case of *E. coli* O157 confirmed on serology and therefore without an isolate for typing (and thus unable to be confirmed as part of the outbreak) who had a history of consuming Dunsyre Blue at the same hotel as one of the confirmed cases during the same time frame that the majority of cases were exposed.

Contamination may not be evenly distributed throughout a batch of cheese and therefore not everyone eating from a contaminated batch would be exposed. During the acidification and

coagulation processes of cheese making, bacteria are trapped within the curds in a relatively uniform but stochastic distribution, thereby creating microscopic environmental niches that fluctuate during the ripening process[74]. Studies on Stilton cheese have shown blue cheese to be complex food matrices and show distinct microenvironments particularly between the white core – a part with limited presence of air and the blue veins[75]. Furthermore, the mould has been shown to affect the pH in different regions of cheese, with experiments showing a differential spatial distribution of bacterial flora within the matrix[76].

During the investigation, a number of cheeses were microbiologically tested for the presence of STEC. The outbreak strain was not detected in any of the cheese tested. This was not unexpected as the samples from hotels/restaurants where cases consumed the cheese were usually taken more than a month after cheese was eaten by cases, so the block of cheese the case consumed from was no longer available for testing. Information provided early in the investigation by Supplier A suggested that two particular batches of Dunsyre Blue C22 and D14 were supplied within the outbreak timeframe to the premises where cases ate (the supplier later withdrew batch level information saying they could not ascertain which batches were supplied to particular customers). No cheese from batch C22 was available for testing as it had all been consumed. The four samples from batch D14 tested negative, but this would not necessarily have been the blocks of cheese consumed by the cases. Failure to isolate the outbreak strain from the suspected cheese is not unique to this outbreak, with similar findings in other food related outbreaks[77] including those associated with cheese[54].

Whilst testing of cheese did not isolate the outbreak strain, STEC organisms and *stx* negative *E. coli* O157 were detected in cheese produced by ECL, demonstrating that pathogens could enter and survive the cheese production process. It is important to note that food safety requirements defined in EU legislation are not predicated on the explicit need to definitively identify the pathogenicity associated with an organism that is detected in a food stuff. Nonetheless, testing undertaken on cheese during the investigations into this outbreak did detect the presence of organisms which had been associated with human illness.

The strains isolated included *E. coli* O unidentifiable[iv]:H20 ST 1308 *stx*2d (F15 of Dunsyre Blue and E24 Lanark Blue); *E. coli* O15:H16 ST 325 *stx2g* and *E. coli* O150:H2 ST3233 *stx1a stx2a* (samples of raw milk) and shiga toxin negative sorbitol fermenting *E. coli* O157:H42 ST7077 (G14, H3, H24 Lanark White). The IMT had to assess the risk to public health and the implementation of appropriate control measures based on the initial microbiological findings, with more detailed analysis of the isolates becoming available later via the WGS results. The IMT concluded that the detection of these organisms represented a risk to public health, on the basis that they were from a faecal source that demonstrated an STEC hazard to the production of these cheese, and also that the strains identified possessed traits that had previously been associated with human illness and therefore took action to mitigate the risk.

Most notably, the identification of *E. coli* O unidentifiable:H20 ST 1308 *stx2d* in Dunsyre Blue (and Lanark Blue) demonstrates that the production process for this cheese was not

[iv] Serotype designation “O unidentifiable” means the WGS does not recognise the O antigen of that strain and this is most likely a novel, as yet undesignated *E.coli* serotype.

effective in eliminating STEC presence in the final product. It is also worth highlighting the presence of *stx2d* in these samples as there is evidence that such strains have been linked to human illness in the absence of attaching and effacing genes (e.g. *eae*)[78].

The IMT also considered the detection of *E. coli* O157 in certain batches of Lanark White presented a potential food safety risk. Whilst this strain was identified as *stx* negative, there is no single or combination of genetic marker(s) that defines the potential of an *E. coli* strain to cause human disease[79], and therefore the absence of *stx* genes does not necessarily guarantee than an *E. coli* O157 strain would not be capable of causing human illness. Whilst the possession and expression of the *stx2* gene correlates strongly with the causation of bloody diarrhoea and HUS[5], *stx*-negative *E. coli* O157 strains have been found in clinical cases in Scotland[8] and elsewhere[80], although they appear to cause less severe disease than do *stx* positive strains. Further to this, *stx* and *eae* negative *E. coli* O157:H42 strains (similar to those isolated from batches G14, H3 and H24 of Lanark White (Table 9)) have been identified in cows indicating that there is potential for such strains to be shed in the faeces of dairy cattle[81] and could therefore contaminate raw milk if controls were deficient.

The fact that multiple strains of STEC could be isolated from cheeses produced using cows' milk from a single supplier is not surprising as multiple serotypes can be isolated at a single point in time from a farm[82]. Indeed there is carriage of multiple STEC strains in individual cattle[83;84]. It is concerning if any of these strains get into milk intended for human consumption, especially if that milk is not going to be pasteurised or the cheese making process does not apply controls which are capable of eliminating or reducing STEC to acceptable levels in the final product (the acceptable level for STEC in a ready to eat food would be absence, due to the low infective dose). The identification of multiple strains in cheeses produced by ECL demonstrated that adequate control measures were not in place to prevent STEC being present in the final ready to eat product. It is also of note, that of the 58 samples of Dunsyre Blue tested by ESS, 27 (46.6%) had *Enterobacteriaceae* counts higher than the SCA and HPA guidelines (Table 9).

During the investigation, the IMT discussed whether sampling of cattle at the dairy farm supplying cows' milk to ECL should be undertaken in order to attempt to identify the outbreak strain. After careful consideration and consultation with veterinary experts, such testing was not recommended. The rationale for this decision was that testing conducted in August/September would be approximately four months after the cheese implicated in the outbreak was produced and therefore such samples could not be considered to reliably reflect the situation in the herd during the spring of 2016. Positivity in herds fluctuates during the year[11] and not all cattle within a positive herd carry STEC at any one time so even contemporaneous testing would have to be on impractically large numbers of animals. In addition, as healthy cattle shed STEC, food management procedures should be based on the assumption that STEC is present in the herd and appropriate measures put in place to deal with this risk, as farmers are unable to take any action that would guarantee STEC negative herds. Some testing of the cows' milk was undertaken, yielding two different STEC positive results, O15:H16 and O150:H2, demonstrating the ability of pathogens from the cattle to get into the milk subsequently to be used in cheese production. It is of note that the *E. coli* O15:H16 was found by WGS to contain a mixture of virulence genes including *stx*2 normally present in STEC and heat-stable (ST) enterotoxin genes typically present in enterotoxigenic *E. coli* (ETEC). *E. coli* with mixed STEC/ETEC virulence genes are referred

to as hybrids. STEC/ETEC hybrid strains have been isolated from human clinical samples and they may represent an emerging threat as a foodborne pathogen[85].

The microbial quality of the raw milk for unpasteurised products is critical. As it is not possible to eradicate STEC from cattle faeces, the risk of contamination of the raw milk supply needs to be controlled through the application of strict hygiene measures during the milking process, alongside regular monitoring to verify that the controls are effective. ECL were relying on dairy hygiene controls to prevent contamination of raw milk but were not testing any samples of the raw milk for generic *E. coli* or *E. coli* O157 (or STEC) to verify that these controls were effective. During investigations, samples of raw milk taken by SLC in October identified STEC (Table 9), demonstrating that ECL's system was not effective in managing and monitoring the microbiological safety of their cow's milk supply.

This outbreak has highlighted the potential of unpasteurised dairy products to pose a risk to public health if adequate control mechanisms are not in place at all stages throughout the production process. Appropriate validation and verification of controls during the production and maturation of cheese is also important in ensuring the safety of the end product. At the initial stage of cheese making, the temperature (30°C) and a_w of milk are ideal for the growth of STEC, with some research suggesting potential for growth during the initial stages of manufacturing[51]. An apparent increase at this stage may also arise from concentration of the bacteria in the curds after drainage of the whey[51]. Acidity is a key factor in ensuring the microbiological safety of cheeses, with the survival and growth of pathogenic bacteria including STEC being particularly influenced by the rate of acidification, however some strains of STEC are acid-resistant[86]. During the ripening and storage of the cheese the behaviour of STEC can also be dependent upon the physiochemical properties of products including temperature, a_w, salt concentration and pH, but STEC can survive the maturation process [87-92]. Additional information on the ability of STEC to survive in unpasteurised cheese is contained within the FSS risk assessment[93]. Managing STEC risks in cheese production is therefore reliant on effective controls, at specific control points in the process, to ensure the safety of the end product. The use of these parameters as food safety control points requires on-going monitoring during production and maturation to verify that they are operating effectively throughout the process. There was insufficient evidence that these parameters had been validated for the production of ECL cheeses or that they were being monitored to verify the effectiveness of their controls in eliminating or reducing STEC throughout production.

In addition to a_w, salt concentration and pH, the testing of end product for the presence of microbiological indicators and pathogens can provide additional verification that controls are operating effectively. However, the use of end product testing cannot, in isolation, guarantee safety due to the uneven distribution of pathogens within products and variability in detection. At the time of the outbreak, ECL's testing regime for cheeses covered a range of relevant microbiological criteria, including generic *E. coli* but did not include tests for *E. coli* O157 or STEC, which is recommended by the SCA Assured code of practice.

STEC testing was only commenced by the company following the outbreak as a means of demonstrating the safety of individual batches of cheese.

20 Conclusion

Extensive investigations concluded that the source of the outbreak was the consumption of an unpasteurised cheese – Dunsyre Blue. This conclusion was based on evidence from epidemiological and food chain investigations and supported by microbiological evidence and deficiencies identified at ECL in the procedures in place for the monitoring and control of STEC. Control of STEC was reliant on receiving pathogen free milk but no processes were in place to validate or monitor this. The investigation isolated potentially pathogenic *E. coli* from two different samples of raw milk taken from the dairy supplying cows' milk to ECL.

The investigation did not isolate the outbreak strain from any of the cheese tested. This was not unexpected as the samples from hotels/restaurants where cases consumed the cheese were usually taken more than a month after the cheese was eaten by cases, so the block of cheese the case consumed from was no longer available for testing. However other potentially pathogenic STEC and *stx* negative *E. coli* O157 were isolated from a number of varieties of cheese produced by ECL demonstrating that pathogenic organisms did enter and survive the cheese production process and were present in the final ready to eat product.

Throughout the investigation the paramount aim of the IMT was the protection of public health. To this end, products considered to pose a risk to the public were withdrawn from the market and the risks communicated to the public and professionals.

The outbreak highlighted a number of issues, as described in the recommendations, which are wider than this specific incident and will be progressed by the relevant agencies.

21 Legal aspects - Procurator Fiscal investigation

In line with national guidance, the death of the three year old child was reported by the treating clinicians to the Procurator Fiscal.

22 Recommendations

A debrief meeting was held in November 2016 with representation from HPS, FSS, FSA, NHS Boards, Local Authorities, SERL and Scientific Service Laboratories. Participants were asked to consider what went well, areas that could be improved and to make recommendations for improvement as appropriate. The following key learning points were identified and related recommendations made:

Learning Point:
There was excellent inter-agency cooperation and participation in the IMT meetings, which was sustained over the duration of the investigation. However this was a fast moving investigation with information being updated on a regular basis and at times communication of the accurate and up-to-date information between all the agencies was challenging.

Recommendation:
FSS is procuring during 2017/18 an incident management software, which can be accessed from anywhere in real time by all members of the IMT. This will provide more efficient coordination of activities/actions and records of decision making.

That in addition to a minute taker, at each of the IMT meetings there is a decision logger, to support the fast turnaround of action notes and minutes.

Learning Point
The trawling questionnaires and subsequent investigations of foods supplied to the premises that cases ate at by EHOs and FSS allowed the rapid identification of the source of the outbreak. However the trawling questionnaire was found to be long and in places repetitive and time consuming to administer.

Recommendation
HPS to progress work reviewing and refining the STEC trawling questionnaire by the summer of 2017.

Learning Point
The establishment of the sub-group of the IMT chaired by FSS to progress the detailed and technical discussions around assurances of processes at the food business worked well. The sub group provided a focused environment for those discussions and reduced the potential length of time of the main IMT meetings. However there needs to be explicit understanding of the Terms of Reference of the sub-group and how this also relates to the statutory obligations of representative agencies.

Recommendation
Under a separate work stream, a group is being established under the Scottish Health Protection Network co-chaired by HPS and FSS to review current guidance on the investigation and control of outbreaks of foodborne disease in Scotland. This group will be asked to consider the potential inclusion of specialised sub-groups of an IMT and consider draft terms of reference.

Learning point
Approximately 30% of STEC from humans identified in Scotland are confirmed by SERL as non-O157 STEC. Scientific Service Public Analyst laboratories in Scotland relying on standard culture methods for the detection of STEC would not have detected these organisms. The adoption of PCR testing for *E. coli* O157 and STEC in food and other environmental samples by Scottish Scientific Service Public Analyst laboratories would provide an effective way to detect shiga toxin genes and aid in the subsequent isolation of the STEC.

The pre-incubation of food samples needs to be considered as the standard broth of mTSB is perhaps unsuitable for some matrices, and non O157 STEC enrichment with BPW may be more appropriate.

Recommendation
The capability and capacity of the Scientific Services Public Analyst laboratories to detect and isolate O157 and non O157 STEC should be reviewed in conjunction with FSS and SERL. The VTEC Action Plan for Scotland[94] includes a recommendation to review the laboratory provision for STEC testing of food in Scotland and a mapping exercise is being undertaken to identify current availability in public and commercial scientific services. This work will be considered as part of the wider review of Scottish Public Analysts in conjunction with strategies to implement WGS in Scotland (see below).

Learning point
Currently in Scotland isolates of *E. coli* O157 are typed using MLVA and non-O157 STEC isolates are sent to GBRU for WGS to determine serogroup. Additionally the comparison of strains across the UK requires the exchange of isolates between SERL and GBRU which can delay the linkage of potential cases in outbreaks.

Recommendation
WGS should be implemented in Scotland by SERL at the earliest opportunity and include provision for the sequencing of clinical and food isolates. SERL has recently completed a successful pilot of WGS and is working towards the implementation of WGS for the routine typing of all STEC isolates during the summer of 2017. The requirement for WGS capacity in Scotland is recognised as not being restricted to STEC organisms and is being progressed through wider National Services Scotland led Reference Laboratory work. It is critical that this work takes full account of official food, water and environmental sampling activities undertaken by the Scottish public analyst network to ensure there is adequate capacity for outbreak investigations and on-going research to improve understanding of the attribution of STEC infection in Scotland.

Learning
The food chain investigation work was hampered by the lack of information held by distributors of the batches of cheese provided to different premises.

Recommendation
FSS and the Scottish Food Enforcement Liaison Committee should consider current requirements for food businesses to identify and record this information and develop best practice guidance during 2017/18.

Learning point
A number of cases were unaware of the type of blue cheese they had eaten in hotels/restaurants when it was served as part of another dish or was on a cheese board and were also unaware that the product was unpasteurised. Current guidance recommends that some unpasteurised cheese products should be avoided by certain high risk individuals including the elderly and pregnant women, to reduce the risk of listeriosis. Although there is a legal requirement for manufacturers to label the products as unpasteurised, there is no legal requirement for labelling at the point of consumption by the consumer (e.g. on a restaurant menu) or at the point of sale to the public (e.g. retail outlets). This restricts the consumer from being able to make an informed choice regarding the consumption of unpasteurised cheese.

The need for adequate labelling of raw cheese at point of consumption was also identified in the VTEC Action Plan for Scotland[94] (recommendation 10.1) and a survey of Local Authorities was undertaken at that time, demonstrating a very mixed picture of labelling at the point of consumption/sale.

Recommendation
During 2017, FSS and the Scottish Food Enforcement Liaison Committee should develop best practice guidance to ensure unpasteurised cheeses are clearly labelled to support consumers in making an informed choice.

Learning point
The SCA Assured Code of Practice does not provide sufficient guidance regarding the validation and verification of food safety management controls for STEC. This incident has also identified that further guidance is required on appropriate testing regimes for STEC (including non-O157 serogroups) in ready to eat foods such as cheese.

Recommendation
During 2017 FSS and the Scottish Food Enforcement Liaison Committee (SFELC) will work with local authorities and the Specialist Cheesemakers Association to strengthen existing guidance and promote an understanding across the sector (particularly small producers) of potential risks associated with STEC and control measures and testing regimes required to manage the risk.

FSS and SFELC have met with the Specialist Cheesemakers Association twice during the first quarter of 2017 to scope out this work.

The Specialist Cheesemakers Association are arranging courses for enforcement authorities in Scotland on the cheese making process; this will support ongoing collaboration and joint understanding of the risks and how they are controlled.

Appendix 1: Abbreviations

a_w	Water activity
CCP	Critical control point
CPHM	Consultant in Health Protection
ECL	Errington Cheese Limited
EHO	Environmental Health Officer
ESS	Edinburgh Council Scientific Services
FBO	Food Business Operator
FSA	Food Standards Agency
FSS	Food Standards Scotland
GBRU	Gastrointestinal Bacteria Reference Unit
HACCP	Hazard Analysis and Critical Control Point
HPS	Health Protection Scotland
HPT	Health Protection Team
MLVA	Multi Locus Variable-number Tandem Repeat Analysis
IID	Infectious Intestinal Disease
IMT	Incident Management Team
ISO	International Organisation for Standardisation
NDNS	National Diet and Nutrition Survey
OCL	Official Control Laboratory
oPRPs	Operational pre-requisite programmes
PAG	Problem Assessment Group
PCR	Polymerase chain reaction
PHE	Public Health England
SALSA	Safe and Local Supplier Approval
SCA	Specialist Cheesemakers Association
SCVPH	Scientific Committee on Veterinary Measures relating to Public Health
SERL	Scottish *E. coli* O157/VTEC Reference Laboratory
SLC	South Lanarkshire Council
SNP	Single-nucleotide polymorphism
STEC	Shiga toxin producing *Escherichia coli*
stx	Shiga toxin gene
UKAS	United Kingdom Accreditation Service
VTEC	Verocytotoxin producing *Escherichia coli*
WGS	Whole Genome Sequencing

Appendix 2: Timeline of outbreak investigation

Date	Event
21 July 2016	HPS notified by SERL of eight confirmed cases of *E. coli* O157 PT21/28 with the same MLVA profile and four cases of PT21/28 for which the MLVA was awaited
22 July 2016	PAG held
22 July 2016	Four cases reported on 21 July for which MLVA awaited confirmed by SERL with outbreak profile.
22 July 2016	HPS Alert about outbreak issued to NHS Boards, Local Authorities, microbiologists and Scottish Government.
26 July 2016	IMT held
26 July 2016	FSS contacted SLC to advise that cheese produced by ECL had a possible link to an outbreak of *E. coli* O157
28 July 2016	IMT held
29 July 2016	IMT held
29 July 2016	ECL advised SLC and FSS that they had instigated a voluntary recall of batches C22 and D14 on the evening of 28 July
29 July 2016	FSS issue product recall notice for batches C22 and D14 of Dunsyre Blue
29 July 2016	HPS media statement issued on behalf of the IMT
29 July 2016	Updated HPS Alert to NHS Boards, Local Authorities, microbiologists, NHS24 and Scottish Government
01 August 2016	IMT held
04 August 2016	IMT held
09 August 2016	FSS chaired sub-group
11 August 2016	IMT held
17 August 2016	FSS chaired sub-group
18 August 2016	IMT held
23 August 2016	FSS chaired sub-group
25 August 2016	IMT held
25 August 2016	IMT advised of provisional positive result for batch F15 of Dunsyre Blue
25 August 2016	Ad hoc IMT core members only
26 August 2016	Ad hoc IMT core members only
30 August 2016	IMT held
30 August 2016	Presumptive positive *stx2* reported for batch E24 of Dunsyre Blue
30 August 2016	FSS chaired sub-group
31 August 2016	FSS chaired sub-group
05 September 2016	IMT held and IMT stood down
05 September 2016	HPS issue media statement on behalf of the IMT including that the IMT had stood down.
08 September 2016	FSS issued product recall information notice for batch E24 of Dunsyre Blue
10 September 2016	FSS issue Food Alert for Action for batch G14 of Lanark White cheese
14 September 2016	Two additional cases confirmed with the outbreak MLVA profile.
14 September 2016	Presumptive positive STEC from batch G12 of Dunsyre Blue and batch H24 of Lanark White
14 September 2016	Ad hoc IMT core members
14 September 2016	FSS issue Food Alert for Action for all batches of Lanark Blue, Lanark White, Dunsyre Blue, Dunsyre Baby, Maisie's Kebbuck and Cora Linn
15 September 2016	IMT held and IMT reconvened

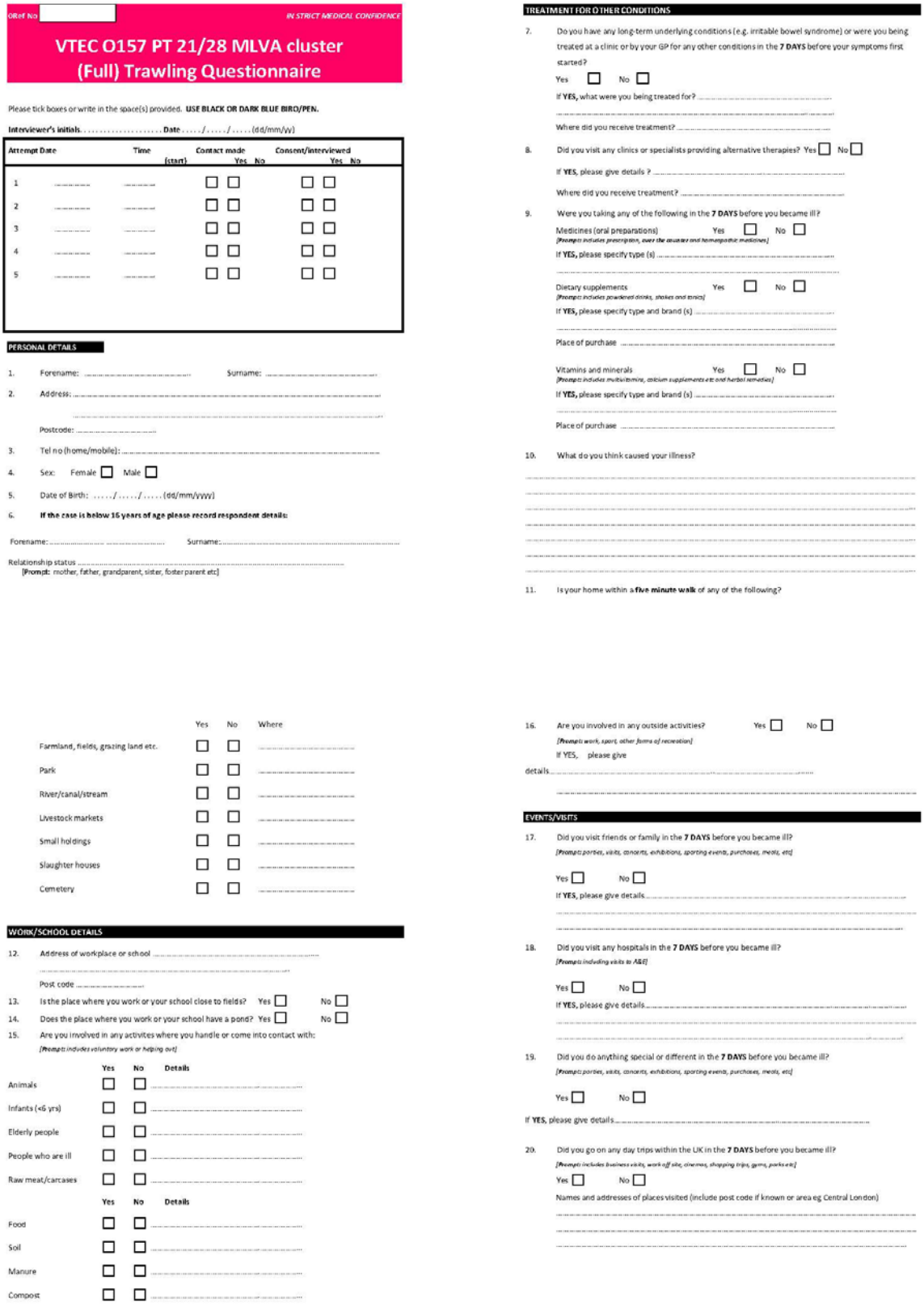
15 September 2016	FSS issue updated Food Alert for Action to also include Sir Lancelot cheese
15 September 2016	HPS issue media release on behalf of the IMT
16 September 2016	FSS chaired sub-group
21 September 2016	IMT held
21 September 2016	FSS chaired sub-group
28 September 2016	IMT held
12 October 2016	FSS chaired sub-group
12 October 2016	IMT held and IMT stood down
28 November 2016	Debrief and lessons learnt meeting held

Appendix 3: Membership of Incident Management Team

Organisation

Health Protection Scotland, National Services Scotland
Food Standards Scotland
South Lanarkshire Council
SERL
Edinburgh Scientific Services
Glasgow Scientific Services
Public Health England
Food Standards Agency
NHS Dumfries & Galloway
NHS Fife
NHS Grampian
NHS Greater Glasgow & Clyde
NHS Lanarkshire
NHS Lothian
NHS Tayside
Aberdeen City Council
Aberdeenshire Council
Angus Council
Dumfries & Galloway Council
Dundee City Council
East Ayrshire Council
Edinburgh City Council
Fife Council
Glasgow City Council
North Ayrshire Council
Perth & Kinross Council
Scottish Government (Observer)

Appendix 4: Trawling Questionnaire



ORef No

IN STRICT MEDICAL CONFIDENCE

VTEC O157 PT 21/28 MLVA cluster (Full) Trawling Questionnaire

Please tick boxes or write in the space(s) provided. **USE BLACK OR DARK BLUE BIRO/PEN.**

Interviewer's initials..... Date...../...../.....(dd/mm/yy)

Attempt	Date	Time (start)	Contact made Yes	Contact made No	Consent/interviewed Yes	Consent/interviewed No
1			☐	☐	☐	☐
2			☐	☐	☐	☐
3			☐	☐	☐	☐
4			☐	☐	☐	☐
5			☐	☐	☐	☐

PERSONAL DETAILS

1. Forename: ..... Surname: .....
2. Address: .....
   Postcode: .....
3. Tel no (home/mobile): .....
4. Sex: Female ☐ Male ☐
5. Date of Birth: ...../...../.....(dd/mm/yyyy)
6. **If the case is below 16 years of age please record respondent details:**

Forename: ..... Surname: .....

Relationship status .....
[Prompt: mother, father, grandparent, sister, foster parent etc]

TREATMENT FOR OTHER CONDITIONS

7. Do you have any long-term underlying conditions (e.g. irritable bowel syndrome) or were you being treated at a clinic or by your GP for any other conditions in the **7 DAYS** before your symptoms first started?
   Yes ☐ No ☐
   If **YES,** what were you being treated for? .....
   Where did you receive treatment? .....
8. Did you visit any clinics or specialists providing alternative therapies? Yes ☐ No ☐
   If **YES,** please give details ? .....
   Where did you receive treatment? .....
9. Were you taking any of the following in the **7 DAYS** before you became ill?
   Medicines (oral preparations) Yes ☐ No ☐
   [Prompt: includes prescription, over the counter and homeopathic medicines]
   If **YES,** please specify type (s) .....
   Dietary supplements Yes ☐ No ☐
   [Prompt: includes powdered drinks, shakes and tonics]
   If **YES,** please specify type and brand (s) .....
   Place of purchase .....
   Vitamins and minerals Yes ☐ No ☐
   [Prompt: includes multivitamin, calcium supplements etc and herbal remedies]
   If **YES,** please specify type and brand (s) .....
   Place of purchase .....
10. What do you think caused your illness?
    .....
11. Is your home within a **five minute walk** of any of the following?

	Yes	No	Where
Farmland, fields, grazing land etc.	☐	☐	.....
Park	☐	☐	.....
River/canal/stream	☐	☐	.....
Livestock markets	☐	☐	.....
Small holdings	☐	☐	.....
Slaughter houses	☐	☐	.....
Cemetery	☐	☐	.....

WORK/SCHOOL DETAILS

12. Address of workplace or school .....
    Post code .....
13. Is the place where you work or your school close to fields? Yes ☐ No ☐
14. Does the place where you work or your school have a pond? Yes ☐ No ☐
15. Are you involved in any activites where you handle or come into contact with:
    [Prompt: includes voluntary work or helping out]

	Yes	No	Details
Animals	☐	☐	.....
Infants (<6 yrs)	☐	☐	.....
Elderly people	☐	☐	.....
People who are ill	☐	☐	.....
Raw meat/carcases	☐	☐	.....

	Yes	No	Details
Food	☐	☐	.....
Soil	☐	☐	.....
Manure	☐	☐	.....
Compost	☐	☐	.....

16. Are you involved in any outside activities? Yes ☐ No ☐
    [Prompt: work, sport, other forms of recreation]
    If YES, please give details.....

EVENTS/VISITS

17. Did you visit friends or family in the **7 DAYS** before you became ill?
    [Prompt: parties, visits, concerts, exhibitions, sporting events, purchases, meals, etc]
    Yes ☐ No ☐
    If **YES,** please give details.....
18. Did you visit any hospitals in the **7 DAYS** before you became ill?
    [Prompt: including visits to A&E]
    Yes ☐ No ☐
    If **YES,** please give details.....
19. Did you do anything special or different in the **7 DAYS** before you became ill?
    [Prompt: parties, visits, concerts, exhibitions, sporting events, purchases, meals, etc]
    Yes ☐ No ☐
    If **YES,** please give details.....
20. Did you go on any day trips within the UK in the **7 DAYS** before you became ill?
    [Prompt: includes business visits, work off site, cinemas, shopping trips, gyms, parks etc]
    Yes ☐ No ☐
    Names and addresses of places visited (include post code if known or area eg Central London)
    .....

21. Did you travel outside the UK in the **7 DAYS** before you became ill?
[Prompts: includes business visits]

Yes ☐ No ☐

What country, and Resort / town did you visit, and what were your travel dates

.....

.....

Date left UK ..... Date returned UK .....

RECREATIONAL

22. Do you have any hobbies or pastimes?
[Prompt: gardening, swimming, hiking, football, stamp collecting etc]

Yes ☐ No ☐

If **YES,** please specify

.....

.....

23. Did you handle any potted plants in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If yes give details [type of plant and where bought if known]

.....

.....

24. Did you handle any cut flowers in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If yes give details [type of flowers and where bought if known]

.....

.....

25. In the **7 DAYS** before you became ill did you carry or handle any manure or compost?

Yes ☐ No ☐

If **YES,** please specify

.....

.....

7. Did you participate in or attend any of the following social/recreational activities in the **7 DAYS** before you became ill?
[Prompt: parties, visits, concerts, exhibitions, sporting events, purchases, meals, etc]

	Yes	No	Details
Parties	☐	☐	.....
Concerts/Theatre	☐	☐	.....
Cinema	☐	☐	.....
Leisure Centre	☐	☐	.....
Other	☐	☐	.....

If **Other**, please give details.....

.....

.....

26. In the **7 DAYS** before you became ill did you visit any of the following?

	Yes	No	If **YES** give details
Parks	☐	☐	.....
Wild or rough ground	☐	☐	.....
Woodlands	☐	☐	.....
Farms	☐	☐	.....
Fields	☐	☐	.....
Grazing land	☐	☐	.....
Beaches	☐	☐	.....
Lakes, ponds etc	☐	☐	.....
Canals, rivers etc	☐	☐	.....
Swimming pools	☐	☐	.....
Allotments	☐	☐	.....
Garden centres	☐	☐	.....
Stables	☐	☐	.....
Campsites	☐	☐	.....

	Yes	No	If **YES** give details
Zoos	☐	☐	.....
Bird sanctuaries	☐	☐	.....
Animal markets	☐	☐	.....
Slaughter houses	☐	☐	.....
Small holdings	☐	☐	.....

CONTACT WITH ANIMALS

27. Do you or your family keep any of the following pet animals?

	Yes	No
Dog(s)	☐	☐
Cat(s)	☐	☐
Fish	☐	☐
Bird(s) *[Prompt: budgies, parrots, pigeons etc]*	☐	☐
Reptile(s) *[Prompt: snakes, lizards, tortoises etc]*	☐	☐
Other animals	☐	☐

Please specify .....
[Prompt: hamsters, rabbits, frogs etc]

28. Did you clean the pets, or their cages, tanks etc? Yes ☐ No ☐

29. Did you feed the pets? Yes ☐ No ☐

30. Are your pets fed in the kitchen? Yes ☐ No ☐

31. Did you come into contact with any animals outside your home?
[Prompt: at a friends house, school etc]

Yes ☐ No ☐

If **YES** please specify. *[Prompt: cats, dogs, hamsters, snakes, terrapins etc]*

.....

32. Did you or any other member of your household buy any of the following products in the **7 DAYS** before you became ill?

	Yes	No		Yes	No
Fishfood	☐	☐	Dog chews	☐	☐
Birdfood	☐	☐	Cat biscuits	☐	☐
Fresh meat/offal	☐	☐	Other	☐	☐

If other, please specify type, name/brand. *[Prompt: dog biscuits, live mice/insects for reptiles etc]*

.....

If **YES** to any, where were the products purchased? *[Prompt: name and location of shop, stall, internet]*

.....

33. Did you ride, touch or care for any horses or ponies in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If **YES** give details [eg xx stables near xx village in Kent etc]

.....

.....

34. Did you visit any places where farm animals or wildlife might be found in the **7 DAYS** before you became ill? [Prompt: parks, farms, woods, zoos etc] Yes ☐ No ☐

If **YES** give details [eg xx zoo or park, or woods near xx village in Kent etc]

.....

.....

35. Did you come into contact with any goats, kids, sheep or lambs in the **7 DAYS** before you became ill?

Yes ☐ No ☐

36. Did you come into contact with any cows or calves,in the **7 DAYS** before you became ill?

Yes ☐ No ☐

37. Did you come into contact with any other farm animals or wildlife in the **7 DAYS** before you became ill? *[Prompt: hens, rabbits, fish, birds etc]* Yes ☐ No ☐

If **YES**, what types of animal did you touch?

.....

.....

38. If you have a garden or access to a communal garden or allotment did you come into contact with any wildlife or their droppings in the **7 DAYS** before you became ill? *[Prompt: squirrels, foxes, hedgehogs, birds etc]*

Yes ☐ No ☐ If **YES**, what types of animal were they?

.....

.....

39. Did you handle or feed any garden birds,in the **7 DAYS** before you became ill?

Yes ☐ No ☐ If **YES**, what type of feed did you use?

Name of brand .....

Place of purchase

.....

FOOD HANDLING AT HOME

Now we are going to ask you about cooking and preparing food at home.

40. Did you handle any of the following raw foods in your kitchen in the **7 DAYS** before you became ill?

	Yes	No	Where bought
Eggs	☐	☐	.....
Sausages	☐	☐	.....
Beefburgers	☐	☐	.....
Minced beef	☐	☐	.....
Other beef *[Prompts: steak, joints etc]*	☐	☐	.....
Lamb	☐	☐	.....
Other pork *[Prompts: chops, joints etc]*	☐	☐	.....
Chicken	☐	☐	.....
Turkey	☐	☐	.....
Other meat *[Prompts: duck, rabbit etc]*	☐	☐	.....
Potatoes	☐	☐	.....
Carrots	☐	☐	.....
Onions	☐	☐	.....
Leeks	☐	☐	.....
Other vegetables *[Prompts: parsnips, cabbage etc]*	☐	☐	.....

If you did handle any vegetables, was there any soil on them? Yes ☐ No ☐

41. Did any other members of your household handle any of the above raw foods in your kitchen in the **7 DAYS** before you became ill?

If **YES** give details *[Prompts: parents, brothers, sisters etc]*

.....

.....

42. Were you involved in any of the following activities the **7 DAYS** before you became ill?

	Yes	No
Washing/cutting vegetables	☐	☐
Making/mixing cakes	☐	☐

If you did make any cakes could you have eaten any of the mix before it was cooked?

Yes ☐ No ☐

43. In which part of your kitchen fridge is raw meat kept?

	Yes	No		Yes	No
Top	☐	☐	Middle	☐	☐
Bottom	☐	☐	Other	☐	☐

If other, please specify *[Prompt: in a designated drawer]*.

.....

44. Is raw meat stored in a freezer? *[Prompt: includes that in a fridge freezer]*.

Yes ☐ No ☐

45. Do the cooks in your family or household use a separate chopping board for cutting raw meat?

Yes ☐ No ☐

46. Is it possible that the cooks in your family used any gelatin in making any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand?

.....

47. Is it possible that the cooks in your family used any milk powder in making any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand? .....

48. Is it possible that the cooks in your family used any whey powder in making any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand? .....

49. Is it possible that the cooks in your family used any flour to make any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand?

.....

50. Is it possible that the cooks in your family used any suet in making any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand? .....

51. Is it possible that the cooks in your family used any beef dripping in making any dishes in the **7 DAYS** before you became ill? Yes ☐ No ☐ If **YES,** what brand?

.....

FOOD HISTORY

We are going to ask you about food eaten in the **7 DAYS** before you became ill.

VENUES

52. Did you have a packed lunch prepared at home? Yes ☐ No ☐

53. Have you eaten lunch at any of the following places in the **7 DAYS** before you became ill?

	Yes	No	Name
At home	☐	☐	
At Work/School	☐	☐	.....
Restaurant	☐	☐	.....
Take away	☐	☐	.....
Pub	☐	☐	.....
Other	☐	☐	.....

54. Did you eat any food (including take-aways and delivered foods) from or in any of the following places in the **7 DAYS** before you became ill?

	Yes	No	Name/Branch
Coffee shop *[Prompts: eg Starbucks, Costas etc]*	☐	☐	.....
Burger bar *[Prompts: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompts: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompts: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompts: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompts: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompts: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompts: eg ferry, theme park etc]*	☐	☐	.....

POULTRY

55. Did you eat any of the following foods in the **7 DAYS** before you became ill?

	Prepared at home	Away from home	No
Chicken pies/pasties	☐	☐	☐
Fried chicken nuggets/portions	☐	☐	☐
Hot chicken *[Prompts: eg roasts, casseroles, curries, etc]*	☐	☐	☐
Cold chicken *[Prompts: eg sandwiches/baguettes, salads etc]*	☐	☐	☐
Chicken liver pâté/parfait	☐	☐	☐
Hot turkey	☐	☐	☐
Cold turkey *[Prompts: eg sandwiches/baguettes, salads etc]*	☐	☐	☐
Hot duck *[Prompts: eg roasts, crispy fried duck etc]*	☐	☐	☐
Cold duck *[Prompts: eg in a wrap/sandwich]*	☐	☐	☐
Other poultry *[Prompts: eg goose, guinea fowl, quail, partridge etc]*	☐	☐	☐

If YES to other please specify .....
[specify eg quail, partridge]

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	
British restaurant	☐	☐	
Chinese restaurant	☐	☐	
Greek restaurant	☐	☐	
Indian restaurant	☐	☐	
Italian restaurant	☐	☐	
Other restaurant	☐	☐	
Hotel	☐	☐	
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	
Pub	☐	☐	
Canteen *[Prompt: eg work, school etc]*	☐	☐	
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stalls etc]*	☐	☐	
Motorway service	☐	☐	
Airport	☐	☐	
Railway station/train	☐	☐	
Petrol station	☐	☐	
Other *[Prompt: eg cinema,ferry, theme park etc]*	☐	☐	

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Cheese shop	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Other	☐	☐	

If other please specify .....
[Prompt: department store, farm shop, ethnic grocer etc]

BEEF

56. Did you eat any of the following foods containing beef in the **7 DAYS** before you became ill?

	Prepared at home	Away from home	No
Burgers	☐	☐	☐
Other minced beef dishes *[Prompt: lasagne/chilly/cottage pie etc]*	☐	☐	☐
Steak	☐	☐	☐
Roast beef (hot)	☐	☐	☐
Beef stew/casserole/curry	☐	☐	☐
Pies and pasties *[Prompt: steak & kidney pie, Cornish pasty etc]*	☐	☐	☐
Cold roast/corned beef etc	☐	☐	☐
Sausages [including hot dogs]	☐	☐	☐
Other beef	☐	☐	☐

If YES to other please specify .....

57. Did you eat any **cold cooked beef** in salads or sandwiches etc, which were made at home in the **7 DAYS** before you became ill? *[Prompt: sliced roast beef, corned beef, tongue, brawn etc]*

Yes ☐ No ☐

If **YES** please specify .....
[Prompt: product type and brand eg corned beef from supermarket X]

If **YES,** was the meat sliced at the counter? Yes ☐ No ☐
[Prompt: at a butchers shop or delicatessen counter]

58. Did you eat any processed beef products which were cooked at home?
[Prompt: cooked chill or ready meals eg lasagne, steak and kidney pudding etc]

Yes ☐ No ☐

If **YES** please specify .....

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	
British restaurant	☐	☐	
Chinese restaurant	☐	☐	
Greek restaurant	☐	☐	
Indian restaurant	☐	☐	
Italian restaurant	☐	☐	
Other restaurant	☐	☐	
Hotel	☐	☐	
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	
Pub	☐	☐	
Canteen *[Prompt: eg work, school etc]*	☐	☐	
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stalls etc]*	☐	☐	
Motorway service	☐	☐	
Airport	☐	☐	
Railway station/train	☐	☐	
Petrol station	☐	☐	
Other *[Prompt: eg cinema,ferry, theme park etc]*	☐	☐	

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Cheese shop	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Other	☐	☐	

If other please specify .....
[Prompt: department store, farm shop, ethnic grocer etc]

PORK

59. Did you eat any of the following foods in the **7 DAYS** before you became ill?

	Prepared at home	Away from home	No
Pork sausages	☐	☐	☐
Bacon	☐	☐	☐
Gammon	☐	☐	☐
Hot pork dishes *[Prompt: roast, chops, casseroles etc]*	☐	☐	☐
Pork pies	☐	☐	☐
Pâté	☐	☐	☐
Salami	☐	☐	☐
Pepperoni	☐	☐	☐
Pork scratchings	☐	☐	☐
Ham *[Not including Parma, Serrano ham etc]*	☐	☐	☐
Dry cured ham *[Parma, Serrano, Bayonne ham etc]*	☐	☐	☐
Luncheon meat	☐	☐	☐

Other ☐ ☐ ☐

If Other please specify .....
[eg cold roast pork, moriadella etc]

If ham, salami or luncheon meat were eaten –

Were any of the products sliced at the shop counter? Yes ☐ No ☐
[Prompt: at a butchers shop or delicatessen counter]

60. Did you eat any processed pork products which were cooked at home?
[Prompt: cooked chill or ready meals eg sweet and sour pork, slimmers meals etc]

Yes ☐ No ☐

If **YES** please specify the brands .....

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennesee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompt: eg cinema,ferry, theme park etc]*	☐	☐	.....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompts department store, farm shop, ethnic grocer etc]

LAMB

61. Did you eat any dishes containing lamb/mutton in the **7 DAYS** before you became ill?
[Prompts: Roast lamb, chops, kebabs, mince, casseroles, curries or stews]

Cooked at home from raw ☐ Ready meals cooked at home ☐

Away from home ☐ No ☐

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennesee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompt: eg cinema,ferry, theme park etc]*	☐	☐	.....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompts department store, farm shop, ethnic grocer etc]

OTHER MEAT PRODUCTS

62. Did you eat any of the following foods in the **7 DAYS** before you became ill?

	Cooked/prepared at home	Away from home	No
Kebabs	☐	☐	☐
Sausages	☐	☐	☐
Biltong	☐	☐	☐
Sausage rolls	☐	☐	☐
Pasties/ meat pies	☐	☐	☐
Scotch eggs	☐	☐	☐
Haggis	☐	☐	☐
Haslet	☐	☐	☐
Brawn	☐	☐	☐
Tripe, liver etc	☐	☐	☐
If **YES** please specify			
Pizza with meat *[Pepperoni etc]*	☐	☐	☐
Rabbit meat	☐	☐	☐
Goat meat	☐	☐	☐
Venison	☐	☐	☐
Gravy *[Prompt: ready made in cartons or plastic packs]*	☐	☐	☐
Other	☐	☐	☐
If **YES** please specify ..... *[eg ready meals, slimmers meals etc]*			

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	
British restaurant	☐	☐	
Chinese restaurant	☐	☐	
Greek restaurant	☐	☐	
Indian restaurant	☐	☐	
Italian restaurant	☐	☐	
Other restaurant	☐	☐	
Hotel	☐	☐	
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	
Pub	☐	☐	
Canteen *[Prompt: eg work, school etc]*	☐	☐	
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	
Motorway service	☐	☐	
Airport	☐	☐	
Railway station/train	☐	☐	
Petrol station	☐	☐	
Other *[Prompt: eg cinema, ferry, theme park etc]*	☐	☐	

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Cheese shop	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Other	☐	☐	

If other please specify .....

FISH & SEAFOOD

62. Did you eat any seafood in the **7 DAYS** before you became ill? *[Prompt: fish, crab, scamp(i, mussels]*

☐ Cooked at home from raw ☐ Ready meals cooked at home

☐ Ready to eat at home ☐ Away from home

☐ No

If **YES**, what type of seafood? .....

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	
British restaurant	☐	☐	
Chinese restaurant	☐	☐	
Greek restaurant	☐	☐	
Indian restaurant	☐	☐	
Italian restaurant	☐	☐	
Other restaurant	☐	☐	
Hotel	☐	☐	
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	
Pub	☐	☐	
Canteen *[Prompt: eg work, school etc]*	☐	☐	
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	
Motorway service	☐	☐	
Airport	☐	☐	
Railway station/train	☐	☐	
Petrol station	☐	☐	
Other *[Prompt: eg cinema, ferry, theme park etc]*	☐	☐	

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Cheese shop	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Other	☐	☐	

If other please specify .....

EGGS

64. Did you eat any of the following foods in the **7 DAYS** before you became ill?

	Prepared at home	Away from home	No
Omelettes/scrambled eggs	☐	☐	☐
Egg sandwiches	☐	☐	☐
Egg salads	☐	☐	☐
Eggs with runny yolks	☐	☐	☐
Eggs with hard yolks	☐	☐	☐
Quiches	☐	☐	☐
Souffles	☐	☐	☐

If handled, eaten or cooked away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	
British restaurant	☐	☐	
Chinese restaurant	☐	☐	
Greek restaurant	☐	☐	
Indian restaurant	☐	☐	
Italian restaurant	☐	☐	
Other restaurant	☐	☐	
Hotel	☐	☐	

Café ☐ ☐ .....
[Prompt: greasy spoons, supermarkets, gyms etc.]

Pub ☐ ☐ .....

Canteen ☐ ☐ .....
[Prompt: eg work, school etc]

Food stalls/vans ☐ ☐ .....
[Prompt: eg lunch van, hot dog stand, market stands etc]

Motorway service ☐ ☐ .....

Airport ☐ ☐ .....

Railway station/train ☐ ☐ .....

Petrol station ☐ ☐ .....

Other ☐ ☐ .....
[Prompt: eg cinema,ferry, theme park etc]

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

Were the eggs sold boxed Yes ☐ No ☐ or loose Yes ☐ No ☐

Were the eggs Lion mark Yes ☐ No ☐

65. Did you eat any other types of eggs in the **7 DAYS** before you became ill?
[Prompt: duck eggs, quails eggs, dried eggs etc.]
Yes ☐ No ☐
If **YES** please specify .....
Purchased from .....

MILK

66. Did you drink (or have with cereal, tea/coffee etc) any milk in the **7 DAYS** before you became ill?
Yes ☐ No ☐

67. If **YES** was the milk?

	Yes	No	Brand
Cows milk	☐	☐	.....
Goats milk	☐	☐	.....
Soya milk	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

68. Was the milk?

	Full fat	Semi-skimmed	Skimmed
Unpasteurised	☐	☐	☐
Pasteurised	☐	☐	☐
Filtered	☐	☐	☐
Sterilised/UHT	☐	☐	☐
Powdered	☐	☐	☐

69. Was it bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Milk round	☐	☐	.....
Other	☐	☐	.....

CHEESE

70. Did you eat any of the following types of cheese in the 7 **DAYS** before you became ill?
[Prompt: includes cheese in salads, sandwiches, burgers etc]

	At home	Away from home	No	Type(s) of cheese / brand
Goats cheese	☐	☐	☐	.....
Processed cheese *[eg Cheese strings,slices]*	☐	☐	☐	.....
Blue cheese *[eg Stilton, Gorgonzola etc]*	☐	☐	☐	.....
Cheese spread *[eg Philadelphia, Dairylea, Roulé etc]*	☐	☐	☐	.....
Other soft cheese *[eg Brie, dolcelatte, cottage cheese etc]*	☐	☐	☐	.....
Hard white cheese *[eg Cheddar, Edam, Feta etc]*	☐	☐	☐	.....
Cooked cheese dishes *[eg pizza, macaroni cheese etc]*	☐	☐	☐	.....

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennesee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompt: eg cinema,ferry, theme park etc]*	☐	☐	.....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompt: department store, farm shop, ethnic grocer etc]

71. Was the cheese prepacked? Yes ☐ No ☐

72. Was the cheese cut in the shop Yes ☐ [Can be both] No ☐

DAIRY PRODUCTS

73. Did you eat or drink any of the following products in the **7 DAYS** before you became ill?

	At home	Away from home	No	Type(s) / brand
Cream *(e.g. sweet or sour)*	☐	☐	☐	.....
Yoghurt	☐	☐	☐	.....
Fromage frais	☐	☐	☐	.....
Butter	☐	☐	☐	.....
Probiotic drinks *[Prompt: eg Yakult, Activia etc]*	☐	☐	☐	.....
Yoghurt drinks *[Prompt: eg Yop]*	☐	☐	☐	.....
Milk drinks *[Prompt: eg cold chocolate milk/milkshakes]*	☐	☐	☐	.....
Smoothies	☐	☐	☐	.....
Other	☐	☐	☐	.....

If Other please specify .....
[Prompt: buttermilk, smetana etc]

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompts: eg Starbucks, Costas etc]*	☐	☐	.....
Burger bar *[Prompts: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompts: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompts: eg KFC, Tennesee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompts: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompts: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompts: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompts: eg cinema, ferry, theme park etc]*	☐	☐	.....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompts: department store, farm shop, ethnic grocer etc]

CAKES & BISCUITS

74. Did you eat any cakes with nuts in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If **YES** please specify .....
[Prompts: ask for type and brand]

75. Did you eat any cream cakes in the **7 DAYS** before you became ill? *[Prompt: cream sponge, eclair, profiterole, other cream cakes]*

Yes ☐ No ☐

If YES please specify .....
[Prompts: ask for type and brand]

76. Did you eat any other type of cakes in the **7 DAYS** before you became ill?
[Prompt: chocolate cake, fresh fruit tart, cooked fruit tart/ pie, cakes with icing, iced buns, Danish pastry, muffin, other]

Yes ☐ No ☐

If **YES** please specify .....
[Prompts: ask for type and brand]

77. Did you eat any kind of biscuits in the **7 DAYS** before you became ill? *[Prompt: flapjacks, cookies, biscuits]*

Yes ☐ No ☐

If **YES** please specify .....
[Prompts: ask for type and brand]

Did the biscuits contain nuts? Yes ☐ No ☐

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompts: eg Starbucks, Costas etc]*	☐	☐	.....
Burger bar *[Prompts: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompts: eg Dominos, Pizza Express etc]*	☐	☐	.....

Kebab shop ☐ ☐ .....
Fish & chip shop ☐ ☐ .....
Fried chicken bar *[Prompts: eg KFC, Tennesee Fried Chicken etc]* ☐ ☐ .....
Bakers shop ☐ ☐ .....
Sandwich bar ☐ ☐ .....
Delicatessen or Farm shop *[not in a supermarket]* ☐ ☐ .....
British restaurant ☐ ☐ .....
Chinese restaurant ☐ ☐ .....
Greek restaurant ☐ ☐ .....
Indian restaurant ☐ ☐ .....
Italian restaurant ☐ ☐ .....
Other restaurant ☐ ☐ .....
Hotel ☐ ☐ .....
Café *[Prompts: greasy spoons, supermarkets, gyms etc.]* ☐ ☐ .....
Pub ☐ ☐ .....
Canteen *[Prompts: eg work, school etc]* ☐ ☐ .....
Food stalls/vans *[Prompts: eg lunch van, hot dog stand, market stands etc]* ☐ ☐ .....
Motorway service ☐ ☐ .....
Airport ☐ ☐ .....
Railway station/train ☐ ☐ .....
Petrol station ☐ ☐ .....
Other *[Prompts: eg cinema, ferry, theme park etc]* ☐ ☐ .....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Cheese shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompts: department store, farm shop, ethnic grocer etc]

Other ☐ ☐ .....

If other please specify .....
[Prompt: eg church fair, friend, abroad etc]

DESSERTS & PUDDINGS

78. Did you eat any of the following types of desserts/puddings in the **7 DAYS** before you became ill?

	At home	Carton	Away from home	No
Mousse (eg chocolate, lemon, strawberry etc)	☐	☐	☐	☐
If yes, details (flavour/type)				
Meringue (including pavlova/baked alaska)	☐	☐	☐	☐
If yes, details (flavour/type)				
Pancakes and crêpes	☐	☐	☐	☐
If yes, details (flavour/type)				
Trifle (with custard)	☐	☐	☐	☐
If yes, details (flavour/type)				
Tiramisu	☐	☐	☐	☐
If yes, details (flavour/type)				
Home made ice cream	☐	☐	☐	☐
If yes, details (flavour/type)				
Other ice cream	☐	☐	☐	☐
If yes, details (flavour/type)				
Ice lollies	☐	☐	☐	☐
If yes, details (flavour/type)				
Sorbets	☐	☐	☐	☐
If yes, details (flavour/type)				
Frozen yoghurt	☐	☐	☐	☐
If yes, details (flavour/type)				
Frozen desserts	☐	☐	☐	☐
If yes, details (flavour/type)				

Milk pudding ☐ ☐ ☐ ☐
[eg rice pudding etc]

If yes, details (flavour/type) .....

Cookie Dough ☐ ☐ ☐ ☐

If yes, details (flavour/type) .....

Other desserts/puddings ☐ ☐ ☐ ☐

If other please specify .....
[Prompt: sticky toffee pudding, banoffee pie, panna cotta etc but not fresh fruit or fruit cocktail]

Did any of the puddings contain cream? Yes ☐ No ☐

Did any of the puddings contain nuts? Yes ☐ No ☐

If eaten away from home were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, garms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other *[Prompt: eg ferry, theme park etc]*	☐	☐	.....

If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Bakers shop	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....
[Prompt: specialist ethnic store etc]

CHOCOLATE & SWEETS

79. Did you eat any bagged chocolate in the **7 DAYS** before you became ill (e.g. Smarties, Maltesers, Mini Eggs, poppets)?

Yes ☐ No ☐

If yes, please specify type, brand and place of purchase:.....

80. Did you eat any wrapped chocolate or chocolate bars (or eggs) in the **7 DAYS** before you became ill (Kit Kat, Milky way, Bounty, Twix, Snickers)?

Yes ☐ No ☐

If yes, please specify type, brand and place of purchase:.....

81. Did you eat any boxed/ tinned chocolate in the **7 DAYS** before you became ill (gift box type e.g Milk Tray, Black Magic, Roses, Quality Street, Celebrations)?

Yes ☐ No ☐

If yes, please specify type, brand and place of purchase:.....

82. Did you eat any chocolate coated nuts products in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If yes, please specify type, brand and place of purchase:.....

83. How was the product packed?

In a box (e.g. gift box) Yes ☐ No ☐

In a bag Yes ☐ No ☐

84. What type of chocolate were the nuts covered with?

Dark chocolate Yes ☐ No ☐

Milk chocolate Yes ☐ No ☐

White chocolate Yes ☐ No ☐

85. Did you eat **any other kind of chocolate** in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If yes, please specify type, brand and place of purchase:.....

86. Did you eat any boiled sweets in the **7 DAYS** before you became ill (i.e. sweets that you can suck on e.g mint humbug, Polo mints , pear drops)?

Yes ☐ No ☐

If **YES**, please specify type, brand and place of purchase:.....

87. Did you eat any other sweets in the **7 DAYS** before you became ill (i.e.nougat, fudge, toffees, jellies)?

Yes ☐ No ☐

If **YES**, please specify type, brand and place of purchase:.....

NUTS AND SEEDS

88. Did you eat any products with the following **nuts** in the **7 DAYS** before you became ill?

	Yes	No	Product/ purchased from
Peanut	☐	☐	.....
Cashew	☐	☐	.....
Brazil	☐	☐	.....
Walnuts	☐	☐	.....
Pine nuts	☐	☐	.....
Sesame	☐	☐	.....
Pistachios	☐	☐	.....
Hazel nuts	☐	☐	.....
Other nuts	☐	☐	.....
Mixed nuts	☐	☐	.....
Other seeds	☐	☐	.....

SNACKFOODS

89. Did you eat any **snackfoods** in the **7 DAYS** before you became ill?
[Prompt: cereal bars, crisps, tortilla chips, bombay mix, etc]

Yes ☐ No ☐

If **YES,** please specify type and brand. [**Prompt:** crisps including flavour, tortilla chips, bombay mix, seedmixes, etc]

.....

90. Were they bought from?

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Delicatessen	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Restaurant/take away	☐	☐	.....
Other	☐	☐	.....

91. In the **7 DAYS** before you became ill did you eat any food that was <u>bought abroad?</u>
[Prompt: bought by yourself or given to you as a gift]

Yes ☐ No ☐

If **YES,** please specify type of food and country of purchase *[Prompt: eg camembert cheese from France etc]*

.....

.....

92. In the **7 DAYS** before you became ill did you eat any regional food items ?
[Prompts: bought by yourself or given to you as a gift maybe such as eccles cakes,welsh cakes, teabies, kendal mint cake,scottish shortbread]

Yes ☐ No ☐

If **YES,** please specify type and brand of food and place of purchase

.....

.....

SANDWICHES, BURGERS & KEBABS

93. Did you eat any sandwiches, rolls, filled baguettes or wraps that were **bought or served** away from home in the **7 DAYS** before you became ill?
[Prompts: includes prepacked sandwiches from shops/railway stations, works/school canteens, rolls at buffet lunches, custom made sandwiches from sandwich bars, pubs etc].

Yes ☐ No ☐

94. If **YES** did you eat any of the following types of sandwich?
[Prompts: Sandwich includes rolls, baguettes, wraps etc. Custom made is non-prepacked sandwiches made to order in sandwich bars, pubs etc.]

	Prepacked	Custom made	Buffet	No
Ham	☐	☐	☐	☐
Beef *[roast beef, corned beef, salt beef, pastrami etc]*	☐	☐	☐	☐
Bacon/BLT	☐	☐	☐	☐
Chicken	☐	☐	☐	☐
Turkey	☐	☐	☐	☐
Other meat *[salami, sausage etc]*	☐	☐	☐	☐
Tuna sandwich	☐	☐	☐	☐
Salmon sandwich *[includes smoked salmon]*	☐	☐	☐	☐
Prawn/other seafood *[crab, crayfish etc]*	☐	☐	☐	☐
Egg mayonnaise	☐	☐	☐	☐
Other egg	☐	☐	☐	☐
Cheese	☐	☐	☐	☐
Other	☐	☐	☐	☐

If Other please specify .....

Hot dogs	☐	☐	.....
Meat kebabs	☐	☐	.....
Chicken kebabs	☐	☐	.....
Sausages	☐	☐	.....
Chips	☐	☐	.....

97. Did any of these include any of the following extras?

	Yes	No		Yes	No
Mayonnaise	☐	☐	Dips	☐	☐
Gravy	☐	☐	Curry sauce	☐	☐
Lettuce	☐	☐	Ketchup	☐	☐
Tomato	☐	☐	Chilli sauce	☐	☐
Cucumber	☐	☐	Gherkins	☐	☐
Onions	☐	☐	Spinach	☐	☐
Other leaves	☐	☐	If other specify.....		

Were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompts: eg Starbucks, Costas etc]*	☐	☐	.....
Burger bar *[Prompts: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompts: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompts: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....

95. Did any of these sandwiches include any of the following extras?

	Yes	No		Yes	No
Mayonnaise	☐	☐	Mustard	☐	☐
Other dressing	☐	☐	Lettuce	☐	☐
Onions	☐	☐	Cucumber	☐	☐
Tomato	☐	☐	Water cress	☐	☐
Spinach	☐	☐	Mustard cress	☐	☐
Bean sprouts	☐	☐	Other leaves	☐	☐
Herbs *[Prompt: coriander, basil,chili etc]*	☐	☐	If **YES**, please specify		

Were the sandwiches bought/served from?

	Yes	No	Name/Brand/Branch/Location
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Supermarket	☐	☐	.....
Mini market	☐	☐	.....
Restaurant/hotel/pub	☐	☐	.....
School/work canteen	☐	☐	.....
Delivery service *[at work or hospital]*	☐	☐	.....
Petrol/service station	☐	☐	.....
Railway station	☐	☐	.....
Airport/plane	☐	☐	.....
Buffet *[cricket club, wedding reception]*	☐	☐	.....
Other	☐	☐	.....

If Other please specify .....

96. Did you eat any of the following foods away from home in the **7 DAYS** before you became ill?

	Yes	No	Where purchased/served
Hamburgers (beef)	☐	☐	.....
Chicken burgers	☐	☐	.....
Chicken nuggets etc	☐	☐	.....
Veggie burgers	☐	☐	.....
Fish burgers *[fillet of fish etc.]*	☐	☐	.....

Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompts: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompts: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompts: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other	☐	☐	.....

SAUCES, PICKLES & DIPS

98. Did you eat any sauces or dips containing yoghurt in the **7 DAYS** before you became ill?
[Prompts: tzaziki, raita, salad dressing etc]

Yes ☐ No ☐

If **YES,** please specify type (s) .....

Was it from a restaurant/hotel/take away Yes ☐ No ☐

If **YES** please specify [can be more than one]:.....
[Prompts: name and location of restaurant/'take away']

.....

99. Did you eat any sauces or dips containing cream in the **7 DAYS** before you became ill?
[Prompts: pasta sauces, cheese dips etc]

Yes ☐ No ☐

If **YES,** please specify type (s) .....

Was it from a restaurant/hotel/take away Yes ☐ No ☐

If **YES** please specify [can be more than one]:.....
[Prompts: name and location of restaurant/'take away']

.....

100. Did you eat any other sauces, pickles or dips in the **7 DAYS** before you became ill?
[Prompt: including those in sandwiches, burgers and kebabs, eg salad dressings, pesto, tomato sauce, soy sauce, Thai fish sauce, piccalilly, Indian pickles, hummus, salsa, curry sauce, chilli sauce etc]

Yes ☐ No ☐

If **YES,** please specify type (s) .....
[Prompt: salad dressing, guacamole, hummus, salsa etc and brand]

.....

Was it away from the home Yes ☐ No ☐

If **YES** please specify [can be more than one]: .....
[Prompt: name and location of restaurant/'take away']

.....

101. Was it a commercial brand Yes ☐ No ☐

If **YES,** please specify type and brand (s) [Can be more than one]: .....
[Prompt: eg Heinz tomato ketchup, HP sauce, Patak's mango pickle, Tesco's hummus etc]

.....

Was it home made Yes ☐ No ☐

If **YES** please specify .....
[Prompt: type of sauce or pickle eg barbecue sauce, lime pickle etc]

Other Yes ☐ No ☐
[Prompt: eg from a local market stall, church sale or friend]

If **YES,** please specify type .....
[Prompt: eg tomato chutney, piccalilly etc]

SALAD VEGETABLES & HERBS

102. Did you eat any of the following raw vegetables in the **7 DAYS** before you became ill?
[Prompt: don't forget salads that you've purced or those in sandwiches, burgers, kebabs and as garnishes with Indian or Chinese meals etc includes lettuce, tomatoes, cucumbers, cress, bean shoots, beetroot, gerkins etc]

	Eaten/prepared at home	Away from home	No	In bag	Loose
Mixed salad leaves	☐	☐	☐	☐	☐
Water cress	☐	☐	☐	☐	☐
Lettuce *[includes rocket, radicchio etc]*	☐	☐	☐	☐	☐
Tomatoes	☐	☐	☐	☐	☐
Spinach	☐	☐	☐	☐	☐
Baby Spinach	☐	☐	☐	☐	☐
Bean sprouts	☐	☐	☐	☐	☐
Other sprouted seeds	☐	☐	☐	☐	☐

[including alfalfa, pea shoots etc.]
.....

Cabbage *[eg cole slaw]*	☐	☐	☐	☐	☐
Cucumber	☐	☐	☐	☐	☐
Peppers	☐	☐	☐	☐	☐
Onions (any)	☐	☐	☐	☐	☐
Mushrooms	☐	☐	☐	☐	☐
Cauliflower	☐	☐	☐	☐	☐
Basil	☐	☐	☐	☐	☐
Parsley	☐	☐	☐	☐	☐
Coriander leaves	☐	☐	☐	☐	☐
Dill	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

If other, specify: .....

If eaten away from home, were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/Vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other	☐	☐	.....

103. If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Green grocers	☐	☐	.....
Chinese grocers	☐	☐	.....
Indian grocers	☐	☐	.....
Greek grocers	☐	☐	.....
Other ethnic grocers *[eg African, Arabic etc]*	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Home delivered boxes	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

104. Did you eat any of the following ready-to-eat products in the **7 DAYS** before you became ill?

	At home	Away from home	No	In bag	Loose
Pasta Salad	☐	☐	☐	☐	☐
Green Salad	☐	☐	☐	☐	☐
Chicken salad	☐	☐	☐	☐	☐
Potato salad	☐	☐	☐	☐	☐
Couscous	☐	☐	☐	☐	☐
Peppers	☐	☐	☐	☐	☐
Coleslaw	☐	☐	☐	☐	☐
Tzatziki	☐	☐	☐	☐	☐
Olives	☐	☐	☐	☐	☐
Sun dried tomatoes	☐	☐	☐	☐	☐
Sauerkraut	☐	☐	☐	☐	☐
Anchovies	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

If other please specify .....

If eaten away from home, were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....

British restaurant	☐	☐
Chinese restaurant	☐	☐
Greek restaurant	☐	☐
Indian restaurant	☐	☐
Italian restaurant	☐	☐
Other restaurant	☐	☐
Hotel	☐	☐
Café *[Prompt: greasy spoons, supermarkets, garms etc.]*	☐	☐
Pub	☐	☐
Canteen *[Prompt: eg work, school etc]*	☐	☐
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐
Motorway service	☐	☐
Airport	☐	☐
Railway station/train	☐	☐
Petrol station	☐	☐
Other	☐	☐

105. If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Green grocers	☐	☐	
Chinese grocers	☐	☐	
Indian grocers	☐	☐	
Greek grocers	☐	☐	
Other ethnic grocers *[eg African, Arabic etc]*	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Home delivered boxes	☐	☐	

Other	☐	☐

If other please specify .....

FRUIT

106. Did you eat any of the following fresh fruit in the **7 DAYS** before you became ill?

	At home	Away from home	No
Apples	☐	☐	☐
Bananas	☐	☐	☐
Grapes	☐	☐	☐
Oranges *[includes satsumas, tangerines etc]*	☐	☐	☐
Cranberries	☐	☐	☐
Blueberries	☐	☐	☐
Bilberries	☐	☐	☐
Strawberries	☐	☐	☐
Raspberrries	☐	☐	☐
Kiwi fruit	☐	☐	☐
Pomegranate	☐	☐	☐
Other	☐	☐	☐

If other please specify .....
[Prompt: eg pineapples, pears, guava etc]

If eaten away from home, were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not ins supermarket]*	☐	☐	

British restaurant	☐	☐
Chinese restaurant	☐	☐
Greek restaurant	☐	☐
Indian restaurant	☐	☐
Italian restaurant	☐	☐
Other restaurant	☐	☐
Hotel	☐	☐
Café *[Prompt: greasy spoons, supermarkets, garms etc.]*	☐	☐
Pub	☐	☐
Canteen *[Prompt: eg work, school etc]*	☐	☐
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐
Motorway service	☐	☐
Airport	☐	☐
Railway station/train	☐	☐
Petrol station	☐	☐
Other	☐	☐

107. If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	
Corner shop/mini mkt	☐	☐	
Delicatessen *[not in a supermarket]*	☐	☐	
Green grocers	☐	☐	
Chinese grocers	☐	☐	
Indian grocers	☐	☐	
Greek grocers	☐	☐	
Other ethnic grocers *[eg African, Arabic etc]*	☐	☐	
Market	☐	☐	
Mobile shop	☐	☐	
Home delivered boxes	☐	☐	

Other	☐	☐

If other please specify .....

108. Did you eat any of the following types of preserved fruit in the **7 DAYS** before you became ill?

	At home	Away from home	No
Raisins/sultanas	☐	☐	☐
Dates	☐	☐	☐
Figs	☐	☐	☐
Desiccated coconut	☐	☐	☐
Candied peel	☐	☐	☐
Glacé cherries	☐	☐	☐
Tinned fruit	☐	☐	☐
Specify ..... *[brand and type eg peaches, pears etc]*			
Dried fruit	☐	☐	☐
Specify ..... *[brand and type eg apricots, apples, fruit bars etc]*			
Jam and other preserves	☐	☐	☐
Specify ..... *[brand and type eg raspberry jam, lime marmalade, cranberry jelly etc]*			

If eaten away from home, were the products bought from? [Can be more than one]

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costas etc]*	☐	☐	
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	
Kebab shop	☐	☐	
Fish & chip shop	☐	☐	
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	
Bakers shop	☐	☐	
Sandwich bar	☐	☐	
Delicatessen or Farm shop *[not ins supermarket]*	☐	☐	

British restaurant	☐	☐

Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other	☐	☐	.....

109. If eaten at home were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Green grocers	☐	☐	.....
Chinese grocers	☐	☐	.....
Indian grocers	☐	☐	.....
Greek grocers	☐	☐	.....
Other ethnic grocers *[eg African, Arabic etc]*	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Home delivered boxes	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

If yes, please specify .....

113. Did you eat any foods which contained any other seeds in the **7 DAYS** before you became ill?

Yes ☐ No ☐

If yes, please specify .....
[Prompt: pumpkin, sunflower, poppy seed]

If other please specify .....

DRINKS

114. Did you have any of the following drinks in the **7 DAYS** before you became ill?

	At home	Carton/Bottle	Away from home	No
Orange juice	☐	☐	☐	☐
Apple juice	☐	☐	☐	☐
Pineapple juice	☐	☐	☐	☐
Grapefruit juice	☐	☐	☐	☐
Fruit smoothie	☐	☐	☐	☐
Yoghurt based (inc. lassi)	☐	☐	☐	☐
High protein milk shakes	☐	☐	☐	☐
Slimmers milk shakes	☐	☐	☐	☐
Other milk shakes (including e.g. nesquik)	☐	☐	☐	☐
Other high protein drinks	☐	☐	☐	☐
Herbal infusions	☐	☐	☐	☐
If **YES** please specify ..... *[Prompt: camomile; mint etc]*				
Iced tea	☐	☐	☐	☐
Chilled coffee	☐	☐	☐	☐
Coffee (inc. cappuccino, latte etc)	☐	☐	☐	☐
Still mineral water	☐	☐	☐	☐
Sparkling mineral water	☐	☐	☐	☐

If drank away from home, were the products bought from? [Can be more than one]

SPICES AND SEEDS

110. Did you eat any foods prepared at home which contained any of the following spices in the **7 DAYS** before you became ill?

	Yes	No
Chilli powder	☐	☐
Cinnamon bark/powder	☐	☐
Coriander seeds/powder	☐	☐
Cumin seeds/powder	☐	☐
Curry powder	☐	☐
Chinese five spice	☐	☐
Ginger root/powder	☐	☐
Nutmeg	☐	☐
Paprika	☐	☐
Pepper	☐	☐
Saffron	☐	☐
Turmeric	☐	☐
Other	☐	☐

Please specify .....
[Prompt: cardamom, star anise, poppy seeds, nigella etc]

111. If **YES** to any of the above were the products bought from? [Can be more than one]

	Yes	No	Name/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Delicatessen *[not in a supermarket]*	☐	☐	.....
Green grocers	☐	☐	.....
Ethnic grocers	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

112. Did you eat any foods which contained sesame seeds in the **7 DAYS** before you became ill?
[Prompt: bread, halva, tahini, houmous]
Yes ☐ No ☐

	Yes	No	Name
Coffee shop *[Prompt: eg Starbucks, Costa etc]*	☐	☐	.....
Burger bar *[Prompt: eg McDonalds, Burger King etc]*	☐	☐	.....
Pizza parlour *[Prompt: eg Dominos, Pizza Express etc]*	☐	☐	.....
Kebab shop	☐	☐	.....
Fish & chip shop	☐	☐	.....
Fried chicken bar *[Prompt: eg KFC, Tennessee Fried Chicken etc]*	☐	☐	.....
Bakers shop	☐	☐	.....
Sandwich bar	☐	☐	.....
Delicatessen or Farm shop *[not in a supermarket]*	☐	☐	.....
British restaurant	☐	☐	.....
Chinese restaurant	☐	☐	.....
Greek restaurant	☐	☐	.....
Indian restaurant	☐	☐	.....
Italian restaurant	☐	☐	.....
Other restaurant	☐	☐	.....
Hotel	☐	☐	.....
Café *[Prompt: greasy spoons, supermarkets, gyms etc.]*	☐	☐	.....
Pub	☐	☐	.....
Canteen *[Prompt: eg work, school etc]*	☐	☐	.....
Food stalls/vans *[Prompt: eg lunch van, hot dog stand, market stands etc]*	☐	☐	.....
Motorway service	☐	☐	.....
Airport	☐	☐	.....
Railway station/train	☐	☐	.....
Petrol station	☐	☐	.....
Other	☐	☐	.....

If **YES** to having any of the above at home were the products bought from?
(Can be more than one)

	Yes	No	Name/Brand/Branch/Location
Supermarket	☐	☐	.....
Corner shop/mini mkt	☐	☐	.....
Health food shops	☐	☐	.....
Delicatessen *(not in a supermarket)*	☐	☐	.....
Green grocers	☐	☐	.....
Ethnic grocers *(eg Indian, Chinese, Polish, African, etc)*	☐	☐	.....
Market	☐	☐	.....
Mobile shop	☐	☐	.....
Other	☐	☐	.....

If other please specify .....

MISCELLANEOUS

115. Did you eat any of the following in the **7 DAYS** before you became ill?

Breakfast cereal Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Margarines and spreads Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Bread/rolls etc Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Crispbreads and crackers Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Peanut butter Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Chocolate spreads *(eg Nutella)* Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Sandwich spreads/pastes/fillers Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Uncooked cookie dough Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Instant soup, noodles (e.g. pot noodle) Yes ☐ No ☐
If **YES,** please specify type and brand (s) .....

Is there anything else that you think it is important for us to know?

.....
.....
.....
.....
.....
.....
.....
.....

THANK YOU FOR YOUR CO-OPERATION

Would it be all right for us to contact you again for additional information? Yes ☐ No ☐

If you have any specific questions about this investigation either now or in the future please call or write to:

Please return questionnaires to Health Protection Scotland, GIZ team
NSS.HPSgiz@nhs.net

Appendix 5: Technical description of Bayesian modelling

Methodology

In a standard 2 by 2 table where the exposure status (Yes/No) of cases and controls is classified the odds ratio measures the strength of the association between exposure and being a case. In a Bayesian analysis the same odds ratio is used but, in addition, prior information on the prevalence of the exposure among cases and controls is included. This prior information is expressed as a probability distribution and represents belief and judgement but can also be based upon data from previous studies. Within a Bayesian analysis the data – the 2 by 2 table – modifies the prior information to produce a posterior distribution for the odds ratio. Often this distribution is skew and so the median is used as a measure of location and the range of 95% of the distribution, known as the credible interval, gives the precision of the odds ratio. If this is wide then there is little information in the data.

Prior distributions can be non-informative – especially flat – meaning that we have no prior idea of the exposure prevalence in cases and controls. This would be represented by a uniform distribution between 0 and 1. Prior information can be informative and this would be represented by a distribution which has single peak but with variation. The stronger the prior belief the smaller the variability.

We used two types of prior information. One was the flat prior which is non informative. The other was based upon estimates from some of the restaurants involved who estimated either from order records or experience that about 5 to 10% of diners order the cheese board. For the general population the percentage eating blue cheese is likely to be lower and we use priors centred upon 1% of the population. A second prior was centred on 3.5% of the population eating blue cheese within the last 7 days based upon data the National Diet and Nutrition survey (Table 4). Sensitivity analysis to the location and shape of the prior information was carried out.

The advantages of the Bayesian method are that it can easily cope with situations where there are small samples and some of the cells in the 2 by 2 table are zero. In this case much of the posterior information is based on the prior information. It can also be used when there is no control information and there is only exposure data on cases. In this case all the information on exposure among non-cases is based solely on the prior information.

There are advantages to the Bayesian approach but there are also disadvantages. In small samples, particularly, the influence of the prior is great and changes to the prior will follow through to the conclusions from the posterior distribution. Over confidence in the prior will lead to over certainty in the posterior and vice versa.

The Bayesian modelling was conducted twice during the early stages of the investigation before all the cases were identified and at the end once information was available for all 26 cases.

Technical description of results

The final Bayesian modelling carried out for the blue cheese consumption eaten outside the home, based upon 17 out of 26 current outbreak cases and 0 out of 21 cases from previous investigations exposed, the median odds ratio from the posterior distribution is 59 (95% credible interval 8, 1721) using flat uniform priors for the probability of being exposed to blue

cheese away from home in both cases and prior cases. Using a more informative prior for the exposure among non-cases of only 10%, ranging from 0% to 40%, gives an odds ratio of 78 (95% Crl 11,2552).

If we ignore the cases from the previous outbreaks and use a prior distribution centred on 1%, ranging from 0-4% for the exposure among non-cases gives a median posterior odds ratio of 270 (95% Crl 39, 7729) and with a prior centred on 1% ranging from 0% to 10% the median posterior odds ratio is 196 (95% Crl 14, 93814). With the updated prior centred on 3.5% and ranging from 0% to 8% the median posterior odds ratio was 78 (95% Crl 16, 264) and with a more dispersed prior ranging from 0 to 16% the median posterior odds ratio was 50 (95% Crl 8,940).

This analysis can be repeated (1) by excluding the two secondary cases, who are both linked to the childcare cluster, (2) excluding all five childcare cluster associated cases and also (3) using the adults only cases in which case the five childcare cluster and one other case are excluded. All that will happen, relative to the all case analysis is that the median odds ratio will get larger, the lower limits will get a little higher and the widths of the credible intervals will increase as the sample sizes are smaller.

These results show the great uncertainty given by the width of the credible interval (the Bayesian equivalent of a confidence interval). Notwithstanding for all reasonable priors the lower limit of the credible interval is well above 1. Furthermore it would be necessary to make a prior assumption that about 30-40% of non-current cases are exposed before the lower limit of the 95% credible interval approaches 1.

Appendix 6: Further testing of ECL cheese after IMT stood down

SLC submitted to ESS 245 samples (5 samples per batch of cheese) from a range of batches of Lanark Blue and Corra Linn in January and February 2017. A range of potentially pathogenic *stx* negative *E. coli* O157 and *stx* positive *E. coli* non O157 were detected in seven batches of Corra Linn (a ewes' milk cheese)

Corra Linn Batch	***E. coli* identification**
B17A	*E. coli* O153-O178:H7 *stx1c* positive ST278
E23A	*E. coli* unidentifiable:H14 *stx2b* positive ST7010
F27A	*E. coli* O8:H9 *stx2e* positive ST23
G7A	*E. coli* O157:H42 *stx* negative ST7077
G20A	*E. coli* O157:H42 *stx* negative ST7077
G25A	*E. coli* O157:H42 *stx* negative ST7077
H1A	*E. coli* O157:H42 *stx* negative ST7077

E. coli O157:H42 stx negative ST7077 had previously been detected in Lanark White batches G14, H3 and H24. WSG results showed these isolates from Lanark White and Corra Linn had the same SNP addresses.

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