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Outbreak of STEC O157:H7 linked to a milk pasteurisation failure at a dairy farm in England, 2019

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Abstract

In November 2019, an outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 was detected in South Yorkshire, England. Initial investigations established consumption of milk from a local dairy as a common exposure. A sample of pasteurised milk tested the next day failed the phosphatase test, indicating contamination of the pasteurised milk by unpasteurised (raw) milk. The dairy owner agreed to immediately cease production and initiate a recall. Inspection of the pasteuriser revealed a damaged seal on the flow divert valve. Ultimately, there were 21 confirmed cases linked to the outbreak, of which 11 (52%) were female, and 12/21 (57%) were either <15 or >65 years of age. Twelve (57%) patients were treated in hospital, and three cases developed haemolytic uraemic syndrome. Although the outbreak strain was not detected in the milk samples, it was detected in faecal samples from the cattle on the farm. Outbreaks of gastrointestinal disease caused by milk pasteurisation failures are rare in the UK. However, such outbreaks are a major public health concern as, unlike unpasteurised milk, pasteurised milk is marketed as ‘safe to drink’ and sold to a larger, and more dispersed, population. The rapid, co-ordinated multi-agency investigation initiated in response to this outbreak undoubtedly prevented further cases.

Introduction

Foodborne gastrointestinal infections are a public health and financial burden on society [1]. Diarrhoeal symptoms are often mild and self-limiting, but can be persistent, blood-stained and accompanied by severe abdominal pain, vomiting and fever. In 2018, there were an estimated 2.4 million cases of foodborne gastrointestinal disease in the UK, of which approximately 16 300 cases received hospital treatment and over 180 deaths were reported (The Burden of Foodborne Disease in the UK 2018: https://www.food.gov.uk/sites/default/files/media/document/the-burden-of-foodborne-disease-in-the-uk_0.pdf). Although the number of cases attributed to Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in the UK are lower than other zoonotic foodborne bacteria (such as *Campylobacter* and *Salmonella* species), STEC O157:H7 is regarded as a priority pathogen because of the relatively high hospitalisation rate and the risk of progression to haemolytic uraemic syndrome (HUS) [2, 3]. HUS is characterised by renal dysfunction, and/or cardiac and neurological complications; it is the leading cause of kidney failure in children and can be fatal [4, 5].

Historically, foodborne outbreaks of STEC O157:H7 in England were mainly associated with contaminated beef and lamb meat and dairy products [6]. Recently, foodborne outbreaks of STEC O157:H7 have been linked to raw vegetables and ready to eat salad items contaminated by animal faeces pre- or post-harvest [7]. However, there is evidence that exposure to raw or undercooked meat and unpasteurised dairy products remain a high risk for consumers [8–11].

The National Enhanced Surveillance System for STEC (NESSS) operates in England [2, 3]. All cases are notified to the UK Health Security Agency (UKHSA, formerly, Public Health England) based on the detection of STEC O157:H7 from patients’ faecal specimens. Members of the local health protection teams (HPTs) co-ordinate the administration of STEC Enhanced Surveillance Questionnaires (ESQs) to all cases to determine likely food and environmental exposures. All isolates of STEC O157:H7 are sequenced and single-nucleotide

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polymorphism (SNP) typing is used to identify single linkage clusters that may be epidemiologically linked [12, 13]. Outbreaks are identified by linking cases based on their epidemiological exposures and/or the SNP typing analysis highlighting a cluster of closely related isolates likely to be from the same point source [14].

In November 2019, an increase in the expected number of cases of STEC O157:H7 resident in the South Yorkshire region were notified to the Yorkshire and Humber HPT (Y&H HPT). An Incident Management Team (IMT) meeting was convened to investigate the outbreak, and initial review of the STEC ESQs revealed that the majority of cases reported door-step delivery of milk from the same dairy farm. Here, we describe the co-ordination of the multi-agency investigation, the key finding from the analysis of the epidemiological and microbiological enhanced surveillance data and highlight recommendations for future practice.

Methods

Case ascertainment

In the UK, faecal specimens from all patients submitted to local hospital microbiology laboratories are tested for the presence of *Salmonella*, *Campylobacter*, *Shigella* spp. and STEC O157:H7. Presumptive isolates of STEC O157:H7 were sent to the UKHSA Gastrointestinal Bacteria Reference Unit (GBRU) for confirmation and typing.

Presumptive cases of STEC were reported directly to UKHSA HPTs by clinical microbiologists at local hospital laboratories and a standardised STEC ESQ (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323423/VTEC_Questionnaire.pdf) was administered to each case either by local health protection professionals or environmental health officers (EHOs). Data from the questionnaires were uploaded to NESSS. NESSS was reviewed to identify any cases with an epidemiological link to the implicated dairy farm. Any cases identified this way, or as having a microbiological link through STEC surveillance processes, were reviewed against the outbreak case definition.

A confirmed case was defined as an individual with STEC O157:H7 infection confirmed by the UKHSA GBRU, with an epidemiological link to the implicated dairy farm and/or as having a microbiological link through STEC surveillance processes, and onset of illness between 1 October 2019 and 31 March 2020.

A possible case was defined as an individual who had symptoms of gastrointestinal illness and were a known contact of a confirmed case but were not a culture confirmed case of STEC O157:H7 either because their specimen tested negative for STEC O157:H7 or they did not submit a faecal sample for microbiological analysis.

Microbiological examination of food samples

Samples of milk from the implicated farm were taken by EHOs during their first inspection of the dairy farm on 21 November 2019. Apart from the Christmas and New Year period, samples of pasteurised whole, semi-skimmed and skimmed milk from the holding tanks and from bottles were obtained from the farm by EHOs at least once each week from the 26 November until 24 March 2020. All samples were collected and transported in accordance with the Food Standards Agency (FSA) Food Law Code of Practice (<https://www.food.gov.uk/enforcement/codes-of-practice/food-law-code-of-practice-2015>). They were transported to the UKHSA Food, Water and Environmental (FW&E)

Microbiology Laboratory, York, in cold boxes (temperature 0–8 °C) and tested within 24 h of collection.

Tests for the detection of STEC including STEC O157:H7, *Salmonella*, *Campylobacter* and *Listeria* species were performed on 25 ml samples of milk. Enumeration of coliform bacteria, *Escherichia coli*, coagulase-positive staphylococci, aerobic colony count and *Listeria* species (including *L. monocytogenes*) was carried out using dilutions of milk samples. Real-time polymerase chain reaction (PCR) was used to examine samples for the presence of STEC O157:H7 based on CEN/ISO TS 13136 (<https://www.iso.org/standard/53328.html>).

Veterinary investigation and microbiological examination of animal faecal specimens

Veterinary Investigation Officers from the Animal and Plant Health Agency (APHA) visited the implicated farm on 30 November 2019 to assess the potential role of the farm animals as the source of infection. Thirty fresh faecal samples were collected from calf, young stock and cow accommodation, and were tested using immuno-magnetic separation culture methodology, and any suspect isolates presumptively identified by latex agglutination test, as described previously [15].

Molecular typing of STEC O157:H7 by whole genome sequencing

Isolates of STEC O157:H7 from human clinical specimens and animal samples were sent to UKHSA GBRU for confirmation by PCR and phage typing, as described previously [16, 17], and whole genome sequencing (WGS) [13].

For WGS, DNA was extracted from cultures for sequencing on the HiSeq 2500 instrument (Illumina, California, USA). High-quality Illumina reads were mapped to the STEC O157:H7 reference genome Sakai (GenBank accession BA000007) using BWA-MEM. SNPs were identified using GATK2 in unified genotyper mode [13]. Core genome positions that had a high-quality SNP in at least one isolate were extracted. SNP positions that were present in at least 80% of isolates were used to derive maximum likelihood phylogenies using the GTRCAT model with 1000 iterations [13].

Genomes were compared with the sequences held in the UKHSA STEC O157:H7 WGS database. This database comprised of genomes from more than 4000 cultures of STEC O157:H7 submitted to GBRU between 1982 and 2019. Hierarchical single linkage clustering was performed on the pairwise SNP difference between all isolates at various distance thresholds ($\Delta 250$, $\Delta 100$, $\Delta 50$, $\Delta 25$, $\Delta 10$, $\Delta 5$, $\Delta 0$) [18]. The result of the clustering is an SNP address that can be used to describe the population structure based on clonal groups. Clusters at the zero, five and 10 SNP level are highlighted for further investigation and are analysed in the context of their nearest neighbours. Isolates of STEC O157:H7 with less than five SNP differences within their core genome were considered closely related and likely to originate from the same source [12–14]. *stx* subtyping was performed, as described previously by Ashton *et al.* 2015 [19].

Results

Epidemiological investigations

Ultimately, 21 cases of STEC O157:H7 PT21/28 were linked to the outbreak, with symptom onset dates ranging from 1 to 28

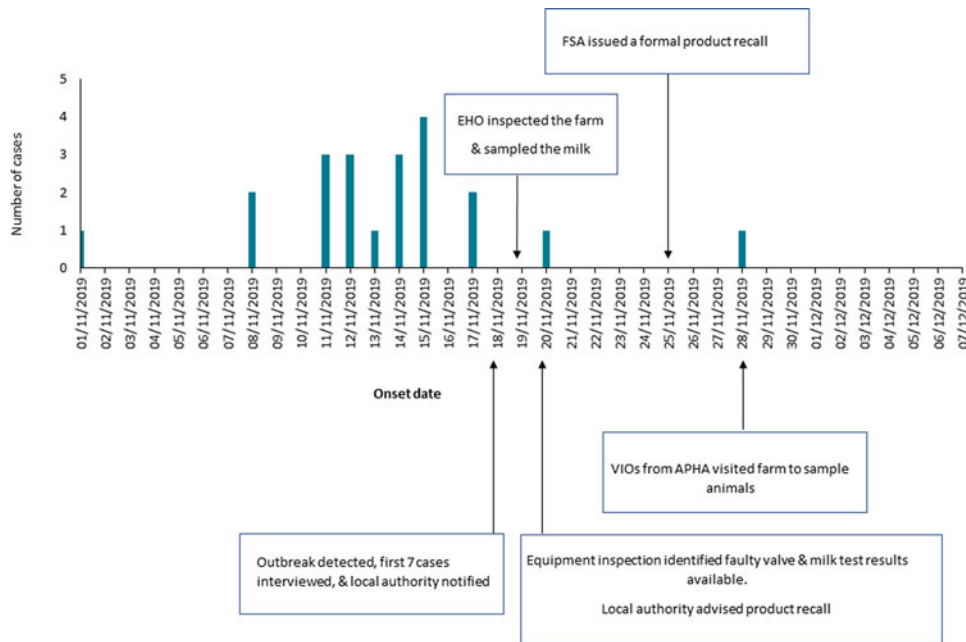


Fig. 1. Number of confirmed cases of STEC O157:H7 in South Yorkshire with dates of onset of symptoms from 01/11/2019 to 30/11/2019 ($n = 21$).

November 2019 (Fig. 1). There were 10 (48%) male and 11 (52%) female cases. Confirmed cases occurred in all age groups, with 12 (57%) cases detected in vulnerable groups, including eight cases aged 65 years old or over, and four cases aged less than 15 years old (Fig. 2). All 21 cases had diarrhoea, of which 17/21 (81%) reported having bloody diarrhoea and/or abdominal pain. Other reported symptoms included nausea ($n = 5$, 25%), vomiting ($n = 3$, 14%) and fever ($n = 3$, 14%). Twelve of 21 (57%) cases were admitted to hospital, and 3/21 (14%) were diagnosed with STEC-HUS. A further five possible cases were identified.

Analysis of the ESQ administered to the patient following the primary notification of diagnosis revealed that 13/21 (62%) cases reported consumption of milk produced on the same dairy farm. Consumption of milk from the dairy farm was subsequently confirmed for an additional five cases during follow-up interviews using a supplementary questionnaire used to explore milk exposures, making a total of 18/21 cases with the same exposure (86%). The remaining three cases did not provide sufficient details of their milk consumption to establish the provider and/or supplier; however, a link to the implicated dairy could not be ruled out. A case-control study was considered in order to determine a statistical association however, recruitment to the control group was too low to be useful to the investigation.

Environmental investigations

The dairy farm was a small family run on-farm producer of pasteurised cow's milk and cream products and an 'Approved Establishment' in accordance with Regulation (EC) No. 853/2004. The dairy Food Business Operator (FBO) supplied milk locally via door-step delivery rounds and other local food retailers and caterers. On 20 November 2019, the local authority received information regarding a public health investigation into confirmed cases of STEC O157:H7 infections in three residents in an adjoining local authority area. Information recorded from

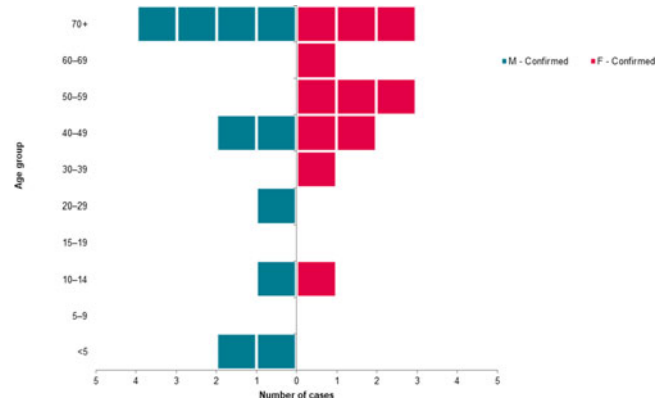


Fig. 2. Age-sex distribution of confirmed cases of STEC O157:H7 in South Yorkshire from 01/11/2019 to 30/11/2019 ($n = 21$).

each of the three cases suggested that all of them had consumed pasteurised milk from the same dairy farm.

EHOs from the local authority visited the dairy farm on 21 November 2019 and recorded that the FBO was not aware of any potential hygiene issues, either with their operating procedures, equipment or staff. However, the EHOs noted that there was a lack of processing records available to inspect, in particular (i) records of checks to ensure the correct operating flow rate of the pasteuriser, (ii) thermograph charts to show the operating and cleaning temperatures achieved and (iii) flow diversion checks and 'events'.

The pasteuriser used in the dairy was last inspected and tested by an independent dairy engineer in March 2019, and no operating faults with the pasteuriser were found at this time. The next inspection was not scheduled until March 2020, however as a precaution, the dairy owner arranged for the pasteuriser to be inspected by an independent dairy engineer the following day.

On the 22 November, the engineer inspected the heat exchanger plate pack and gaskets, as failure of this plate pack is a known cause of phosphatase failures. No faults were found with the heat exchanger plate pack however, the engineer found a damaged rubber seal located on the flow divert valve of the pasteuriser. The purpose of the flow divert valve is to divert milk that has not been adequately heat treated back into the pasteuriser to be heated again. The faulty seal resulted in pasteurised milk being contaminated by unpasteurised (raw) milk intermittently leaking through the broken seal.

Test results of the milk samples

Prior to the outbreak, milk samples from the implicated dairy farm has been submitted for microbiological testing at the UKHSA FW&E laboratory in York on two occasions in 2019, once in July and once in November. The test results for samples of milk and cream submitted to the UKHSA FW&E laboratory on 13 November 2019 were described as 'Satisfactory'. Although the level of phosphatase in the skimmed milk sample was legally compliant, it was higher than expected and resampling was recommended.

On 21 November 2019, samples of milk that were produced that morning were submitted to the FW&E laboratory for testing for phosphatase activity, Enterobacteriaceae counts and presence of STEC O157:H7. On 22 November 2019, colleagues at the FW&E laboratory in York notified the local authority that the sample of whole milk submitted the day before had failed the phosphatase test, indicating either that the milk had not been adequately pasteurised or that pasteurised milk had been contaminated by raw milk.

Control measures

The local authority immediately notified the dairy FBO of the result and advised them to cease any further milk processing. They were also advised to start a product recall for milk and cream products placed on the market since 21 November 2019. The food business operators accepted a voluntary prohibition agreement, requiring them to immediately cease production and not to resume until they had confirmed that the repairs carried out on the pasteuriser had been effective and that any milk subsequently produced was safe for human consumption.

The dairy FBO submitted samples of milk on 25 November 2019 which passed the phosphatase test, however, as recommended by the IMT, they remained closed. Additional sampling of the milk on a weekly basis was co-ordinated by the local authority and carried out at the UKHSA FW&E laboratory in York. On 27 November 2019, the FSA issued a formal product recall notice for all of milk and cream products produced by the dairy farm that were currently in circulation (Product Recall Information Notice Ref. PRIN-50-2019). The dairy FBO re-commenced production and distribution on 17 December 2019. The EHOs continued to take further samples to monitor the microbiological quality of the milk on a weekly basis until 24 March 2020.

Follow-up milk sampling investigations

On 26 and 27 November 2019, a total of 20 samples were submitted to the FW&E laboratory, York for testing, including five samples each of skimmed, semi-skimmed and whole milk and five

samples of raw milk from the bulk tank. The raw milk samples sent on 27 November 2019 were negative for STEC, including STEC O157:H7. All phosphatase results were satisfactory. However, test results showed that 14/15 samples had levels of Enterobacteriaceae that exceed legislative compliance limits for process hygiene.

Between 25 November 2019 and 25 March 2020, EHOs took a minimum of 15 samples of milk from the dairy once every week (except over the Christmas holiday period). Samples of raw milk, cream and environmental swabs were also taken and processed at the FW&E laboratory in York. In addition to the unsatisfactory levels of Enterobacteriaceae found in samples taken on 26 November 2019, unsatisfactory levels of Enterobacteriaceae were detected in the samples taken on 3 and 12 December 2019, as well as 2, 6, 13 and 22 January, and 18 and 25 February 2020. Dates where all samples tested had satisfactory levels of Enterobacteriaceae were 10, 17 and 19 December 2019, 20 January, 4 and 11 February and 3, 10, 17 and 24 March 2020. All samples taken since the damaged rubber seal on the divert valve was replaced on Saturday 23rd November 2019 were satisfactory for phosphatase activity, indicating that the pasteurisation process was operating effectively.

Animal sampling investigations

E. coli serogroup O157 was isolated from 6/30 fresh faecal samples taken from cattle accommodation on the farm. Of these six isolates, three were confirmed as STEC O157:H7 PT21/28. The remaining three isolates were identified as *E. coli* O157:H12 and were Shiga toxin negative.

Analysis of WGS data

The outbreak strain was identified as STEC O157:H7 PT21/28 harbouring *stx* subtypes *stx2a* and *stx2c*. Phylogenetic analysis showed that all 21 isolates from the human cases and the three cattle isolates fell within the same five SNP single linkage cluster (Fig. 3). These results indicate that the cattle on the farm were the source of the human infections, most likely via the consumption of unpasteurised milk contaminated with cattle faeces.

There were four additional sequences in the archive database that were closely related to the outbreak strain from cases identified in the months and years prior to the incident (Fig. 3). All four cases reported travelling to the Y&H region prior to onset of symptoms, however detailed histories of their food and/or environmental exposures while in Y&H were not available.

Discussion

Previous reviews of foodborne outbreaks in England have highlighted the risks associated with consumption of raw drinking milk (RDM), and milk sold as pasteurised but contaminated with raw milk, as a result of pasteurisation failures [20–23]. Of the nine milkborne outbreaks of STEC O157:H7 documented between 1992 and 2000, five were linked to the consumption of RDM and four to pasteurisation failures [21]. Small dairy farms that bottled their own milk were identified as a major problem due to the lack of regular microbiological testing of their products. Following an 11-year period (2003–2013) where no milkborne outbreaks were reported, a recent review highlighted an increase in outbreaks of gastrointestinal illness, including STEC O157:H7, caused by consumption of RDM [9]. None of these recent milkborne outbreaks

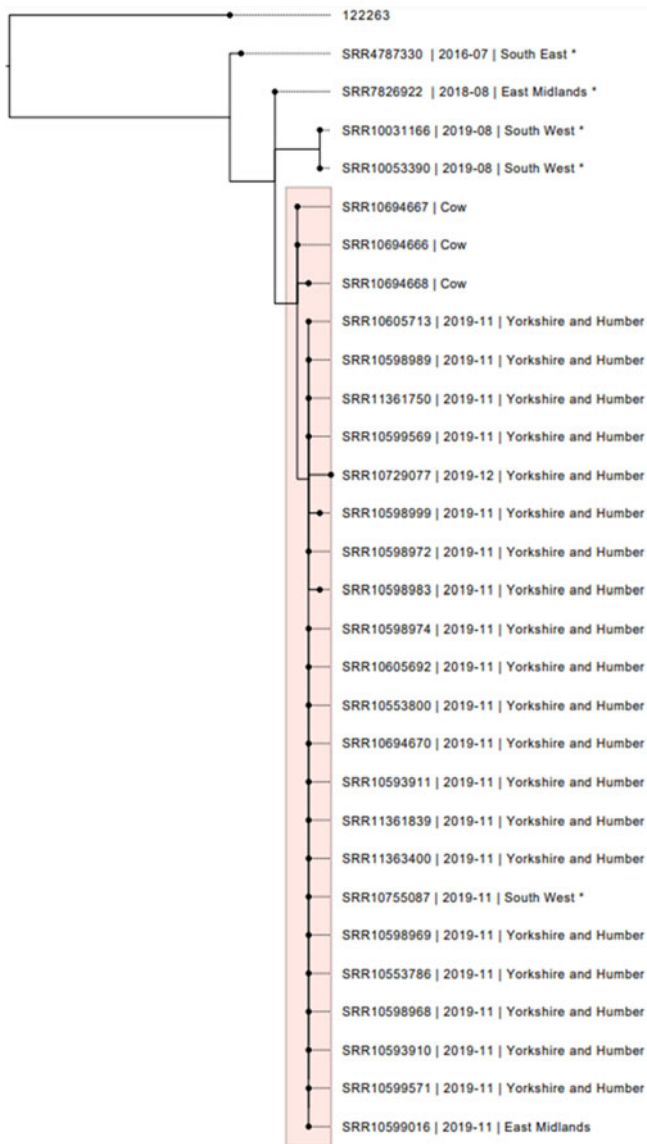


Fig. 3. Phylogeny of isolates of STEC O157:H7 belonging to the same five SNP single linkage cluster. Nodes of the tree are labelled with short read archive accession numbers (SRRs), the month the case was detected and their region of residence. The isolates from the human cases ($n=21$) and cattle on the farm ($n=3$) linked to the outbreak are highlighted. *Case not resident in Y&H but had travelled to Y&H region prior to onset of symptoms 122 263 is a closely related reference isolate used to root the tree.

were linked to pasteurisation failures. Restrictions on the sale of RDM only permit direct sale at the "farm gate" to the final consumer (and not via an intermediate retailer), limiting the size and geographical distribution of milkborne outbreaks caused by RDM. Outbreaks caused by pasteurisation failures are concerning as the geographical distribution of the product may be widespread, affecting a larger number of people [20].

Despite the fact the dairy linked to the current outbreak was a small, local business and the catchment area was relatively contained, there was a large number of households on the customer list. The underlying cause of the contamination of the milk was a faulty valve in the pasteuriser, and it is likely that intermittent contamination of the milk would have continued if the fault had not been identified. Therefore, it is likely that the rapid, co-ordinated response of the IMT, leading to the identification

of the faulty valve within 48 h of the outbreak being detected, contributed to preventing further cases. The early detection of the outbreak based on case notification to the local HPT by colleagues in the local hospital laboratories triggered a request to the EHOs from the local authority to visit the farm to inspect the business operation and to take samples of the milk for microbiological testing. The failure of the phosphatase test carried out at FW&E laboratory in York proved that the milk had failed the pasteurisation process, and this was instrumental in providing the IMT with the evidence they needed to suspend production and initiate a recall. It is concerning that without the phosphatase test result it was unlikely that the epidemiological evidence alone would have been deemed sufficient to enable the IMT to provide a case for suspending production.

Although the dairy recommenced production in December 2019, the local authority continued to test milk samples from the dairy, and results indicated intermittent unsatisfactory levels of Enterobacteriaceae in the milk until March 2020. Unsatisfactory results are defined as levels of Enterobacteriaceae that are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/363146/Guidelines_for_assessing_the_microbiological_safety_of_ready-to-eat_foods_on_the_market.pdf).

Following unsatisfactory Enterobacteriaceae results in milk samples, the legal requirement placed on the FBO is to investigate the cause in order to prevent recurrence and, specifically, to check the efficiency of heat treatment and the quality of the raw material (Article 7 2073/2005). Further sampling of the product is then recommended to verify that any corrective actions taken have been successful. However, unsatisfactory Enterobacteriaceae results are not sufficient to halt production or to initiate recall action.

Despite the extensive testing, the causative agent was not detected in any of the milk samples during the investigation. STEC are notoriously difficult to detect and isolate from food samples, including dairy products [8, https://www.foodstandards.gov.scot/downloads/MPI_FSS_STEC_in_Raw_Cheese_Review_10082018.pdf]. The infectious dose of STEC O157:H7 is low and studies indicate that consumption of 10–100 viable bacteria can result in an infection [24]. Furthermore, food samples contaminated by animal faeces may contain high levels of other species of bacteria that hamper the detection of the target pathogen. It is also known that following the onset of symptoms, diagnosis of the illness and investigation of potential source can take several days, if not weeks. As a result, it is often unlikely that the batch of food consumed by a case is available for testing. During the investigation described here, the detection of the causative agent was further confounded by the intermittent nature of the contamination event; for example, only a small proportion of the milk bottles may have been contaminated. Failure to detect the organism in a food vehicle does not provide evidence that the implicated vehicle is not the source of an infection. The detection of the pathogen in food samples should not be considered necessary to prove a link when the epidemiological data provide good evidence of an association. In recent years, PCR has been successfully used to detect STEC O157:H7 in food samples during outbreaks in the UK, most often where the vehicle is a meat product where the level of contamination may be higher [11, 25] and/or if the sampling strategy was sufficiently extensive and rigorous [26], and/or if the sampling was initiated and actioned in a timely manner [10].

Although the outbreak strain was not isolated from the milk, it was isolated from faecal samples collected from the cattle located

on the dairy farm, providing microbiological evidence of link between the dairy farm and the outbreak. Despite this finding there was reluctance by the FBO to accept this as evidence of a link between the outbreak cases and the milk as the vehicle of infection. Questions were raised as to why only a sub-set of the customers reported symptoms. This phenomenon can be explained partly by low levels of the pathogen in the milk and the intermittent nature of contamination. Moreover, not all individuals exposed to STEC are symptomatic, as evidenced by the detection of STEC in asymptomatic contacts of cases tested to minimise person-to-person transmission [2, 3].

As we were unable to recruit the sufficient controls to perform a statistical test of association between the cases and consumption of milk from the implicated dairy, we questioned whether the causative agent could have come from any other source. However, the combination of the descriptive epidemiology, the faulty pasteuriser and the molecular typing data identifying the outbreak strain in the cattle on farm was deemed sufficient evidence of a link to implicate the dairy farm as the source of the outbreak. No two *E. coli* bacteria can have the same DNA sequence by chance, so the isolates of STEC O157:H7 from the human cases and the cattle that have the same DNA sequence must have come from the same source, in this example, the same herd of cows on the dairy farm. Individuals linked to a foodborne outbreak do not always report consumption of the implicated product, and these cases are sometimes used to deflect attention away from the suspected vehicle [14, 27]. However, if a person who is apparently unconnected to the outbreak is infected with the outbreak strain, it is likely that they are linked in some way but that the connection is obscured. For example, the individual may have either consumed the milk but did not remember, or they were not aware of the name of the dairy farm the milk originated from [8]. Alternatively, they may not have consumed the milk but may have been exposure as a result of direct contact with the animals on the farm, or the farm environment, or they had close contact with another person who had consumed the milk.

Outbreaks of gastrointestinal disease caused by milk pasteurisation failures are rare in the UK; the outbreak described here was the first recorded incident in England for over two decades [9, 20–23]. However, such outbreaks are a major public health concern because, unlike RDM, the milk is sold to the consumer as safe to drink and to a larger and more dispersed population. There is good evidence from this investigation, and from previous outbreaks, that cattle in the UK are at risk of being colonised with pathogenic variants of STEC O157:H7 that have the potential to cause severe clinical outcomes, including STEC-HUS [8, 10, 14]. Consequently, when investigating outbreaks of STEC potentially linked to dairy products, it may be prudent to implement public health actions and interventions based on robust epidemiological analysis, regardless of the microbiological test results from sampling of the implicated food vehicle. We also recommend performing microbiological testing of faecal samples from animals located on the implicated farm [8, 10]. The causative agent is likely to be present in animal faeces in higher numbers than the food vehicle, thus facilitating detection and providing microbiological evidence of an epidemiological link.

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Conflict of interest. There are no conflicts of interest.

Data availability statement. FASTQ reads from all sequences in this study can be found at the PHE Pathogens BioProject at the National Center for Biotechnology Information (accession number: PRJNA315192).

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