Original Paper

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A genetically related cluster of *Salmonella Typhimurium* cases in humans associated with ruminant livestock and related food chains, United Kingdom, August 2021–December 2022

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Abstract

Following an outbreak of *Salmonella Typhimurium* in Wales in July 2021 associated with sheep meat and offal, further genetically related cases were detected across the UK. Cases were UK residents with laboratory-confirmed *Salmonella Typhimurium* in the same 5-single-nucleotide polymorphism (SNP) single-linkage cluster with specimen date between 01/08/2021–2031/12/2022. We described cases using routine (UK) and enhanced (Wales only) surveillance data. Exposures in cases in Wales were compared with non-Typhimurium *Salmonella* case–controls. Environmental Health Practitioners and the Food Standards Agency investigated supply chains of food premises reported by ≥2 cases. Animal, carcass, and environmental samples taken for diagnostic or monitoring purposes for gastrointestinal pathogens were included in microbiological investigations. We identified 142 cases: 75% in England, 23% in Wales and 3% in Scotland. Median age was 32 years, and 59% were male. Direct contact with sheep was associated with becoming a case (aOR: 14, 95% CI: 1.4–145) but reported by few (6/32 cases). No single food item, premises, or supplier linked all cases. Multi-agency collaboration enabled the identification of isolates in the same 5-SNP single-linkage cluster from a sheep carcass at an English abattoir and in ruminant, wildlife, poultry, and environmental samples, suggesting multiple vehicles and pathways of infection.

Introduction

Salmonella Typhimurium outbreaks, UK

Non-typhoidal salmonellosis is caused by the enteric pathogen *Salmonella enterica*, a species that includes over 2,600 different serovars [1]. In the United Kingdom (UK), over 8,000 cases of salmonellosis are reported annually [2], with the most commonly reported serovars in humans being *Salmonella Enteritidis* and *S. enterica subsp. enterica serovar Typhimurium* (*S. Typhimurium*). The majority of reported outbreaks of *S. Typhimurium* are considered to be associated with foodborne transmission [3]. Since 2000, vehicles of reported *S. Typhimurium* outbreaks have included: lettuce (n = 3), duck eggs (n = 2), cooked ham (n = 1), pre-packaged salad (n = 1), pre-packaged sandwiches (n = 1), lamb (n = 1), suspected raw lamb liver (n = 1), and chocolate products (n = 1) [2, 4–6].

Whole genome sequencing (WGS) of all *Salmonella* isolates received by the UK Health Security Agency (UKHSA) (formerly Public Health England) was implemented in April 2014, which improved the detection of outbreaks of gastrointestinal illness (GI) [7, 8]. Isolates are grouped using a single-linkage clustering approach based upon single-nucleotide polymorphism (SNP) differences, enabling the identification of linked cases even when epidemiological links are obscured [7–9]. A 5-SNP threshold denotes high genetic relatedness and identifies cases likely associated with a common source [8, 9].

Point-source outbreak, Cardiff, Wales

A previously described outbreak of 22 cases of genetically related *S. Typhimurium* was detected in Cardiff, Wales, in July 2021 following an Eid al-Adha barbecue celebration [5]. Isolates belonging to the same 5-SNP single-linkage cluster were first detected in Mid Wales in April 2018 and intermittently thereafter [5]. The clinical severity of cases in the barbecue outbreak was high, with 55% of cases reporting attendance at emergency health services and 27% requiring admission [5]. Contaminated sheep meat and lamb liver consumed raw were identified as likely vehicles of infection.

Wider investigations, UK

Further *S. Typhimurium* infections belonging to the same 5-SNP single-linkage cluster to those in the point-source barbecue outbreak were subsequently identified. Here, we describe investigations into the national expansion of the cluster of *S. Typhimurium* that was linked with ruminant livestock and related food chains in the UK.

Methods

Epidemiological investigation

Descriptive study

A case was defined as a resident in the UK with laboratory-confirmed *S. Typhimurium* belonging to the same 5-SNP single-linkage cluster as the barbecue outbreak strain according to the UKHSA SNP pipeline and with a specimen date between 1 August 2021 and 31 December 2022. Barbecue attendees were excluded from analyses given their known source of infection [5].

For cases in England and Wales, routine surveillance data collected by the UKHSA Gastrointestinal Bacteria Reference Unit were used to obtain demographic and laboratory information (date of data extraction: 3 January 2023). Environmental Health Practitioners and Health Protection teams collected questionnaire information via telephone interview for all cases in Wales (as is routine) and a subset of cases in England (not routine) on exposures in the week before symptom onset, or positive specimen date if the former was missing. Data were collected on symptoms, hospital admission, travel, contacts, food history, household water supplier, pets, contact with animals and/or birds, swimming, and contact with faeces. Trawling questionnaires were used by national teams in the UKHSA and Public Health Wales (PHW) for a subset of cases using purposive sampling, prioritizing by most recent date of symptom onset, to obtain further exposure information via telephone interview. Trawling questionnaires were used to generate hypotheses pertaining to potential vehicles of infection and premises for investigation.

For cases in Scotland, routine surveillance data collected by Public Health Scotland were used to obtain demographic and laboratory information (date of data extraction: 17 March 2023).

Intelligence on localized outbreaks in England was received through personal communication with incident directors in regional UKHSA teams.

Case–case study

PHW compared exposures in cases from Wales with those of individuals notified with non-Typhimurium *Salmonella* (defined as 'case–controls') [4] with sample date between 1 August 2021 and 16 May 2022 to test associations between case status and exposures.

The ratio of case–controls to cases was 3:1. Case–controls were selected using simple random sampling. Secondary cases (defined as onset of illness ≥24 h after illness in a household with a primary case, or close contact with an individual reporting diarrhoea or vomiting) and cases who had travelled abroad in the week prior to symptom onset were excluded.

For cases, information from routine or trawling questionnaires was supplemented using an enhanced questionnaire which was based on the findings of trawling questionnaires. Interviews were conducted by the PHW Field Epidemiology Team by telephone and included detailed questions relating to the week prior to the earliest of symptom onset or specimen date. For case–controls, data were obtained from routine questionnaires on individuals with reported *Salmonella* infection (response rate: 50%).

Data analysis

Cases were summarized by time, place, and person. Continuous data were assessed for normality using histograms and the Shapiro–Wilk test [10]. Skewed data were summarized with the median and interquartile range (IQR). Categorical variables were presented in frequency tables and missing data summarized. Pearson's chi-squared [11], Fisher's exact [12] and Wilcoxon rank-sum tests [13] were used to compare differences in all cases and trawled cases, and cases and case–controls by demographic and clinical variables to assess comparability. Exposure attack rates were calculated.

Odds of exposures among cases and case–controls with 95% confidence intervals (95%CI) were calculated using logistic regression. Observations with missing values were excluded from statistical analysis. Stratified analysis was used to assess for potential confounding and effect modification. A multivariable model was created using forward-stepwise logistic regression, with each variable retained if significant ($p < 0.05$) in the likelihood ratio test [14].

Analysis was carried out using Stata v14.2 [15], and visualizations were created using RStudio v4.2.2 [16].

Environmental and microbiological investigations

Tracing supply chains and environmental sampling

Environmental Health Practitioners investigated selected premises (e.g., butchers, restaurants, cafés) reported by ≥2 cases in questionnaires. The Food Standards Agency (FSA) then made enquiries to trace supply chains to source (e.g., abattoirs). Suppliers were prioritized for inspection by the FSA if supplying ≥2 premises under investigation. Environmental swabs were taken from implicated suppliers (e.g., equipment and carcasses). Specimens were cultured for bacterial pathogens including *Salmonella* spp., and all *Salmonella* isolates were typed and sent to the Gastrointestinal Bacteria Reference Unit for confirmation and sequencing. Information on routine microbiological sampling of carcasses (in accordance with Regulation (EC) No 2073/2005 [17]) undertaken by food business operators at abattoirs and corrective enforcement action was provided by the FSA.

Animals and animal environments

The Animal and Plant Health Agency (APHA) surveillance system comprises diagnostic, serotyping, and WGS data from animal and post-mortem samples submitted as part of disease investigations, described in detail elsewhere [18]. Any *Salmonella* identified in species for which *Salmonella* is reportable under the zoonosis order is received at the *Salmonella* National Reference Laboratory at APHA Weybridge for further characterization by WGS and, on occasion, by conventional serotyping methods in parallel.
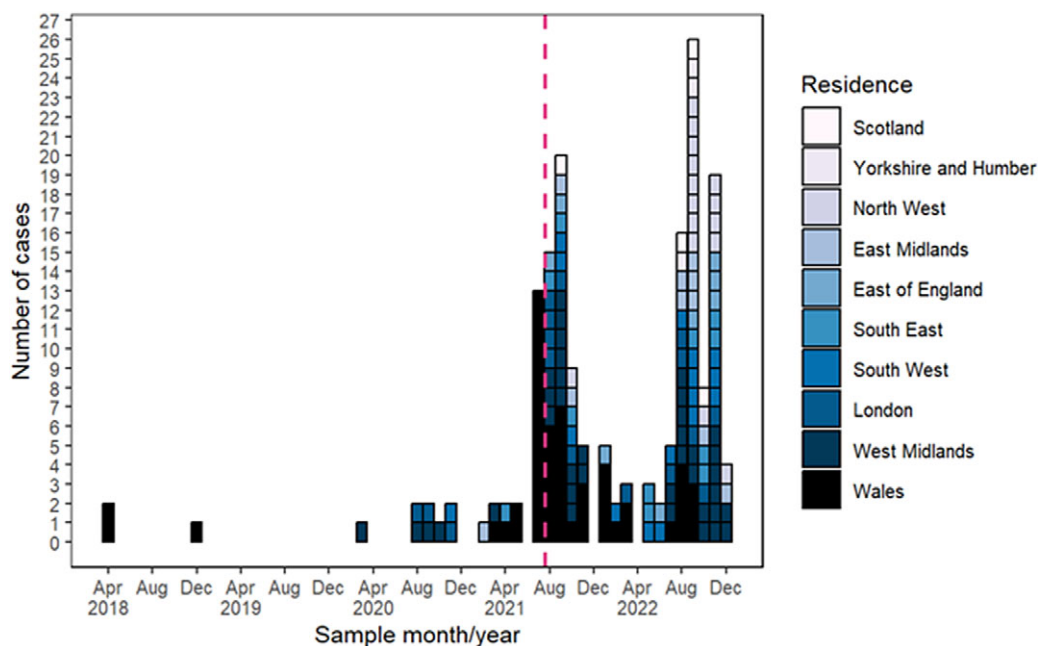
Figure 1. Epidemic curve of all cases in *Salmonella Typhimurium* five-single-nucleotide polymorphism single-linkage cluster, England, Wales, and Scotland, Apr 2018-Dec 2022 (*N* = 170). Date of barbeque outbreak indicated by vertical pink dotted line.

Data were obtained from the APHA surveillance system for samples with specimen date between 1 August 2021 and 31 December 2022.

Whole genome sequencing (WGS) and phylogenetic analysis

Isolates were sequenced on an Illumina sequencing platform, as described previously [19, 20]. Sequences were analysed to determine the sequence type, serotype, and SNP type.

Illumina reads were mapped to *S. Typhimurium* reference genome (AE006468) using BWA v0.7.12 and Samtools v1.1 [21, 22]. High-quality variants (SNPs) were identified using GATK v2.6.5 in unified genotyper mode [23]. High-quality core-genome SNPs (>90% consensus, minimum depth 10×, GQ ≥30) were extracted from SnapperDB v0.2.8 [8]. IQtree v2.0.4 [24] was used to derive the maximum-likelihood phylogeny of the isolates. Visualization and annotation of the phylogeny was performed through the iTol platform.

Ethical approval

Ethical approval was not required as this study was conducted as part of an outbreak public health response. All data containing patient identifiable information was handled and stored in compliance with the Data Protection Act (2003) and GDPR (2018).

Results

Epidemiological investigation

Descriptive study

Routine surveillance. From 1 August 2021 to 31 December 2022, 142 cases were identified in the UK, of which 11 (8%) were known to be part of a localized outbreak associated with raw drinking milk in Northwest England between September to December 2022.

Cases peaked in September following the point-source outbreak in July 2021 (Figure 1). The median age of cases was 32 (IQR: 5–57 years), and over half (59%, 81/137) were male (Figure 2). Cases were geographically dispersed across the UK, with 106 (/142, 75%) in England, 32 (23%) in Wales and four (3%) in Scotland. The incidence of cases was highest in Powys in Mid Wales (8 per 100,000 population) (Figure 3). Among cases with available information, fewer than five (/31) in England were hospitalized, and seven (/32, 22%) in Wales. No deaths due to infection with *Salmonella* were reported.

Trawling questionnaires. Between August and September 2021, 19 trawling questionnaires were completed: 11 for cases resident in England and eight in Wales. Compared to all cases, cases who completed a trawling questionnaire were younger (median: 5 years, *p* = 0.04). The majority were of White (9/19, 47%) or (8/19, 42%) Asian British ethnicity. Median symptom duration was 14 days (IQR: 10–28 days).

Eating any meat and eating outside of the home were commonly reported exposures (both 15/19, 79%). Chicken (14/19, 74%) was consumed by most cases while lamb and mutton were reportedly eaten by few (<5/19, <26%). Approximately half of cases reported washing raw meats in their household (11/19, 58%) and adhering to a halal diet (i.e., meat processed as prescribed in accordance with the Islamic faith) (9/19, 47%).

Epidemiological investigation

Analytical study

Among cases in Wales, 32 cases were eligible for inclusion in the analytical study. Cases (median: 14 years, IQR: 3–44) were younger on average than case–controls (median: 38 years, IQR: 16–58, *p* < 0.03) (Table 1). Most cases were male (19/31, 61%) but no statistical difference by sex was observed compared to case–controls (*p* = 0.3). Cases were more frequently identified in Cardiff (10/32,
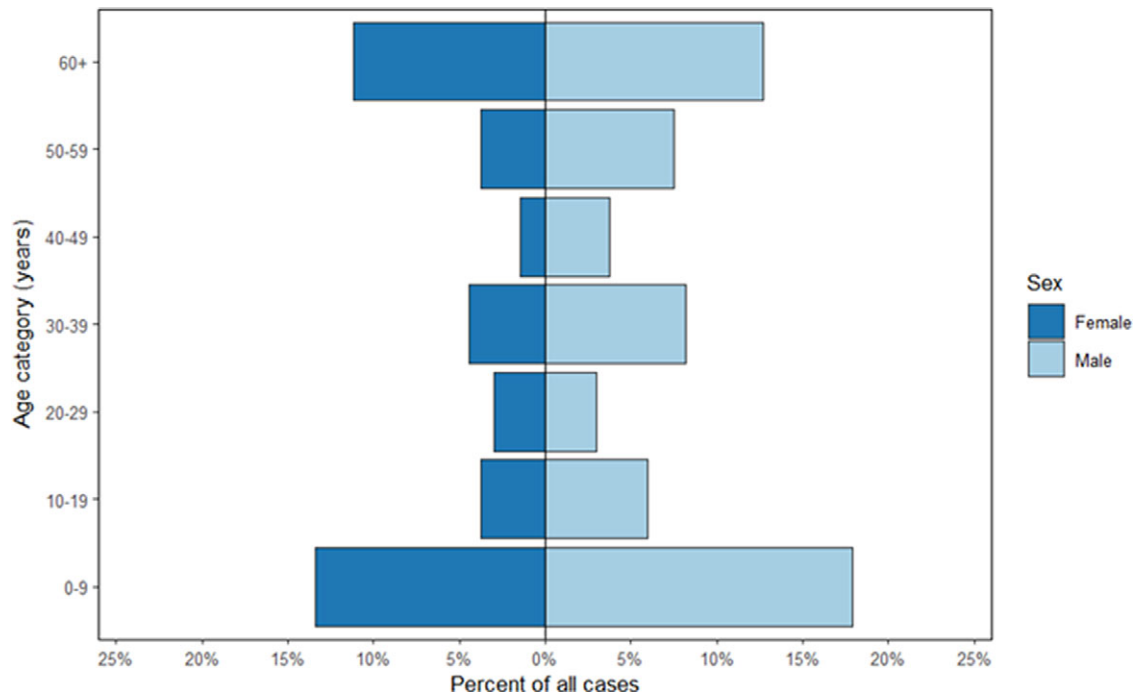
Figure 2. Age group (years) and sex of cases in *Salmonella Typhimurium* five-single nucleotide polymorphism single-linkage cluster, England, Wales, and Scotland, Aug 2021-Dec 2022 (*N* = 137). Excludes cases with unknown sex (*n* = 5).
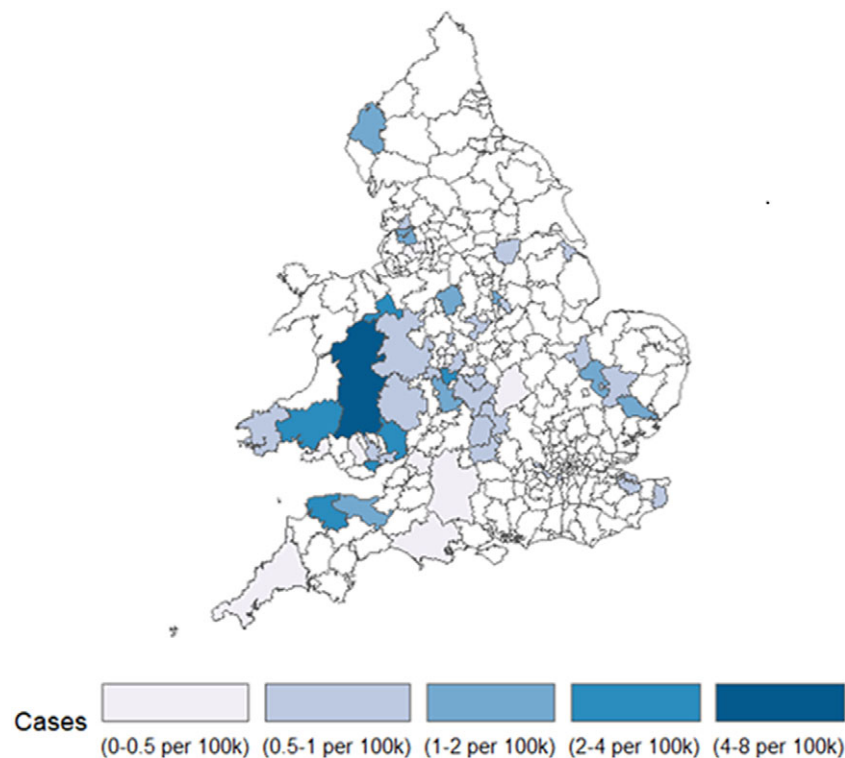

Figure 3. Incidence map of cases in *Salmonella Typhimurium* five-single-nucleotide polymorphism single-linkage cluster per 100,000 population by residential local authority, England and Wales, Aug 2021-Dec 2022 (*N* = 101). Excludes cases with insufficient address data (*n* = 41).

31%), the capital city of Wales (19/86, 20%, *p* = 0.001), than case–controls. Case–controls had infection with *Salmonella Enteritidis* (30/96, 31%), Infantis (21/96, 22%) or one of 28 other serovars. Fewer case–controls reported bloody stools (6/86, 7% of case–controls vs. 8/28, 29% of cases, *p* < 0.01).

Exposure data were missing for up to 10% (10/96) of case–controls (Table 2). In univariate analysis, illness was associated with direct contact with sheep or lambs (OR: 21.3, 95%CI: 2.5–183), any direct animal contact (OR: 3.3, 95%CI: 1.3–8.4), swimming pool use (OR: 3.1, 95%CI: 1.01–9.7), and young age (≥60

Table 1. Selected demographic, laboratory, and clinical characteristics of Salmonella non-Typhimurium controls (*N* = 96) and cases of *Salmonella Typhimurium* in five-single-nucleotide polymorphism single-linkage cluster (*N* = 32), Wales, Aug 2021–Dec 2022

Characteristic	Case–control, N = 96[a]	Case, N = 32[a]	*p*-value
Median age (IQR)	38 years (16, 58 years)	14 years (3, 44 years)	0.033
Male	47 (*49%*)	19 (*61%*)	0.3
Unknown	1	1	
Symptoms			
Diarrhoea	75 (*87%*)	27 (*96%*)	0.3
Abdominal pain	57 (*66%*)	20 (*71%*)	0.6
Fever	47 (*55%*)	19 (*68%*)	0.2
Vomiting	37 (*43%*)	8 (*29%*)	0.2
Nausea	33 (*38%*)	9 (*32%*)	0.6
Bloody stools	6 (*7%*)	8 (*29%*)	0.006
Unknown	10	4	

[a]*n* (%).
p < 0.05

vs. 10–19 years, OR: 0.08, 95%CI: 0.01–0.70). After adjusting for age and sex, the association between direct contact with sheep or lambs and illness remained (adjusted OR: 14.0, 95%CI: 1.4–145) but was reported by few (6/32 cases, 19%) (Figure 4).

Environmental and microbiological investigation

Tracing supply chains
No single food item, premises, or supplier was identified as a common link between all cases. Livestock were identified to have usually passed from farms through markets, before being batched together with animals from other farms and taken to abattoirs for slaughter. Abattoirs frequently received livestock from different suppliers, some had changed ownership in recent years, and invoice trails were difficult to follow.

Three abattoirs (A, B, and C) were identified as suppliers to premises reported by ≥2 cases. Abattoir A was a large red meat slaughterhouse which supplied butchers throughout England and Wales. The FSA took investigation and enforcement action against this abattoir after poor hygiene practices were identified in July and August 2021, including unhygienic storage of tools, bunching of carcasses (which can result in cross-contamination), and high faecal contamination of carcasses. Of all investigated abattoirs, Abattoir A was the most common supplier to implicated premises, with known links to eight premises reported by cases (Figure 5). Abattoir B was another large red meat slaughterhouse operating in England. Four premises were linked to Abattoir B. Abattoir C was a small abattoir operating in Wales with links to five premises, which were all also supplied by Abattoir A (2), B (1), or A and B (2).

Animals and animal environment sampling
S. Typhimurium isolates within the 5-SNP single-linkage cluster were identified in 47 samples from 36 premises in England and Wales submitted to the APHA for diagnostic and monitoring purposes. This included 20 samples from cattle, nine from sheep, eight from chickens, five from dogs and one from a swan. Three samples of raw pet food and one sample of mixed oil seeds intended for poultry feed were also identified in the same 5-SNP cluster.

Environmental sampling
Environmental samples from Abattoir A and B were positive for *Salmonella* spp. but did not belong to the same 5-SNP single-linkage cluster. No sampling was undertaken at Abattoir C due to its low throughput.

An isolate belonging to the same 5-SNP cluster was identified in a sheep carcass sample in Abattoir D in England in December 2021. This isolate was collected as part of routine sampling.

Phylogenetic analysis

The 5-SNP single-linkage cluster reflected some diversity (Figure 6). Highly genetically related clusters were observed for previously reported incidents, indicating common sources of infection. Isolates from animal, food, and human samples were distributed across the phylogeny.

Discussion

We provide evidence of a highly genetically related cluster of *S. Typhimurium* among humans that was associated with ruminant livestock and related food chains in the UK. The evidence for this is threefold. Firstly, individuals who had contact with sheep or lambs were 14 times more likely to become infected compared to case–controls drawn from other *Salmonella* serovars. However, fewer than one in five cases reported this exposure and there were no obvious epidemiological links between the remaining, geographically dispersed cases. It is therefore likely that the cause of infection was not only ovine, as is consistent with the isolation of the outbreak strain from wide ranging samples, including wildlife, poultry, and animal environments. Secondly, the three abattoirs identified as suppliers to premises attended by multiple cases in trace back investigations were red meat slaughterhouses, one of which had enforcement action taken against them by the FSA for process hygiene failures between July–August 2021. Thirdly, an isolate in the same 5-SNP single-linkage cluster was identified from a sheep carcass sample at another abattoir in December 2021.

Control measures exist along the farm to fork pathway to limit zoonotic and foodborne transmission of pathogens from livestock via food products to people. Control failures which occur upstream in the food chain can cascade and increase the likelihood of contamination at multiple points later in the farm to fork pathway. In April 2018, the first cases in the *S. Typhimurium* cluster were detected in Mid Wales among individuals living on a sheep farm [5]. The Incident Management Team was informed through personal communication that then, in October 2020, an isolate belonging to the same 5-SNP single-linkage cluster was isolated from an employee at a lamb and mutton supplier, Abattoir D, in England. In December 2021, the strain was linked to this abattoir again after it was isolated from a sheep carcass. Previously, this abattoir had been implicated in a similar *S. Typhimurium* outbreak that was linked to cull ewes and investigated between July 2017 and August 2019 [25, 26]. While Abattoir D was not identified as a supplier to premises attended by cases in our trace back investigations, it is possible that connections were missed due to the complexity of the distribution network and unavailability of samples and detailed food histories for all cases. Similarly, isolates from samples taken from Abattoir A or B did not belong to the same 5-SNP single-linkage cluster.

Table 2. Univariate and multivariable analysis of selected exposures of cases in *Salmonella Typhimurium* five-single-nucleotide polymorphism single-linkage cluster (*N* = 32) and Salmonella non-Typhimurium case–controls (*N* = 96) reported in the week prior to symptom onset, Wales, Aug 2021–Dec 2022

	Descriptive		Univariate			Multivariable		
Exposure	Case, *N* = 32[a]	Case–control, *N* = 96[a]	OR[b]	95% CI[b]	*p*-value	OR[b]	95% CI[b]	*p*-value
Age category (years)								
<9	7 (22%)	23 (24%)	0.37	0.12, 1.12	0.08	0.42	0.13, 1.35	0.14
10–19	14 (44%)	17 (18%)	–	–		–	–	–
20–29	<5 (<16%)	12 (12%)	0.41	0.11, 1.54	0.2	0.32	0.07, 1.42	0.13
30–39	<5 (<16%)	10 (10%)	0.25	0.05, 1.30	0.1	0.31	0.06, 1.70	0.18
40–49	<5 (<16%)	10 (10%)	0.37	0.09, 1.59	0.2	0.46	0.10, 2.05	0.31
50–59	<5 (<16%)	9 (9.4%)	0.14	0.02, 1.21	0.07	0.16	0.02, 1.50	0.11
≥60	<5 (<16%)	15 (16%)	0.08	0.01, 0.70	0.02	0.11	0.01, 0.95	0.04
Male[c]	19 (61%)	47 (49%)	1.61	0.71, 3.67	0.3	1.50	0.61, 3.67	0.37
Direct contact with sheep or lambs	6 (19%)	<5 (<6%)	21.3	2.47, 183	0.005	14.0	1.36, 145	0.03
Food item								
Lamb	<5 (<16%)	13 (15%)	0.58	0.16, 2.19	0.4	–	–	–
Beef	8 (25%)	32 (37%)	0.57	0.23, 1.40	0.2	–	–	–
Chicken	14 (44%)	46 (53%)	0.68	0.30, 1.53	0.4	–	–	–
Pork	<5 (<16%)	13 (15%)	0.58	0.16, 2.19	0.4	–	–	–
Seafood	5 (16%)	21 (24%)	0.58	0.20, 1.68	0.3	–	–	–
Salad (loose)	9 (28%)	32 (37%)	0.66	0.27, 1.60	0.4	–	–	–
Salad (bagged)	5 (16%)	20 (23%)	0.61	0.21, 1.79	0.4	–	–	–
Fruit	15 (47%)	46 (53%)	0.77	0.34, 1.73	0.5	–	–	–
Unpasteurized milk	0 (0%)	<5 (<6%)	–	–	–	–	–	–
Eggs	10 (31%)	37 (43%)	0.60	0.26, 1.42	0.2	–	–	–
Cold meats	6 (19%)	28 (33%)	0.48	0.18, 1.30	0.15	–	–	–
Dairy	11 (34%)	43 (50%)	0.53	0.23, 1.22	0.13	–	–	–
Other exposure								
Ate at commercial food vendor	13 (41%)	40 (47%)	0.79	0.35, 1.79	0.6	–	–	–
Overnight away from home (UK)	<5 (<16%)	8 (9.3%)	0.65	0.13, 3.23	0.6	–	–	–
Direct contact with any animal	12 (38%)	13 (15%)	3.33	1.32, 8.37	0.011	–	–	–
Used a swimming pool	7 (22%)	7 (8.1%)	3.12	1.01, 9.70	0.049	–	–	–
Private water supplier	<5 (<16%)	10 (12%)	0.51	0.11, 2.45	0.4	–	–	–
Outdoor activity	22 (69%)	64 (74%)	0.76	0.31, 1.84	0.5	–	–	–
Unknown	0	10	–	–	–	–	–	–

[a]*n* (%).
[b]CI, confidence interval; OR, odds ratio.
[c]Excludes unknown gender (*n* = 1).

However, both abattoirs were linked to multiple premises identified in trace back investigations and Abattoir A had enforcement action taken against them during the study period for breaches in hygiene practices. These findings are indicative of upstream control measure failures and provide biologically plausible mechanisms for the potential amplification of contamination in the human food chain from ruminant products.

As well as connections to Abattoir D, our investigation had another important commonality with the 2017–2019 outbreak of *S. Typhimurium* [25]. As previously described [5], reported cases of *S. Typhimurium* increased in July 2021 after 22 individuals linked to

an Eid al-Adha barbecue were exposed to contaminated sheep meat and a raw lamb liver dish. Eid al-Adha, meaning 'festival of sacrifice', is one of the most important festivals in the Muslim calendar [27]. In some countries, Muslims may sacrifice an animal for meat during Eid al-Adha, usually sheep [27]. In the *S. Typhimurium* outbreak in the UK that occurred between 2017–2019, rises in cases were linked to the cull ewe meat supply chain [25]. Historically, increased demand for cull ewes in the UK has been associated with the Muslim festival of Ramadan [28]. It is uncommon for *S. Typhimurium* to cause clinical disease in sheep, but host resistance can decrease and bacterial shedding can increase with stress
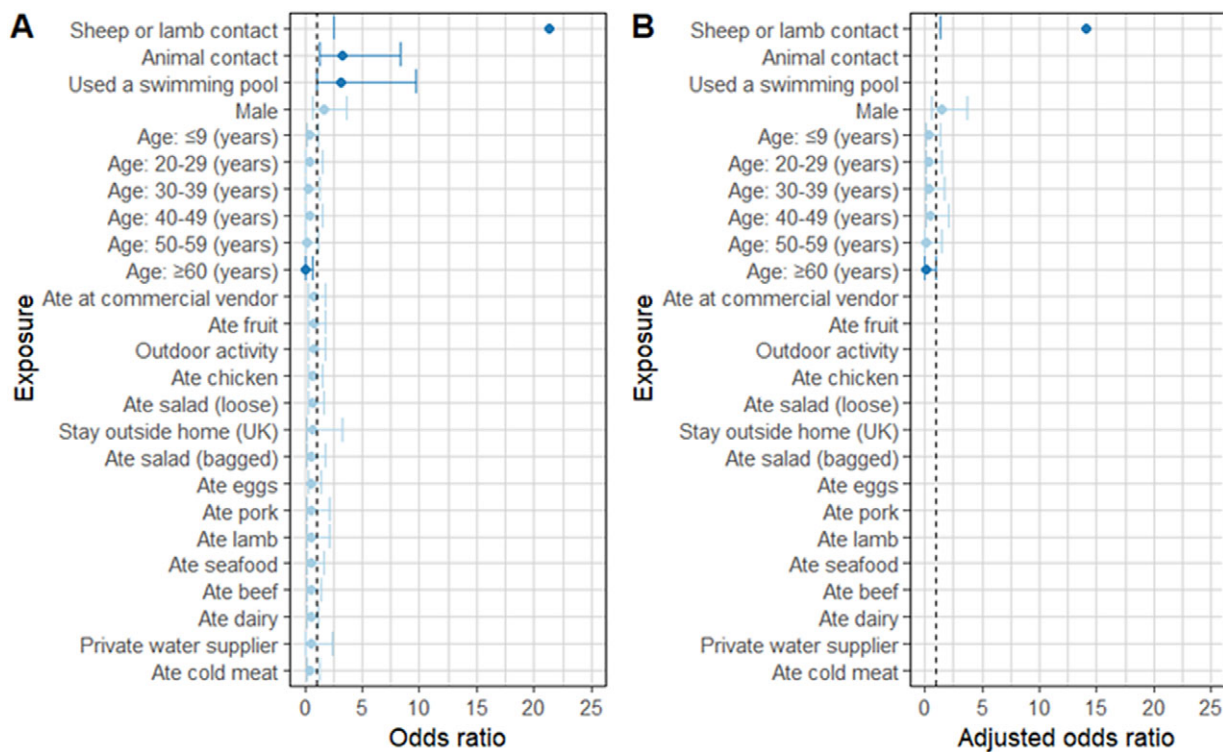
Figure 4. Forest plot of crude (a) and adjusted (b) odds of selected exposures reported by cases in *Salmonella Typhimurium* five-single-nucleotide polymorphism single-linkage cluster (*N* = 32) in the week prior to symptom onset compared to Salmonella non-Typhimurium case–controls (*N* = 96), Wales, Aug 2021-Dec 2022. Reference for age group is 10–19 years. Dark blue colour used to denote statistical significance (*p* < 0.05). Error bars not shown for 95% confidence intervals which include odds ratios >25.
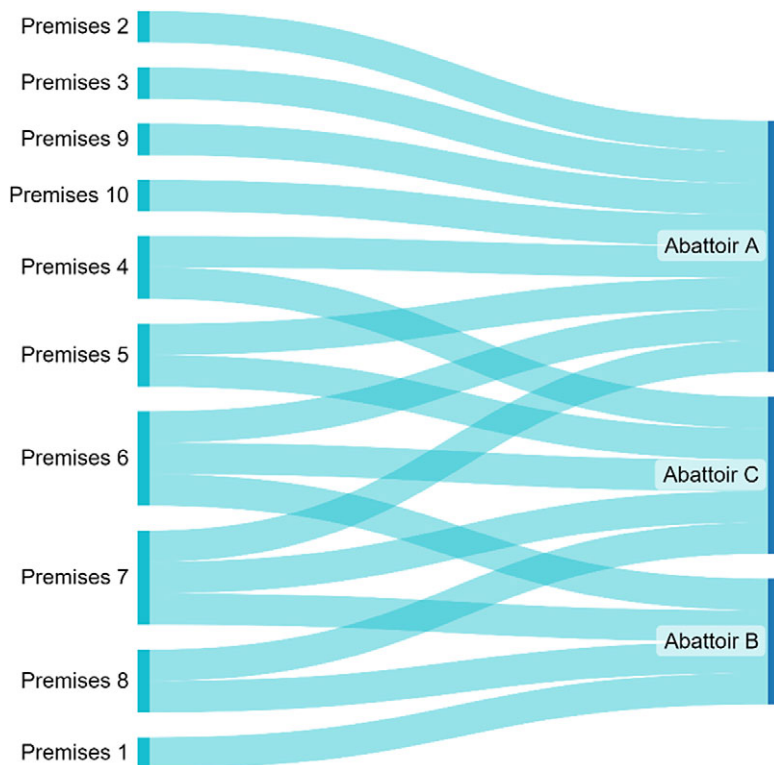
Figure 5. Flow diagram of food supply chain trace back investigations for premises reported by cases in *S. Typhimurium* five-single-nucleotide polymorphism single-linkage cluster, England and Wales, 2021.
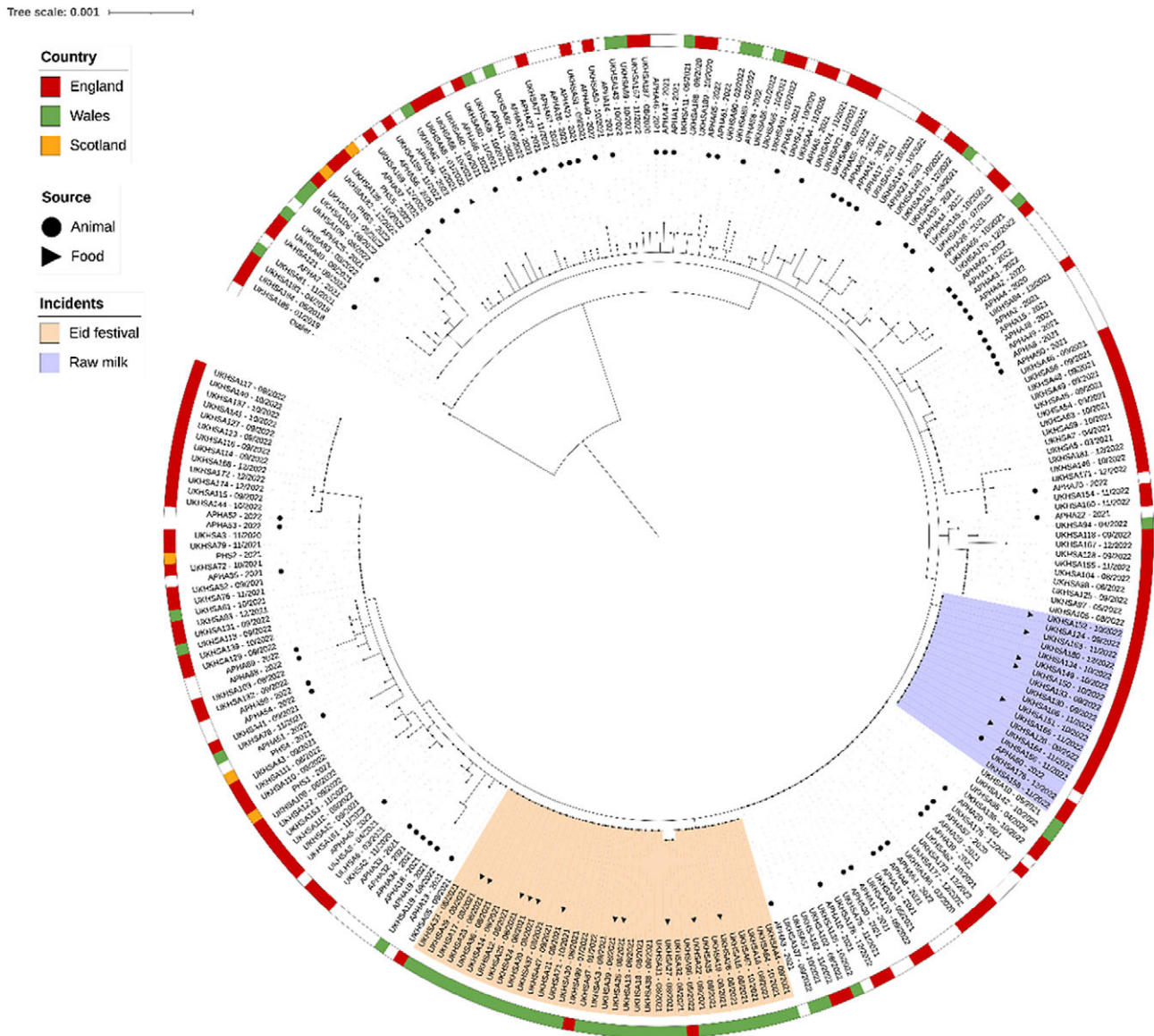
Figure 6. Maximum-likelihood phylogenetic tree of *S. Typhimurium* isolates belonging to a common five-single nucleotide polymorphism single-linkage cluster (*N* = 265).

and in the extremes of age [29]. Farms which mix and move large numbers of livestock, such as lambs or cull ewes, are said to be at high risk from *Salmonella* spp. and other diseases for this reason [30]. Supply chain and environmental contamination due to *Salmonella* spp. may therefore increase around the time of heightened demand for ruminant livestock which, when amplified by warmer summertime conditions, may increase the risk of an outbreak in humans.

Control measures

Animal production

The identification of isolates in the 5-SNP single-linkage cluster in numerous animal sources, including wildlife and poultry, was indicative of likely widespread environmental contamination and spread to other red meat sources. In response to the cluster, the APHA increased targeted communication to industry partners and the veterinary community to promote best practice in biosecurity.

This included reinforcing the requirement that visibly unwell livestock should not be sent for slaughter. Producers were also reminded of their duty to correctly complete food chain information documentation [31].

Slaughter and processing

Regulation (EC) No 2073/2005 states that food business operators have a legal responsibility to ensure that unacceptable quantities of microorganisms are not present in foodstuffs intended for human consumption [17]. For products marketed as to be eaten cooked, such as mutton and lamb, low quantities of *Salmonella* spp. are deemed acceptable permitting the review of animal origin, operator process controls and slaughter hygiene. The FSA enforced this action in Abattoir A and D.

Preparation and consumption

Proportional action was required to reduce the bacterial load in the food chain, but action was also required in the processes thereafter

to prevent further contamination, especially as not all cases in the cluster reported livestock contact or were knowingly exposed to raw meat products. Health promotion activities taken in response to the barbecue point-source outbreak are described in detail elsewhere [5]. Additional measures introduced at the level of households following the ongoing detection of cases included a social media campaign run by the FSA and PHW in 2022 promoting safe barbecuing. The campaign was informed by ongoing engagement between Environmental Health Practitioners and affected North African networks to identify appropriate modifications, such as language translation, to reach specific communities.

Onwards transmission

A 'Warn and Inform' communication was issued to Directors of Public Health, medical directors, and primary care clinicians in Wales to raise awareness of the cluster and re-iterate the need for stool samples for individuals presenting with diarrhoea. Laboratory staff in Wales conducted enhanced notification of cases indicative for *S. Typhimurium* to Health Protection Teams over the study period to rapidly alert them to possible cases. Additionally, a weekly report of trends in *Salmonella* spp. was developed by the PHW Field Epidemiology Team to monitor potential exceedances in the period surrounding Eid-al-Adha and other religious festivals, when demand for ruminant livestock products might have been heightened, in 2022. The intended effect was to improve early case detection and thus, reduce onwards person-to-person transmission.

Limitations

At the animal production level, we were not able to elucidate the extent of involved supply chains. Similarly, we did not identify a direct link throughout the farm to fork pathway. Challenges in identifying connections between the different levels of the supply chain were compounded by the limited availability of livestock samples for microbiological testing. No information was collected for the supply chain of foods consumed by case–controls and food supply chain information was also minimal for cases, both due to resource constraints and because specific address data for premises attended was often missing. Traceback investigations were therefore largely based on information provided in trawling questionnaires, for which 19 were completed. The detection of *Salmonella* spp. in sheep and carcass samples is often as part of clinical investigations of disease as, at time of writing, ruminants are not subject to national disease control plans in the same way that poultry are [18]. Abattoir sampling was likely particularly low during the study period as routine abattoir inspections had been paused during the COVID-19 pandemic, resulting in a higher workload as restrictions eased. There was also no sampling for *Salmonella* spp. at implicated premises because this was not deemed proportionate; multiple butchers and restaurants were reported so the source of infection was thought to be further upstream. Delay between symptom onset, sampling and sequencing results likely influenced cases' ability to recall exposure histories, including foods consumed and premises visited, when questioned. To limit recall bias, cases were prioritized for questionnaires according to most recent symptom onset date. Nevertheless, these factors likely impaired our ability to trace the infection to potentially many sources.

Additionally, enhanced surveillance data were only available routinely for cases in Wales, reducing power for statistical analysis and representativeness. Case–controls were selected from individuals infected with a non-Typhimurium serovar of *Salmonella* and the data collection method for obtaining exposure information differed from that of cases. The use of cases as controls is considered an appropriate comparison group for some outbreaks of GI illness [4]. However, exposures usually associated with salmonellosis, particularly the more common serovars among case–controls (*Salmonella Enteritidis* and *Salmonella* Infantis), such as poultry and eggs, may have been over-represented in the case–control group. This could have biased associations towards the null. However, the aim of our study was to elucidate exposures that were different to other common exposures to *Salmonella* serovars, such as poultry, and it was assumed that using case–controls would limit recall bias given that they too had been ill.

Conclusion

WGS-based surveillance facilitated the identification of a likely association between UK ruminant livestock product production and a wider cluster of *S. Typhimurium* that would likely not have been linked through epidemiological investigation alone. The result was a highly complex and multifactorial investigation, through which sheep were identified as one of likely many sources of infection. Similarities with a previous incident were also identified during the investigation [25], including a potential association between periods of increased demand on the ruminant livestock food supply chain and GI illness. After implementing control measures along the farm to fork pathway and once the incidence of cases had stabilized at a low level, the incident was closed in May 2023.

A multi-agency workshop was convened in June 2023 to discuss commonalities in recent *S. Typhimurium* investigations linked to ruminant livestock food chains. Here, it was agreed that data sharing, such as of human and animal sequencing information, between agencies had greatly assisted outbreak investigations. It was therefore recommended that considerations be made to how these data can be shared proactively for routine surveillance to identify and respond to animal product-related outbreaks of human disease before they can escalate.

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