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

3 Rachel Merrick<sup>1,2</sup>, Caisey Pulford<sup>3</sup>, Polani Rubeshkumar<sup>2</sup>, Parnam Seyan<sup>2</sup>, Laia Fina<sup>2</sup>, Clare Sawyer<sup>2</sup>, Nicole  
4 Pacchiarini<sup>2</sup>, Carrie Pollock<sup>4</sup>, Jonathan Lighthill<sup>4</sup>, Tina Potter<sup>4</sup>, Nathan Harvey<sup>4</sup>, Kara Thomas<sup>4</sup>, Daniel Lloyd<sup>4</sup>,  
5 Iulia Gherman<sup>4</sup>, Adrienne Mackintosh<sup>5</sup>, Joanna Lawes<sup>5</sup>, Lucy Snow<sup>5</sup>, Alison Waldram<sup>3</sup>, Lesley Larkin<sup>3</sup>, Sooria  
6 Balasegaram<sup>3</sup>, Anais Painset<sup>3</sup>, Jacquelyn McCormick<sup>3</sup>, Richard Elson<sup>3</sup>, Lynda Browning<sup>6</sup>, Christopher Williams<sup>2</sup>,  
7 Rachel Andrew<sup>2</sup>, Susan Mably<sup>2</sup> and Daniel Thomas\*<sup>2</sup> on behalf of the Incident Management Team

- 8 1. UK Field Epidemiology Training Programme (UKFETP), UK Health Security Agency, United Kingdom  
9 2. Public Health Wales, United Kingdom  
10 3. UK Health Security Agency, United Kingdom  
11 4. Food Standards Agency, United Kingdom  
12 5. Animal and Plant Health Agency, United Kingdom  
13 6. Public Health Scotland, United Kingdom

14 **\*Corresponding author:** daniel.thomas@wales.nhs.uk

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17 **SUMMARY**

18 Following an outbreak of *Salmonella* Typhimurium in Wales in July 2021 associated with sheep meat  
19 and offal, further genetically related cases were detected across the UK. Cases were UK residents  
20 with laboratory-confirmed *Salmonella* Typhimurium in the same 5-single nucleotide polymorphism  
21 (SNP) single-linkage cluster with specimen date between 01/08/2021-31/12/2022. We described  
22 cases using routine (UK) and enhanced (Wales only) surveillance data. Exposures in cases in Wales  
23 were compared with non-Typhimurium *Salmonella* case-controls. Environmental Health  
24 Practitioners and the Food Standards Agency investigated supply chains of food premises reported  
25 by  $\geq 2$  cases. Animal, carcass, and environmental samples taken for diagnostic or monitoring  
26 purposes for gastrointestinal pathogens were included in microbiological investigations. We  
27 identified 142 cases: 75% in England, 23% in Wales and 3% in Scotland. Median age was 32 years and  
28 59% were male. Direct contact with sheep was associated with becoming a case (aOR: 14, 95%CI:  
29 1.4-145) but reported by few (6/32 cases). No single food item, premises or supplier linked all cases.  
30 Multi-agency collaboration enabled the identification of isolates in the same 5-SNP single-linkage  
31 cluster from a sheep carcass at an English abattoir and in ruminant, wildlife, poultry, and  
32 environmental samples, suggesting multiple vehicles and pathways of infection.

33

34

35 **INTRODUCTION**

36 ***Salmonella* Typhimurium outbreaks, UK**

37 Non-typhoidal salmonellosis is caused by the enteric pathogen *Salmonella enterica*, a species that  
38 includes over 2600 different serovars [1]. In the United Kingdom (UK), over 8000 cases of  
39 salmonellosis are reported annually [2], with the most commonly reported serovars in humans being  
40 *Salmonella* Enteritidis and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.*  
41 Typhimurium). The majority of reported outbreaks of *S.* Typhimurium are considered to be  
42 associated with foodborne transmission [3]. Since 2000, vehicles of reported *S.* Typhimurium  
43 outbreaks have included: lettuce (n=3), duck eggs (n=2), cooked ham (n=1), pre-packaged salad  
44 (n=1), pre-packaged sandwiches (n=1), lamb (n=1), suspected raw lamb liver (n=1), and chocolate  
45 products (n=1) [2,4-6].

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

53 **Point-source outbreak, Cardiff, Wales**

54 A previously described outbreak of 22 cases of genetically related *S.* Typhimurium was detected in  
55 Cardiff, Wales in July 2021 following an Eid al-Adha barbecue celebration [5]. Isolates belonging to  
56 the same 5-SNP single-linkage cluster were first detected in Mid Wales in April 2018 and  
57 intermittently thereafter [5]. The clinical severity of cases in the barbecue outbreak was high, with  
58 55% of cases reporting attendance at emergency health services and 27% requiring admission [5].  
59 Contaminated sheep meat and lamb liver consumed raw were identified as likely vehicles of  
60 infection.

61

62 **Wider investigations, UK**

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

67 **METHODS**

68 **Epidemiological investigation**

69 *Descriptive study*

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

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

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

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

#### 91 *Case-case study*

92 PHW compared exposures in cases from Wales with those of individuals notified with non-  
93 Typhimurium *Salmonella* (defined as “case-controls”) [4] with sample date between 1 August 2021  
94 and 16 May 2022 to test associations between case-status and exposures. The ratio of case-controls  
95 to cases was 3:1. Case-controls were selected using simple random sampling. Secondary cases  
96 (defined as onset of illness  $\geq 24$  hours after illness in a household with a primary case, or close  
97 contact with an individual reporting diarrhoea or vomiting) and cases who had travelled abroad in  
98 the week prior to symptom onset were excluded.

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

#### 105 *Data analysis*

106 Cases were summarised by time, place, and person. Continuous data were assessed for normality  
107 using histograms and the Shapiro-Wilk test [10]. Skewed data were summarised with the median  
108 and interquartile range (IQR). Categorical variables were presented in frequency tables and missing  
109 data summarised. Pearson's Chi-squared [11], Fisher's exact [12] and Wilcoxon rank-sum tests [13]

110 were used to compare differences in all cases and trawled cases, and cases and case-controls by  
111 demographic and clinical variables to assess comparability. Exposure attack rates were calculated.

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

117 Analysis was carried out using Stata v14.2 [15] and visualisations were created using RStudio  
118 v4.2.2 [16].

## 119 **Environmental and microbiological investigations**

### 120 *Tracing supply chains and environmental sampling*

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

### 130 *Animals and animal environments*

131 The Animal and Plant Health Agency (APHA) surveillance system comprises diagnostic, serotyping,  
132 and WGS data from animal and post-mortem samples submitted as part of disease investigations,  
133 described in detail elsewhere [18]. Any *Salmonella* identified in species for which *Salmonella* is

134 reportable under the zoonosis order is received at the *Salmonella* National Reference Laboratory at  
135 APHA Weybridge for further characterisation by WGS and, on occasion, by conventional serotyping  
136 methods in parallel.

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

### 139 ***Whole Genome Sequencing (WGS) and phylogenetic analysis***

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

142 Illumina reads were mapped to *S. Typhimurium* reference genome (AE006468) using BWA  
143 v0.7.12 and Samtools v1.1 [21,22]. High-quality variants (SNPs) were identified using GATK v2.6.5 in  
144 unified genotyper mode [23]. High-quality core-genome SNPs (>90% consensus, minimum depth  
145 10×, GQ ≥30) were extracted from SnapperDB v0.2.8 [24]. IQtree v2.0.4 [25] was used to derive the  
146 maximum likelihood phylogeny of the isolates. Visualisation and annotation of the phylogeny was  
147 performed through the iTol platform.

### 148 **Ethical approval**

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

## 152 **RESULTS**

### 153 **Epidemiological investigation**

#### 154 *Descriptive study*

##### 155 *1. Routine surveillance*

156 From 1 August 2021 to 31 December 2022, 142 cases were identified in the UK, of which 11 (8%)  
157 were known to be part of a localised outbreak associated with raw drinking milk in Northwest

158 England between September to December 2022. Cases peaked in September following the point-  
159 source outbreak in July 2021 (Figure 1). The median age of cases was 32 (IQR: 5-57 years) and over  
160 half (59%, 81/137) were male (Figure 2). Cases were geographically dispersed across the UK, with  
161 106 (/142, 75%) in England, 32 (23%) in Wales and four (3%) in Scotland. The incidence of cases was  
162 highest in Powys in Mid Wales (8 per 100000 population) (Figure 3). Among cases with available  
163 information, fewer than five (/31) in England were hospitalised, and seven (/32, 22%) in Wales. No  
164 deaths due to infection with *Salmonella* were reported.

## 165 2. Trawling questionnaires

166 Between August and September 2021, 19 trawling questionnaires were completed: 11 for cases  
167 resident in England and eight in Wales. Compared to all cases, cases who completed a trawling  
168 questionnaire were younger (median: 5 years,  $p=0.04$ ). The majority were of White (9/19, 47%) or  
169 (8/19, 42%) Asian British ethnicity. Median symptom duration was 14 days (IQR: 10-28 days).

170 Eating any meat and eating outside of the home were commonly reported exposures (both  
171 15/19, 79%). Chicken (14/19, 74%) was consumed by most cases while lamb and mutton were  
172 reportedly eaten by few (<5/19, <26%). Approximately half of cases reported washing raw meats in  
173 their household (11/19, 58%) and adhering to a halal diet (*i.e.*, meat processed as prescribed in  
174 accordance with the Islamic faith) (9/19, 47%).

## 175 Epidemiological investigation

### 176 Analytical study

177 Among cases in Wales, 32 cases were eligible for inclusion in the analytical study. Cases (median: 14  
178 years, IQR: 3-44) were younger on average than case-controls (median: 38 years, IQR: 16-58,  $p<0.03$ )  
179 (Table 1). Most cases were male (19/31, 61%) but no statistical difference by sex was observed  
180 compared to case-controls ( $p=0.3$ ). Cases were more frequently identified in Cardiff (10/32, 31%),  
181 the capital city of Wales (19/86, 20%,  $p=0.001$ ), than case-controls. Case-controls had infection with

182 *Salmonella* Enteritidis (30/96, 31%), Infantis (21/96, 22%) or one of 28 other serovars. Fewer case-  
183 controls reported bloody stools (6/86, 7% of case-controls vs. 8/28, 29% of cases,  $p<0.01$ ).

184 Exposure data were missing for up to 10% (10/96) of case-controls (Table 2). In univariate  
185 analysis, illness was associated with direct contact with sheep or lambs (OR: 21.3, 95%CI: 2.5-183),  
186 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)  
187 and young age ( $\geq 60$  vs. 10-19 years, OR: 0.08, 95%CI: 0.01-0.70). After adjusting for age and sex, the  
188 association between direct contact with sheep or lambs and illness remained (adjusted OR: 14.0,  
189 95%CI: 1.4-145) but was reported by few (6/32 cases, 19%) (Figure 4).

## 190 **Environmental and microbiological investigation**

### 191 *1. Tracing supply chains*

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

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

205 abattoir operating in Wales with links to five premises, which were all also supplied by Abattoir A (2),  
206 B (1) or A and B (2).

### 207 2. *Animals and animal environment sampling*

208 *S. Typhimurium* isolates within the 5-SNP single-linkage cluster were identified in 47 samples from  
209 36 premises in England and Wales submitted to the APHA for diagnostic and monitoring purposes.  
210 This included 20 samples from cattle, nine from sheep, eight from chickens, five from dogs and one  
211 from a swan. Three samples of raw pet food and one sample of mixed oil seeds intended for poultry  
212 feed were also identified in the same 5-SNP cluster.

### 213 3. *Environmental sampling*

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

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

### 219 ***Phylogenetic analysis***

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

### 223 **DISCUSSION**

224 We provide evidence of a highly genetically related cluster of *S. Typhimurium* among humans that  
225 was associated with ruminant livestock and related food chains in the UK. The evidence for this is  
226 threefold. Firstly, individuals who had contact with sheep or lambs were 14 times more likely to  
227 become infected compared to case-controls drawn from other *Salmonella* serovars. However, fewer

228 than one in five cases reported this exposure and there were no obvious epidemiological links  
229 between the remaining, geographically dispersed cases. It is therefore likely that the cause of  
230 infection was not only ovine, as is consistent with the isolation of the outbreak strain from wide  
231 ranging samples, including wildlife, poultry, and animal environments. Secondly, the three abattoirs  
232 identified as suppliers to premises attended by multiple cases in trace back investigations were red  
233 meat slaughterhouses, one of which had enforcement action taken against them by the FSA for  
234 process hygiene failures between July-August 2021. Thirdly, an isolate in the same 5-SNP single-  
235 linkage cluster was identified from a sheep carcass sample at another abattoir in December 2021.

236 Control measures exist along the farm to fork pathway to limit zoonotic and foodborne  
237 transmission of pathogens from livestock via food products to people. Control failures which occur  
238 upstream in the food chain can cascade and increase the likelihood of contamination at multiple  
239 points later in the farm to fork pathway. In April 2018, the first cases in the *S. Typhimurium* cluster  
240 were detected in Mid Wales among individuals living on a sheep farm [5]. The Incident Management  
241 Team was informed through personal communication that then, in October 2020, an isolate  
242 belonging to the same 5-SNP single-linkage cluster was isolated from an employee at a lamb and  
243 mutton supplier, Abattoir D, in England. In December 2021, the strain was linked to this abattoir  
244 again after it was isolated from a sheep carcass. Previously, this abattoir had been implicated in a  
245 similar *S. Typhimurium* outbreak that was linked to cull ewes and investigated between July 2017  
246 and August 2019 [26,27]. While Abattoir D was not identified as a supplier to premises attended by  
247 cases in our trace back investigations, it is possible that connections were missed due to the  
248 complexity of the distribution network and unavailability of samples and detailed food histories for  
249 all cases. Similarly, isolates from samples taken from Abattoir A or B did not belong to the same 5-  
250 SNP single-linkage cluster. However, both abattoirs were linked to multiple premises identified in  
251 trace back investigations and Abattoir A had enforcement action taken against them during the  
252 study period for breaches in hygiene practices. These findings are indicative of upstream control

253 measure failures and provide biologically plausible mechanisms for the potential amplification of  
254 contamination in the human food chain from ruminant products.

255 As well as connections to Abattoir D, our investigation had another important commonality  
256 with the 2017-2019 outbreak of *S. Typhimurium* [26]. As previously described [5], reported cases of  
257 *S. Typhimurium* increased in July 2021 after 22 individuals linked to an Eid al-Adha barbecue were  
258 exposed to contaminated sheep meat and a raw lamb liver dish. Eid al-Adha, meaning “festival of  
259 sacrifice”, is one of the most important festivals in the Muslim calendar [28]. In some countries,  
260 Muslims may sacrifice an animal for meat during Eid al-Adha, usually sheep [28]. In the *S.*  
261 *Typhimurium* outbreak in the UK that occurred between 2017-2019, rises in cases were linked to the  
262 cull ewe meat supply chain [26]. Historically, increased demand for cull ewes in the UK has been  
263 associated with the Muslim festival of Ramadan [29]. It is uncommon for *S. Typhimurium* to cause  
264 clinical disease in sheep, but host resistance can decrease and bacterial shedding can increase with  
265 stress and in the extremes of age [30]. Farms which mix and move large numbers of livestock, such  
266 as lambs or cull ewes, are said to be at high risk from *Salmonella* spp. and other diseases for this  
267 reason [31]. Supply chain and environmental contamination due to *Salmonella* spp. may therefore  
268 increase around the time of heightened demand for ruminant livestock which, when amplified by  
269 warmer summertime conditions, may increase the risk of an outbreak in humans.

## 270 *Control measures*

### 271 1. *Animal production*

272 The identification of isolates in the 5-SNP single-linkage cluster in numerous animal sources,  
273 including wildlife and poultry, was indicative of likely widespread environmental contamination and  
274 spread to other red meat sources. In response to the cluster, the APHA increased targeted  
275 communication to industry partners and the veterinary community to promote best practice in  
276 biosecurity. This included reinforcing the requirement that visibly unwell livestock should not be

277 sent for slaughter. Producers were also reminded of their duty to correctly complete food chain  
278 information documentation [33].

## 279 2. *Slaughter and processing*

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

## 285 3. *Preparation and consumption*

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

## 295 4. *Onwards transmission*

296 A “Warn and Inform” communication was issued to Directors of Public Health, medical directors, and  
297 primary care clinicians in Wales to raise awareness of the cluster and re-iterate the need for stool  
298 samples for individuals presenting with diarrhoea. Laboratory staff in Wales conducted enhanced  
299 notification of cases indicative for *S. Typhimurium* to Health Protection Teams over the study period  
300 to rapidly alert them to possible cases. Additionally, a weekly report of trends in *Salmonella* spp. was

301 developed by the PHW Field Epidemiology Team to monitor potential exceedances in the period  
302 surrounding Eid-al-Adha and other religious festivals, when demand for ruminant livestock products  
303 might have been heightened, in 2022. The intended effect was to improve early case detection and  
304 thus, reduce onwards person-to-person transmission.

#### 305 *Limitations*

306 At the animal production level, we were not able to elucidate the extent of involved supply chains.  
307 Similarly, we did not identify a direct link throughout the farm to fork pathway. Challenges in  
308 identifying connections between the different levels of the supply chain were compounded by the  
309 limited availability of livestock samples for microbiological testing. No information was collected for  
310 the supply chain of foods consumed by case-controls and food supply chain information was also  
311 minimal for cases, both due to resource constraints and because specific address data for premises  
312 attended was often missing. Traceback investigations were therefore largely based on information  
313 provided in trawling questionnaires, for which 19 were completed. The detection of *Salmonella* spp.  
314 in sheep and carcass samples is often as part of clinical investigations of disease as, at time of  
315 writing, ruminants are not subject to national disease control plans in the same way that poultry are  
316 [18]. Abattoir sampling was likely particularly low during the study period as routine abattoir  
317 inspections had been paused during the COVID-19 pandemic, resulting in a higher workload as  
318 restrictions eased. There was also no sampling for *Salmonella* spp. at implicated premises because  
319 this was not deemed proportionate; multiple butchers and restaurants were reported so the source  
320 of infection was thought to be further upstream. Delay between symptom onset, sampling and  
321 sequencing results likely influenced cases' ability to recall exposure histories, including foods  
322 consumed and premises visited, when questioned. To limit recall bias, cases were prioritised for  
323 questionnaires according to most recent symptom onset date. Nevertheless, these factors likely  
324 impaired our ability to trace the infection to potentially many sources.

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

### 336 *Conclusion*

337 WGS-based surveillance facilitated the identification of a likely association between UK ruminant  
338 livestock product production and a wider cluster of *S. Typhimurium* that would likely not have been  
339 linked through epidemiological investigation alone. The result was a highly complex and  
340 multifactorial investigation, through which sheep were identified as one of likely many sources of  
341 infection. Similarities with a previous incident were also identified during the investigation [26],  
342 including a potential association between periods of increased demand on the ruminant livestock  
343 food supply chain and GI illness. After implementing control measures along the farm to fork  
344 pathway and once the incidence of cases had stabilised at a low level, the incident was closed in May  
345 2023.

346 A multi-agency workshop was convened in June 2023 to discuss commonalities in recent *S.*  
347 *Typhimurium* investigations linked to ruminant livestock food chains. Here, it was agreed that data  
348 sharing, such as of human and animal sequencing information, between agencies had greatly  
349 assisted outbreak investigations. It was therefore recommended that considerations be made to

350 how these data can be shared proactively for routine surveillance to identify and respond to animal  
351 product-related outbreaks of human disease before they can escalate.

### 352 **Acknowledgements**

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360 taking the time to share their personal information for the purposes of this investigation.

### 361 **Authors' contributions**

362 RA, SM and DT jointly chaired the Incident Management Team. DT led the Incident Management  
363 Team Epidemiology Subgroup. RM, RP, DT, LL, SB, and CW designed the case-case study. CP, JM, LL  
364 and RE provided data for cases in England and Wales. LB provided data for cases in Scotland. AM, JL,  
365 and LS led and provided data on animal and animal environment investigations. CPO, JLi, TP, and NH  
366 led and provided data on supply chain trace back and environmental investigations. KT, DL, and IG  
367 provided input on risk assessments for the Food Standards Agency. RM performed the data analysis.  
368 RP created the food supply chain flow diagram. AP created the phylogeny, with input from CP, JM,  
369 RM, and LS. RM and DT drafted the manuscript. AP drafted the whole-genome sequencing and  
370 phylogenetic analysis methods and results sections. All authors reviewed the manuscript and  
371 approved the final version.

### 372 **Declaration of interest**

373 Authors RP, PS, LF, CS, NP, RS, CW, RA, SM and DT are employed by Public Health Wales. Authors  
374 RM, CP, LL, SB, AP, JM and RE are employed by the UK Health Security Agency. Author LB is  
375 employed by Public Health Scotland. Authors AM, JL, and LS are employed by the Animal and Plant  
376 Health Agency. Authors CPo, JLi, TP, NH, KT, DL, and IG are employed by the Food Standards Agency.

### 377 **Data availability statement**

378 The sequencing data that support the findings of this study are openly available in Enterobase  
379 (<https://enterobase.warwick.ac.uk>), reference number [34].

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467 **Tables**

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

<b>Characteristic</b>	<b>Case-control, N = 96<sup>1</sup></b>	<b>Case, N = 32<sup>1</sup></b>	<b>p-value</b>
<b>Median age (IQR)</b>	38 years (16, 58 years)	14 years (3, 44 years)	0.033
<b>Male</b>	47 (49%)	19 (61%)	0.3
Unknown	1	1	
<b>Symptoms</b>			
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	
<sup>1</sup> n (%)			

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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

Exposure	Descriptive		Univariate			Multivariable		
	Case, N = 32 <sup>1</sup>	Case-control, N = 96 <sup>2</sup>	OR <sup>2</sup>	95% CI <sup>2</sup>	p-value	OR <sup>2</sup>	95% CI <sup>2</sup>	p-value
<b>Age category (years)</b>								
<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
<b>Male<sup>3</sup></b>	19 (61%)	47 (49%)	1.61	0.71, 3.67	0.3	1.50	0.61, 3.67	0.37
<b>Direct contact with sheep or lambs</b>	6 (19%)	<5 (<6%)	21.3	2.47, 183	0.005	14.0	1.36, 145	0.03
<b>Food item</b>								
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	-	-	-
Unpasteurised 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	-	-	-

Exposure	Descriptive		Univariate			Multivariable		
	Case, N = 32 <sup>1</sup>	Case-control, N = 96 <sup>2</sup>	OR <sup>2</sup>	95% CI <sup>2</sup>	p-value	OR <sup>2</sup>	95% CI <sup>2</sup>	p-value
<b>Other exposure</b>								
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	-	-	-	-	-	-

<sup>1</sup>n (%)

<sup>2</sup>OR = Odds Ratio, CI = Confidence Interval

<sup>3</sup>Excludes unknown gender (n=1)

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