

A multi-country *Salmonella* Enteritidis phage type 14b outbreak associated with eggs from a German producer: 'near real-time' application of whole genome sequencing and food chain investigations, United Kingdom, May to September 2014

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Citation style for this article:

Inns T, Lane C, Peters T, Dallman T, Chatt C, McFarland N, Crook P, Bishop T, Edge J, Hawker J, Elson R, Neal K, Adak GK, Cleary P, on behalf of the Outbreak Control Team. A multi-country *Salmonella* Enteritidis phage type 14b outbreak associated with eggs from a German producer: 'near real-time' application of whole genome sequencing and food chain investigations, United Kingdom, May to September 2014. *Euro Surveill.* 2015;20(16):pii=21098. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21098>

Article submitted on 12 November 2014 / published on 23 April 2015

We report an outbreak of *Salmonella* Enteritidis phage type 14b (PT14b) in the United Kingdom (UK) between May and September 2014 where Public Health England launched an investigation to identify the source of infection and implement control measures. During the same period, outbreaks caused by a *Salmonella* Enteritidis strain with a specific multilocus variable-number tandem repeat analysis (MLVA) profile occurred in other European Union Member States. Isolates from a number of persons affected by the UK outbreak, who had initially been tested by MLVA also shared this particular profile. Cases were defined as any person infected with *S. Enteritidis* PT14b, resident in England or Wales and without history of travel outside of this geographical area during the incubation period, reported from 1 June 2014 onwards, with a MLVA profile of 2-11-9-7-4-3-2-8-9 or a single locus variant thereof. In total, 287 cases met the definition. Food traceback investigations in the UK and other affected European countries linked the outbreaks to chicken eggs from a German company. We undertook whole genome sequencing of isolates from UK and European cases, implicated UK premises, and German eggs: isolates were highly similar. Combined with food traceback information, this confirmed that the UK outbreak was also linked to a German producer.

Introduction

The adoption of vaccination and other measures in management of poultry production has led to a reduction in the number of *Salmonella enterica* serovar Enteritidis infections in the United Kingdom (UK) [1,2]. Despite this reduction, there have been several outbreaks of

S. Enteritidis phage type 14b (PT14b) in the UK which have been associated with chicken eggs originating from outside the UK [3-5].

At the beginning of June 2014, Public Health England (PHE) was alerted to an outbreak of *S. Enteritidis* PT14b in a hospital in central England. Following this, outbreaks of *S. Enteritidis* PT14b cases were detected in the North West and South of England. In addition, PHE was alerted through the Epidemic Intelligence Information System (EPIS) to six *Salmonella* outbreaks in France associated with eggs from a German producer and an Austrian *Salmonella* outbreak, both countries reporting matching multilocus variable-number tandem repeat analysis (MLVA) profiles of 2-11-9-7-4-3-2-8-9 [6]. Following the detection of an exceedance in the cumulative number of *S. Enteritidis* PT14b cases in England and Wales (Figure 1), some of whom reported the same MLVA profile, PHE launched a national investigation; the first national outbreak control team meeting was held on 6 August 2014. The investigation was undertaken to identify the source of infection and implement control measures to prevent further cases.

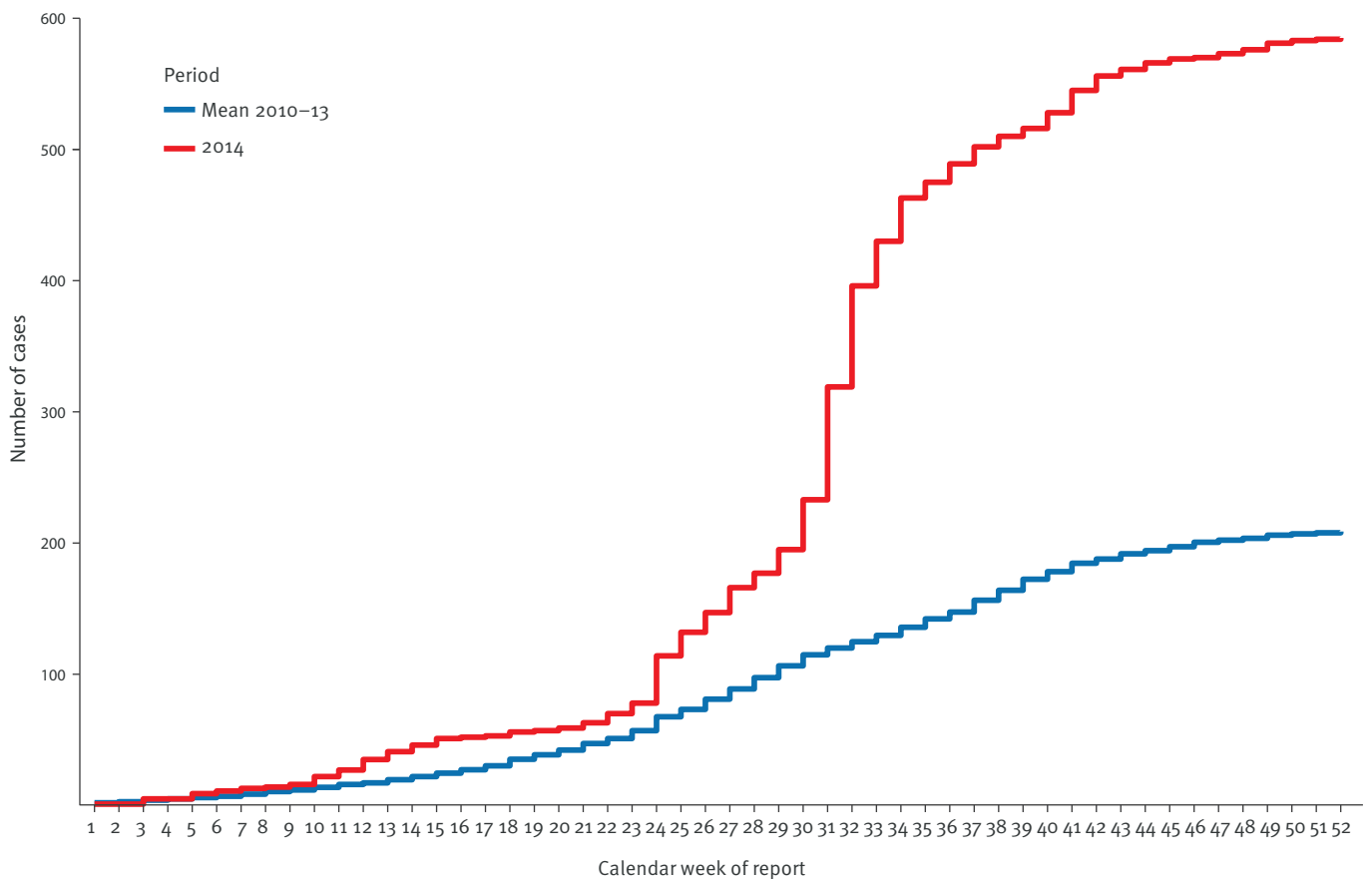
Methods

Epidemiological investigations

Case ascertainment was from statutory notifications of cases of *Salmonella* infection. A case was defined as any person infected with *S. Enteritidis* PT14b, resident in England or Wales and without history of travel outside of this geographical area during the incubation period (usually up to 72 hours) [7], reported on or

FIGURE 1

Cumulative count by calendar week, of *Salmonella* Enteritidis PT14b cases in England and Wales, 2014 compared with the 2010–2013 mean



after 1 June 2014, with a MLVA profile of 2–11–9–7–4–3–2–8–9, or a single locus variant thereof (the MLVA outbreak profile). Cases were interviewed using local questionnaires to ascertain foods eaten in the five days before onset of symptoms. These questionnaires differed depending on where in the UK the case was interviewed. Shops, restaurants and other food outlets reported by cases were identified and when a certain premise was related to several cases, environmental or food samples were taken, where possible.

Food traceback investigations

In addition to the information received through EPIS, Rapid Alert System for Food and Feed (RASFF) notifications were issued on 9 July 2014 (France), 31 July 2014 (Austria) and 1 August 2014 (France). These notifications linked *S. Enteritidis* outbreaks in France and Austria to chicken eggs from Company X in Germany. Subsequent updates to the RASFF notifications indicated that the outbreaks were caused by *S. Enteritidis* PT14b. The MLVA results of clinical isolates from France were first reported on 14 August 2014. Food chain investigations involved obtaining information on the supply of eggs from Company X to UK distributors and tracing onward supply from these distributors to other UK companies. In addition, food chain investigations were conducted in England and Wales to trace supplies

of chicken and chicken eggs consumed by cases to their source, where possible.

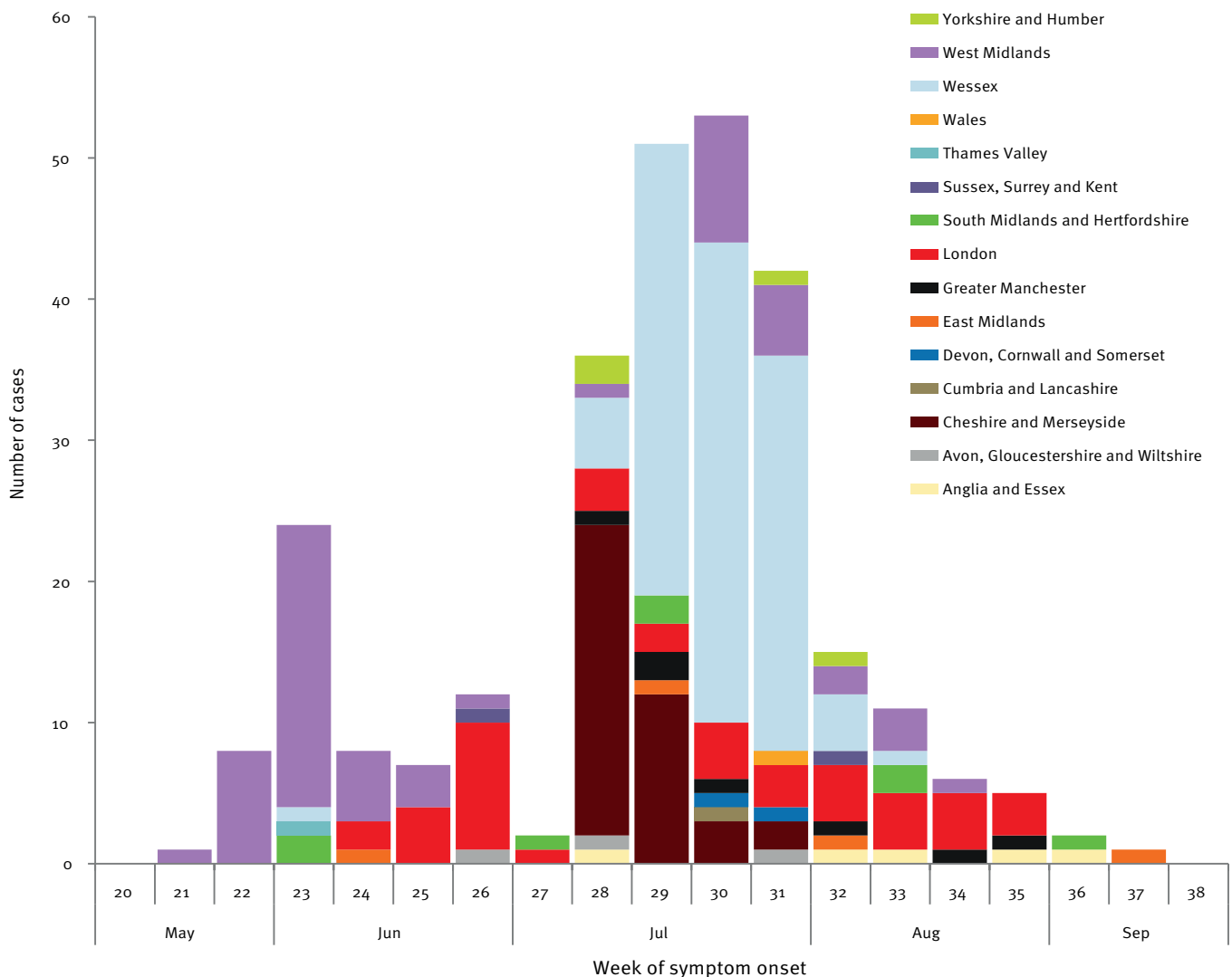
Microbiological investigations

S. Enteritidis strains conformed to the recognised pattern for phage PT14b as described in the current schemes [8]. Isolates were further characterised by MLVA typing [9] and whole genome sequencing (WGS). Sequencing was carried out by the PHE Genome Sequencing Unit using Nextera library preparation and the Illumina HiSeq 2500 in fast run mode according to manufacturers' instructions.

High quality Illumina reads were mapped to the *S. enterica* Enteritidis reference genome (GenBank accession number: AM933172) using BWA-MEM [10]. Single nucleotide polymorphisms (SNPs) were then identified using GATK2 in unified genotyper mode [11]. The core genome is defined as nucleotide positions that are shared between the reference strain and all other strains in the analysis. Core genome positions that had a high quality SNP (>90% consensus, minimum depth 10x, GQ≥30) in at least one strain were extracted and RaxML used to derive the maximum likelihood phylogeny of the isolates [12]. FASTQ reads from all sequences in this study can be found at the PHE Pathogens

FIGURE 2

Distribution of *Salmonella* Enteritidis phage type 14b, by calendar week of symptom onset and Public Health England centre of residence, England and Wales, week 20–38 2014 (n=284)^a



^a Symptom onset dates were not available for three of the 287 outbreak cases.

BioProject at the National Center for Biotechnology Information (accession: PRJNA248792).

International investigations

As international communications had identified that cases had also occurred in other European Union (EU) Member States (Germany and Luxembourg), WGS was undertaken on isolates from outside the UK. This included four isolates from France comprising two from human cases and two from eggs originating from Company X. One human isolate was received from Luxembourg. Six isolates were received from Austria, all from humans. Fourteen isolates were received from Germany; five from humans, one from a cake and eight from eggs from Company X (six from one Company X site, two from another Company X site).

Results

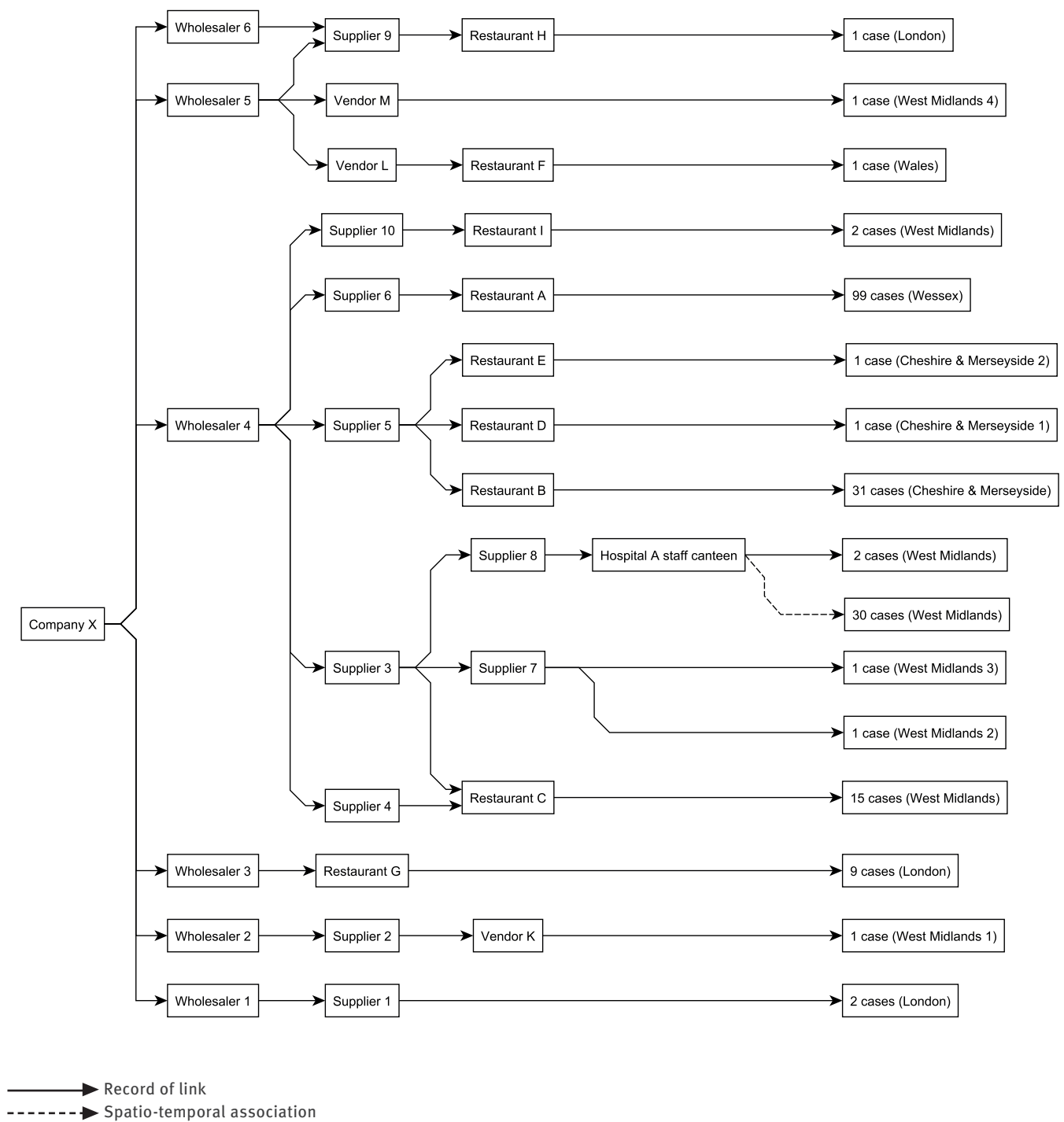
Outbreak description

In total, 287 cases met the definition; ages ranged from <1 to 92 years (median 29), 151 (53%) were male. Seventy-eight (27%) cases were reported to have been hospitalised (of whom 61 were not thought to have acquired their infection while in hospital). Symptom onset dates ranged from 25 May 2014 to 7 September 2014. The week of symptom onset is shown in Figure 2; this also includes information on the residence of cases. A number of outbreak cases were associated with specific premises which are briefly described below.

Between 25 May and 18 June 2014, 32 cases (patients, staff and visitors) were linked to a single hospital in

FIGURE 3

Egg supply network showing links between Company X and *Salmonella* Enteritidis phage type14b cases in England and Wales, 2014



central England (hospital A), of whom 17 had spent the whole incubation period in the hospital. *Salmonella* infection was considered to be a contributory factor in the cause of death for one patient.

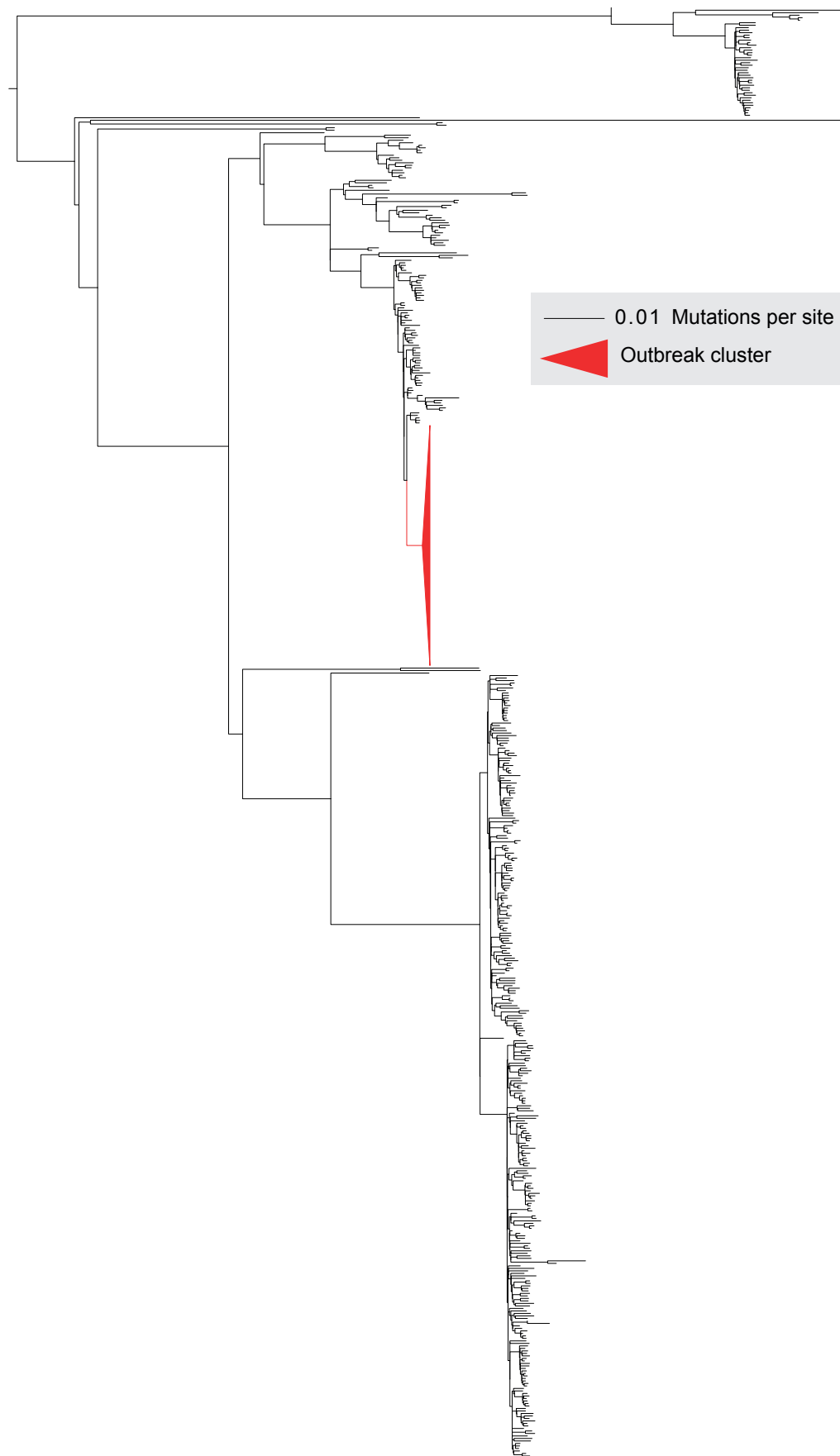
Ninety-nine cases were linked to restaurant A in southern England. Cases reported eating at the restaurant between 11 July and the closure of the restaurant on 24 July to improve food hygiene practices.

Between 8 and 19 July 2014, 31 cases were linked to restaurant B in North West England.

Fourteen cases were linked to restaurant C in central England. Cases reported symptom onsets between 24 July and 6 August 2014. The restaurant closed voluntarily between 7 and 13 August 2014. One further case occurred after this on 1 September.

FIGURE 4

Phylogeny of *Salmonella* Enteritidis isolates, including representative isolates from England and Wales (n = 484) sequenced between January 2012 and September 2014, together with outbreak isolates from Austria, France, Germany, Luxembourg and the United Kingdom, occurring from May to September 2014 (n = 332)



Following epidemiological and environmental investigations, *Salmonella* was isolated from a catering trolley on one of the wards of hospital A. *Salmonella* was also isolated from food samples (cooked chicken and pork) and an environmental sample (a cleaning cloth) taken at the restaurant B premises. At restaurant C, a dishcloth, a swab from a vegetable preparation sink and a sample of egg-containing vegetarian noodles all tested positive for *Salmonella*. The food and environmental samples from hospital A, restaurants B and C which were positive for *Salmonella* were typed as *S. Enteritidis* PT14b, with the same MLVA profile as cases. Six eggs of Spanish origin were sampled from restaurant C on 12 August 2014; all tested negative for *Salmonella*.

Food chain investigations

In total, 198 of 287 (69%) cases could be plausibly linked to eggs supplied by one company, Company X (Figure 3). Thirty-two cases ate at premises where eggs with a Company X egg stamp number were observed; 166 cases were linked to premises that were plausibly supplied by Company X at the time of exposure according to information from suppliers. Restaurants A, B and C were all supplied with eggs originating from Company X, as was one outlet at hospital A (although most cases did not report eating food from this outlet). It appeared that the majority of cases in England may have acquired the infection via catering services, rather than from eggs obtained from retail establishments.

Company X has four sites, three in Germany, one in the Czech Republic, all operationally independent. All four sites use young chickens (pullets) from two sites, one in Germany and one in the Czech Republic. The last delivery to the UK from all three German sites was on the 17 July 2014; the last delivery of eggs from the Czech Republic site was on 1 September 2014.

Microbiology

The human isolates from France, Austria, Germany and Luxembourg all had the outbreak MLVA profile. The eggs from Company X shared the outbreak MLVA profile. Initial WGS results were available by 26 August 2014 for both UK (including 20 environmental samples and the 287 clinical isolates) and non-UK samples. The WGS results showed that the 332 clinical and environmental samples from Austria, France, Germany, Luxembourg and the United Kingdom clustered phylogenetically into a tight cluster on the *S. Enteritidis* phylogeny (Figure 4). Within the outbreak cluster the minimum SNP distance between strains was 0 and the maximum 23 SNPs. Within the hospital outbreak A and the restaurant outbreaks A, B and C the mode SNP distance was 0 SNPs with no clinical isolate differing by greater than 2 SNPs. Implicated environmental isolates were either identical or a single SNP away from a clinical isolate. Clinical isolates from the rest of Europe clustered within clinical isolates from England

and Wales as did all eight isolates from eggs from Company X.

Control measures

Following the reported French outbreaks, investigations at one of the German sites found *Salmonella* in chicken faeces and dust, along with eggs positive for *Salmonella* with the MLVA outbreak profile; investigations at another German site also found eggs positive for the outbreak strain. At both sites public health control measures were taken in August 2014, these included ensuring that affected eggs were properly processed before human consumption.

In the UK, premises associated with clusters were investigated by environmental health officers to ensure compliance with food hygiene guidelines. As the available evidence indicated that potentially affected eggs had been supplied to catering establishments in the UK, on 22 August 2014 the FSA sent letters to UK local authorities which asked them to contact catering establishments in their area and reiterate FSA advice on how to cook and prepare eggs safely. In the letters, the FSA asked local authorities to look in catering establishments for eggs with the three egg stamp numbers relating to Company X's German premises, but no reports of finding these eggs were received. On 22 August 2014, caterers were also reminded of the guidelines for the safe handling of eggs via the FSA website [13]. As the available evidence suggested that the potentially affected eggs had been distributed to catering, rather than retail establishments in the UK, it was not necessary to recall them from consumers. Enhanced infection control procedures were introduced in hospital A to reduce the risk of person to person spread.

Discussion

We present WGS data which provide a clear link between isolates from humans, eggs and environmental samples from premises associated with clusters of cases in an outbreak affecting several EU Member States. This, along with the egg supply network information and information from investigations in other European countries, provides compelling evidence to support the hypothesis that this outbreak was associated with eggs from a German producer (Company X).

This outbreak demonstrated the importance of MLVA which was used to identify this multi-country *Salmonella* outbreak, and the use of WGS which further confirmed the findings. WGS allows improved discrimination between isolates, and adds a new dimension to descriptive epidemiology in the form of phylogenetic relationships [14]. WGS has previously been used for the prospective surveillance of *Salmonella* [15] and to confirm a multi-country *Salmonella* outbreak in Europe [16], but here it was used for the first time in 'near real-time' to define a multi-country *Salmonella* outbreak and inform public health control measures.

During this investigation, no eggs supplied by Company X were found in the UK for testing; this most likely reflects the delay between egg consumption, symptom onset, phage typing, food history taking and egg sampling. The delay between egg consumption and sampling is usually greater than the shelf life of eggs which is typically 26 days (Mark Jones, AHVLA, personal communication, 24 September 2014), making it inherently unlikely that eggs identified at catering premises during this outbreak investigation were the ones consumed by cases.

An interesting aspect of the WGS results is that within the outbreak cluster there was a maximum genetic distance of 23 SNPs. Within each of the restaurant outbreaks (A-C) and the hospital outbreak, strains differed between 0-2 SNPs. We hypothesise that, while the outbreak cluster formed a monophyletic group, these differences between point source outbreaks were due to eggs that had a degree of *S. Enteritidis* variation at the source of the contamination in the various Company X sites.

We present genetic and food supply information which support the hypothesis that this multinational *S. Enteritidis* PT14b outbreak was associated with eggs from a German producer. This investigation demonstrates the importance of European cooperation when investigating complex food supply networks. Information, both official and informal, from other European countries was important in both detecting the outbreak and ensuring that public health actions in the UK were as timely as possible. We therefore recommend greater use of the RASFF and EPIS systems to exchange intelligence on outbreaks and contaminated foodstuffs both between and within European countries.

Being able to sequence isolates from German eggs made the genetic evidence linking this source to UK cases more compelling. We therefore recommend that EU Members States support measures to create a framework to ensure that public health control measures are enhanced by the exchange of pathogen sequencing information.

Members of the Outbreak Control Team

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Acknowledgements

We gratefully acknowledge the contribution of Environmental Health Officers in undertaking food traceback investigations. We also acknowledge those involved in the investigations of outbreaks in Hospital A and restaurants A, B and C. We would like to thank Wolfgang Rabsch, Simon Le Hello, Joel Mossong and Christian Kornschober for sending isolates from Germany, France, Luxembourg and Austria, respectively.

Conflict of interest

None declared.

Authors' contributions

Epidemiological investigations were conducted by CL, CC, NM, PCr, JH, RE, KN and PCI. Microbiological investigations were co-ordinated by TD and TP. Food traceback investigations were coordinated by TB and JE. The manuscript was drafted by TI. All authors commented and agreed upon the final manuscript.

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