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Key role of whole genome sequencing in resolving an international outbreak of monophasic *Salmonella* Typhimurium linked to chocolate products

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Abstract

Background In February 2022, the United Kingdom (UK) detected a cluster of monophasic *Salmonella* Typhimurium based on whole genome sequencing (WGS). Subsequently, several countries reported cases belonging to this cluster. Epidemiological, microbiological and traceability investigations pointed toward a chocolate food business operator (FBO) in Belgium. We describe the magnitude of the outbreak, investigations performed and control measures taken.

Methods Cases were ascertained based on internationally agreed case definitions and interviewed about food consumption prior to disease onset. Analytical epidemiological studies were conducted by the UK and Ireland. The Belgian food safety authority (FSA) coordinated microbiological and traceability investigations.

Results A total of 456 cases (61% female), belonging to two genetically different WGS clusters, in 14 countries of the European Union, the UK, Switzerland, Canada and the United States were linked to the outbreak, between December 2021 and June 2022. 87% of cases were younger than 10 years. Brand A chocolate eggs, marketed for children, were reported as consumed by 168 cases (80%) with information. Analytical studies in the UK and Ireland showed a significantly higher odds of disease associated with consumption of brand A chocolate products. Retrospective investigations by the FSA revealed that routine samples (raw materials, intermediate, semi-finished and finished products as well as environmental and rinse oil samples) taken by the FBO linked to the Brand A products between December 2021 and January 2022 had tested positive for salmonella. Nine isolates were submitted to EnteroBase and matched with human isolates from both WGS clusters. The authorization for production was temporarily withdrawn on 8 April 2022 and all products of brand A were recalled worldwide, followed by a decrease in cases.

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Conclusions A multi-country salmonellosis outbreak linked to chocolate occurred in the months before Easter 2022. International collaboration between stakeholders from epidemiological, microbiological and food safety entities with rapid sharing of WGS results from human and nonhuman isolates were key in containing the outbreak. Implementation of routine WGS on human and nonhuman isolates will strengthen public health responses to future outbreaks. The magnitude of the outbreak underlines the importance of timely and open communication of FBOs to FSAs in case of salmonella detection.

Keywords Salmonellosis, Monophasic *Salmonella* Typhimurium, Outbreak, Whole genome sequencing, Chocolate products

Background

Salmonellosis is a common foodborne illness, with 65,208 cases and 1,014 outbreaks registered in 2022 in the European Union (EU) [1]. The serovars most frequently reported in recent years in human cases were *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium [2]. Monophasic *S.* Typhimurium typically exhibits resistance to multiple antibiotics (ampicillin, streptomycin, sulfonamides and tetracycline). This is likely to be related in large measure to antimicrobial use and associated resistance in livestock, although this resistance has a limited impact on human healthcare as salmonella gastroenteritis generally does not require antimicrobial treatment and other treatment options are available [3, 4].

Chocolate products have caused several outbreaks of salmonellosis in the past [5–8]. Chocolate is a low-moisture food, in which salmonella bacteria can persist for extended periods of time [9, 10]. In addition, the high fat content of chocolate products potentially increases the thermal resistance of salmonella bacteria and provides protection against acidic conditions in the stomach [11–13]. Therefore, even low numbers of salmonella bacteria in chocolate products can result in illness.

Here we describe an international outbreak of monophasic *S.* Typhimurium, with unusual antibiotic resistance patterns, linked to chocolate products manufactured at a single plant of a food business operator (FBO) in Belgium. The initial phase of the outbreak has been described in detail elsewhere [3]. In February 2022, the United Kingdom (UK) detected a genomic cluster of human cases of monophasic *S.* Typhimurium, which was not closely related to any other cluster identified by the UK and had genotypic markers of an unusual and extensive antibiotic resistance pattern. This resistance pattern was not a major clinical concern as other antimicrobial options were available when treatment was required, but was helpful in the case finding phase [3]. On 17 February 2022, an alert was published by the UK via EpiPulse, the information exchange platform hosted by the European Centre for Disease Prevention and Control (ECDC) [14]. Multiple EU member states responded with reports of cases potentially linked to the same cluster. The initial exploratory case interviews suggested a link with the consumption of specific chocolate products, produced by a single FBO. A notification was published via the Rapid Alert System for Food and Feed (RASFF) and via the Early Warning and Response System (EWRS) by the European Commission on 25 March 2022 to alert relevant food safety and public health authorities [15]. The consumption of chocolate products produced by the specific FBO was reported by 88 cases (87%) and food chain investigations in different countries all lead to a single production plant in Belgium [3]. Further microbiological investigations revealed the existence of two microbiologically distinct clusters related to the same FBO. The Belgian food safety authority (Federal Agency for the Safety of the Food Chain, FASFC) was informed on 1 April 2022 and withdrew authorization from the production plant on 8 April 2022.

Methods

Epidemiological and microbiological investigations

An international outbreak response team was convened by the ECDC in March 2022, and case finding was performed based on agreed outbreak case definitions in affected European countries (UK, Austria, Belgium, Czechia, Denmark, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Norway, Slovenia, Spain and Sweden) [16]. Whole genome sequencing (WGS) and core genome multilocus sequence typing (cgMLST) were used to define confirmed outbreak cases whereas probable cases were defined based on the serotype (monophasic *S.* Typhimurium) combined with a specific multilocus variable number tandem repeat analysis (MLVA) and/or antimicrobial resistance (AMR) profile (Table 1). Probable and confirmed cases were subsequently contacted to document exposure to food items including chocolate products.

Cases were interviewed for demographic characteristics, clinical details and food consumption patterns, including suspected chocolate products, within seven days prior to disease onset. The case information collected in each affected country combined with microbiological information was gathered in a central database

Table 1 Case definitions used to define probable and confirmed cases of the two monophasic Salmonella Typhimurium clusters, December 2021—June 2022

	Cluster 1	Cluster 2
Probable case	A laboratory-confirmed monophasic *S.* Typhimurium case with symptom onset on or after 1 October 2021 *(date of sampling or date of receipt by the laboratory if date of symptom onset is not available)*	
	AND	
	By phenotypical testing expressing resistance to ampicillin/amoxicillin, kanamycin/gentamicin, trimethoprim/co-trimoxazole (trimethoprim-sulfamethoxazole) and chloramphenicol	By phenotypical testing expressing resistance to ampicillin/amoxicillin, kanamycin and tetracyclines
	OR	
	MLVA profile 3-11-14-NA-0211	MLVA profile 3-8-10-NA-0211
Confirmed case	A laboratory-confirmed monophasic *S.* Typhimurium case after 1 October 2021 *(date of sampling or date of receipt by the laboratory if date of symptom onset is not available)*	
	AND	
	One of the following laboratory criteria: • clustering with any of the representative outbreak isolates by the national cgMLST pipeline within five AD; • clustering in a centralised WGS analysis within five ADs in a single linkage analysis; • belonging to the cgMLST HC5_296366 according to the EnteroBase scheme; • falling into the 5-SNP single linkage cluster 1.1.1.124.6096.7575.% (t5.7575) (eburst group 1), according to the UKHSA pipeline; • clustering according to a national SNP pipeline within five SNPs.	One of the following laboratory criteria: • clustering with any of the representative outbreak isolates by the national cgMLST pipeline within five AD; • clustering in a centralised WGS analysis within five ADs in a single linkage analysis; • belonging to the cgMLST HC5_298160 according to the EnteroBase scheme; • falling into the 5-SNP single linkage cluster 1.1.1.2765.6144.7643.% (t5:7643) (eburst group 1), according to the UKHSA pipeline; • clustering according to a national SNP pipeline within five SNPs.

MLVA multilocus variable number tandem repeat analysis, *cgMLST* core genome multilocus sequence typing, *AD* allelic differences, *WGS* whole genome sequencing, *SNP* single nucleotide polymorphism, *UKHSA* UK Health Security Agency

coordinated and maintained by the ECDC for compilation, analysis and reporting. In addition, analytical epidemiological studies were conducted by the UK and Ireland to test internationally generated hypotheses regarding the potential outbreak vehicle.

In the UK, a case–control study was performed, with controls recruited via a market research panel and frequency matched by age (in age groups 0–4 and 5–10 years) and sex, based on the distribution of the confirmed UK cases. Cases were interviewed by phone, and controls completed an online questionnaire that was targeted to the hypotheses raised following trawling questionnaires at the time of investigation (consumption of chocolate or chicken products). Age, sex, processed chicken and exposures with a low p-value for illness in univariate analysis ($p < 0.2$) for which at least 30% of cases were exposed, were included in the multivariable logistic regression analyses. As the study was being conducted at the time of product recall, sensitivity analysis was performed by excluding cases and controls recruited after recall.

A matched case-case study was conducted in Ireland. Cases belonging to the same WGS cluster, were matched by age (±2 years for cases ≤ 10 years and ± 5 years for cases > 10 years) and sex with cases of other gastrointestinal diseases (Shiga toxin-producing *Escherichia coli*, *Campylobacter*, *Cryptosporidium*, *Shigella*, *Salmonella* other than the organism of concern) notified within the same two-week period. The detailed methods and results of this study are published elsewhere [17].

FASFC investigations

Microbiological and traceability investigations were coordinated by the Belgian FASFC [18], and supported by traceback investigations by food safety authorities in affected countries. Immediately after initial contact between the UK and Belgian authorities, a first visit to the implicated Belgian plant was made on 1 April 2022. Two different brands of chocolate products (Brand A and Brand B) were produced on separate production lines at this plant. Microbiological results from the FBO self-checking sampling system were requested. In addition, the FASFC collected official samples including environmental swabs, raw materials (milk powder, butter, chocolate, lecithin), finished products at the plant as well as

Table 2 Number of confirmed monophasic *Salmonella* Typhimurium outbreak cases, by cluster and by reporting country, December 2021—June 2022

Country	Cluster 1	Cluster 2	Total
Austria	7	7	14
Belgium	41	25	66
Canada	1	0	1
Czech Republic	1	0	1
Denmark	2	2	4
France	111	10	121
Germany	29	5	34
Ireland	16	1	17
Italy	0	1	1
Luxembourg	2	0	2
Netherlands	2	1	3
Norway	1	0	1
Slovenia	1	0	1
Spain	2	1	3
Sweden	5	0	5
Switzerland	47	2	49
United Kingdom	121	7	128
United States	1	0	1
Total	**390**	**62**	**452**

products collected from shops and products collected from patients/customers. Food and environmental samples were tested for salmonella by the Belgian National Reference Laboratory (NRL) for Foodborne Outbreaks at the Belgian Institute of Health (Sciensano), using standard ISO 6579–1:2017 method and real-time PCR [19]. The National Reference Centre for Salmonella, a different laboratory at Sciensano, performed WGS on human isolates [20] as well as on isolates from food and environmental samples.

Results

Epidemiological and microbiological investigations

As of 15 July 2022, a total of 456 confirmed cases were linked to the outbreak in the European Union (EU), the UK, Switzerland, Canada and the United States (Table 2).

The first reported date of onset was 12 December 2021, in a case in the UK. The peak number of cases occurred in the week of 4 April 2022, followed by a rapid decrease and the last case was reported on 16 June 2022 (Fig. 1).

The majority of cases were young children (62% younger than 5 years and 87% younger than 10 years), 61% were females and the hospitalization rate was 38%. No deaths were reported. Of the interviewed cases for whom information was available (n = 223), 211 (95%) reported consumption of various types of chocolate

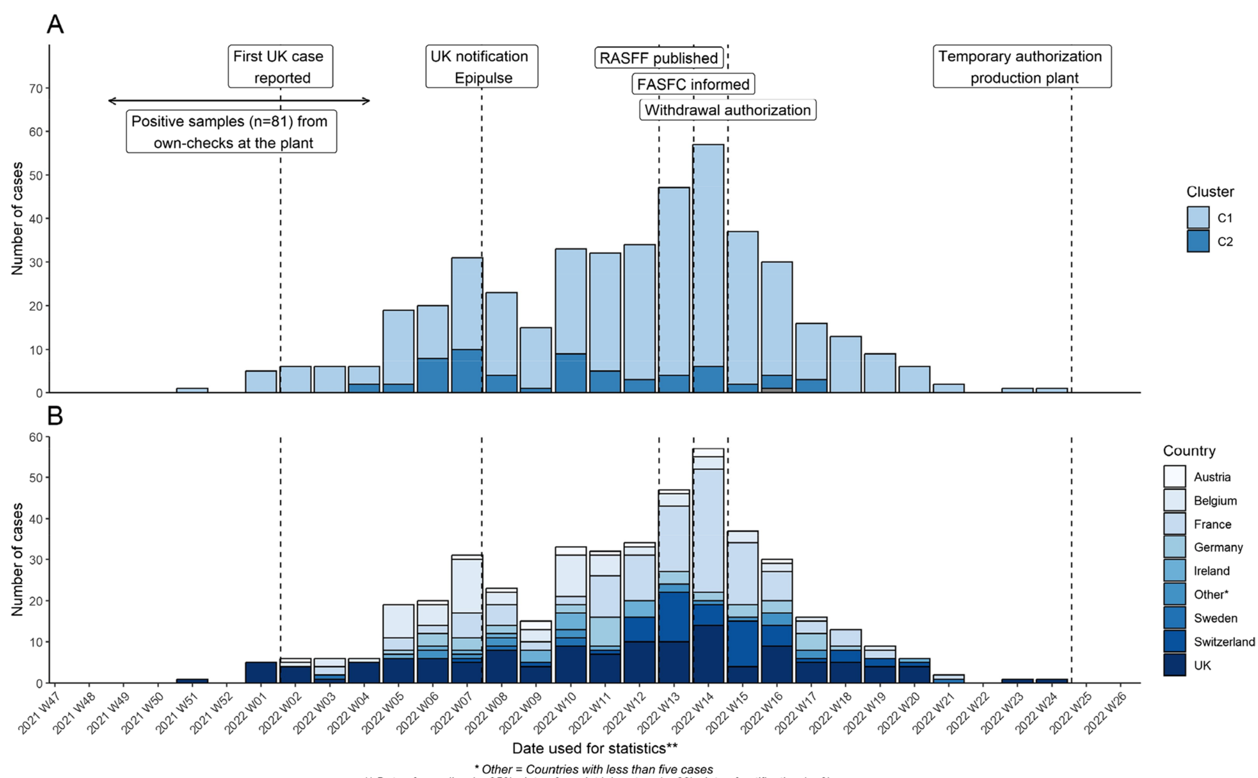

Fig. 1 Timeline of key events and confirmed monophasic *Salmonella* Typhimurium outbreak cases by cluster (**A**) and by country (**B**), December 2021—June 2022

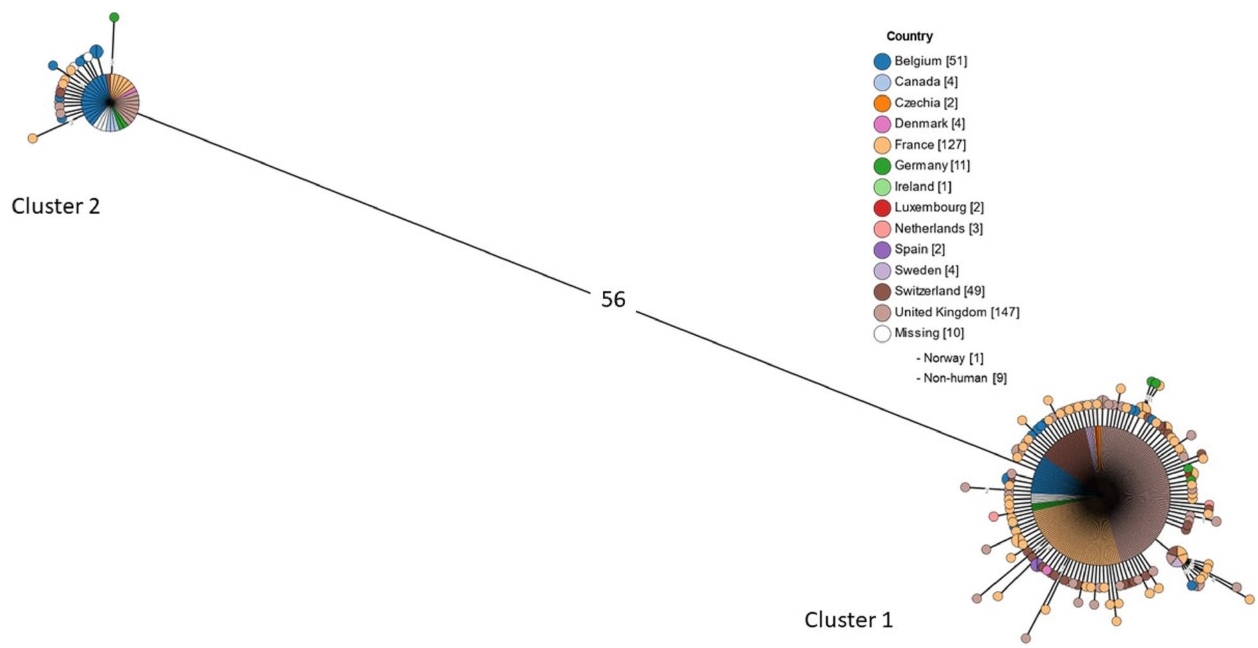

Fig. 2 cgMLST based minimum spanning tree for the monophasic *S.* Typhimurium isolates available on EnteroBase ($n=415$) as of 10 April 2024

products produced by the implicated FBO in the seven days prior to disease onset [21]. The highest consumption rates among these cases were recorded for Brand A chocolate eggs (n = 168, 80%) but other Brand A products were also frequently consumed by cases. The Brand A chocolate eggs are a product marketed primarily for children in the three to ten year-old age group.

The UK case–control study demonstrated that cases who had eaten Brand A chocolate eggs had an adjusted odds ratio (aOR) of 55.0 (95% confidence interval (CI) 9.9–306.4). Other items which showed an association with illness were Brand A bars (aOR = 6.4, 95% CI 1.4–28.0), Brand B chocolate (aOR = 10.9, 95% CI 1.6–72.5) and fresh or frozen chicken products (aOR = 7.3, 95% CI 1.6–33.9). This strong association with Brand A chocolate eggs and similar evidence provided by the descriptive and food chain investigations, supported the recall of Brand A products [22].

The results of the matched case–control study (case-case method) performed in Ireland, showed a significantly higher odds of disease due to the outbreak strain of monophasic *S.* Typhimurium versus other gastrointestinal diseases with a consumption of at least one of the recalled chocolate products of Brand A produced at the Belgian plant (matched OR = 10.5, 95% CI: 1.2–88.6) [17].

On 1 April 2022, analysis of the initial genomic cluster reported by the UK in EnteroBase, identified a match with four identical nonhuman strains, originating from samples taken by the FBO as part of their self-checking system and submitted to EnteroBase [23]. At the same time another cluster of human cases of monophasic *S.* Typhimurium with an uncommon MLVA profile was investigated in Belgium. WGS results obtained by different European countries confirmed the existence of this second distinct cluster, closely related to five nonhuman isolates in EnteroBase, deposited by the same laboratory as the four nonhuman strains in the first cluster and originating from the same Belgian plant, during the same time period (Fig. 1).

Using cgMLST, human strains in the respective clusters showed little genetic diversity, with 61% of the cases within the clusters being indistinguishable by cgMLST (0 allelic differences) and 25% of the strains having only 1 allelic difference. The two clusters were, however, clearly genetically distinct (56 allelic differences), each presenting a different and uncommon, MLVA and AMR profile (Fig. 2).

Cluster 1, MLVA profile 3–11–14-NA-0211, expressed resistance to ampicillin/amoxicillin, kanamycin/gentamycin, trimethoprim/co-trimoxazole and chloramphenicol. Cluster 2, MLVA profile 3–8–10-NA-0211, expressed resistance to ampicillin/amoxicillin, kanamycin and tetracyclines (Table 1).

FASFC investigations

The investigations coordinated by the Belgian FASFC revealed that 81 samples taken by the FBO between 3 December 2021 and 25 January 2022 had tested positive for salmonella. These samples originated from raw materials (3 samples), finished (8), semifinished (39) and intermediate products (21), environmental swabs (7) and rinse oil samples (3). Of these, nine isolates were submitted for molecular typing to an external laboratory, corresponding to the four (cluster 1) and five (cluster 2) nonhuman isolates identified in EnteroBase that matched the two human clusters. The four isolates in cluster 1 were detected from an environmental swab (preparation area), an intermediate and a finished product. The five isolates in cluster 2 originated from an intermediate product as well as from the anhydrous milk fat (AMF) circuit (filter, residual material from the equipment, spout) [21]. Because several of the FBO samples taken from the AMF circuit tested positive for salmonella, this system was considered to be the most likely point of contamination by the FBO. The AMF was provided to the Belgian plant by an Italian supplier. The Italian Food Authorities performed investigations at this supplier in April 2022 on the request of the Belgian FASFC, but salmonella was not detected in the samples taken.

The Belgian NRL for Foodborne Outbreaks analyzed 229 official samples collected by the Belgian FASFC at the plant, seven of which, all from finished products stored at the plant (Brand A chocolate eggs and Brand A mini chocolate eggs), were positive for monophasic *S*. Typhimurium. Further WGS of four isolates sampled from these products matched with both clusters described above (one isolate matched cluster 1 and three isolates matched cluster 2).

Outbreak control measures

Following the first detection of salmonella in self-check samples at the plant on 15 December 2021, mitigation measures were taken by the FBO, including discarding the affected batches and cleaning and temporarily closing the implicated production lines as more samples tested positive. The production lines were restarted following deep cleaning and obtaining negative samples and with bypass of the AMF tank. In the weeks following this restart, additional samples (from the environment, intermediate products, semifinished products) tested positive and further cleaning was performed. The company maintained a heightened level of sampling until 13 March 2022 [21]. Following the RASFF alert on 25 March 2022, recalls of implicated products were undertaken from 2 April 2022 by food safety authorities in the UK and Ireland, followed by recalls in other countries in the following days (4–7 April) [24].

The Belgian FASFC withdrew authorization for production at the Belgian plant of the implicated FBO on 8 April 2022, due to concerns regarding consumer safety. On the same day, the company recalled all Brand A products manufactured at the Belgian plant, regardless of production date and batch number, involving more than 110 countries worldwide.

After extensive cleaning, investments in new equipment and review of the hazard analysis and critical control point (HACCP) system, the implicated plant received a new authorization for production on 17 June 2022 [25]. This was temporarily granted under strict conditions of enhanced quality controls and increased testing. In addition, unannounced inspections took place in the three months following authorization. A final authorization was granted on 17 September 2022.

Discussion

We describe an extensive international salmonellosis outbreak in December 2021—April 2022 with 456 cases in 17 countries within and outside the EU. The number of reported cases belonging to this outbreak is an underestimation of the real incidence as the majority of the cases will not be captured because of underascertainment and underreporting [26]. Only laboratory-confirmed cases meeting the outbreak case definition were included. However, many additional cases likely occurred for which laboratory confirmation was not available. Moreover, various affected countries lacked the capacity to routinely perform WGS on *Salmonella enterica* isolates, but the unusual AMR and MLVA profiles helped to identify probable cases in these countries. The large extent of the outbreak can be explained by several factors: first, chocolate products are widely geographically distributed, and second, the long shelf life of 225–270 days of the implicated products increased the risk of a prolonged and widespread outbreak. The peak of the outbreak occurred just before Easter on 17 April 2022, a period during which more chocolate products are typically purchased. The proximity of the incident to Easter added substantially to the risk posed by this outbreak, due to the potentially large amount of implicated products being purchased and stored by children's relatives ahead of Easter. Public messaging to highlight this fact and to encourage consumers to check for stored products at home was necessary to minimize this risk. Moreover, the products involved in this outbreak were primarily marketed to young children, as reflected in the age profile of cases. The use of rapidly applied exploratory and targeted case questionnaires enabled these important characteristics to be identified at an early stage.

The rapid sharing of microbiological and epidemiological information via established platforms such as EpiPulse, and the concerted collaboration between different stakeholders including laboratories, public health authorities, epidemiological services and food safety authorities, nationally and internationally, allowed for a joint and coordinated investigation, and prompt implementation of measures. The outbreak control measures involved a temporary withdrawal of the authorization for production at the implicated production plant and an extensive recall of products in more than 110 countries worldwide. As illustrated in the epidemic curve, the control measures taken in week 14 led to a rapid decrease in the number of cases in the following weeks. The rapid alert and initial recalls were based on information from case interviews. However, subsequent timely analytical studies in Ireland and the UK, were essential to provide further confirmatory evidence regarding the source, to support and justify the initial decisions, to increase the extent of recall and to differentiate between a wide variety of chocolate brands and product types.

Some countries have routinely implemented WGS as a subtyping method for *Salmonella enterica*, while WGS is performed only to confirm a cluster based on MLVA among human isolates in other countries, such as Belgium at the time of the outbreak. The routine application of WGS as a standard typing method for *Salmonella enterica* has proven to be more discriminatory in linking human cases, especially for commonly reported serovars (*S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium) [27]. The advantage of combined human-food WGS analysis was illustrated in this outbreak. Fortunately, WGS was performed on several *Salmonella enterica* isolates from the implicated plant and the sequences were available in a public database, allowing comparison with human WGS results. The sharing of this information at an international level provided additional strong evidence pointing toward the FBO that was already suspected based on the epidemiological and traceability investigations.

The high degree of relatedness between human and environmental isolates supported the hypothesis that the Belgian plant was the most likely source of contamination. Combined with epidemiological evidence, these findings proved crucial for triggering rapid and effective control measures. The comparison of human and nonhuman isolates via WGS also enabled linking of the second cluster of cases to contamination within the plant. Since 2023, the Belgian FASFC has routinely performed WGS on isolates (from food and animals for *Salmonella enterica*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli*) obtained via control programmes under the European regulation 2017/625 [28]. In addition to the implementation of routine sequencing, actions are currently ongoing to evolve toward a national infrastructure including tools for processing genomic information, data exchange and combined genomic-epidemiological analyses using data from human and agri-food isolates. These actions will result in strengthening the public health response in Belgium regarding local outbreaks and potential cross-border health threats of infectious diseases. Similar initiatives are already implemented or ongoing in other EU/European Economic Area countries, as well as in the UK and Switzerland [29–32].

Two distinct strains (different MLVA profiles, different AMR profiles and two distinct genomic clusters separated by 56 allelic differences) were detected in this outbreak simultaneously. Such polyclonal outbreaks are challenging even when WGS laboratory surveillance is routinely implemented. Incorporating strains and WGS information from positive samples from FBOs into a more integrated One-Health surveillance system would facilitate outbreak investigations. It is mandatory for FBOs to apply a self-checking system consisting of sampling schedules, temperature checks, cleaning procedures and incident registration. In line with the European Regulation, FBOs are committed informing the competent authorities if they consider or have reason to believe that a food that is brought on the market may be injurious to human health [33, 34]. In this outbreak, several FBO samples from raw products, finished, semifinished and intermediate products, environmental swabs and rinse oil samples tested positive for *Salmonella enterica*. However, according to the risk analysis performed by the company, all possibly contaminated batches had been destroyed between December 2021 and January 2022. The company concluded that there was no further risk for the public. For this reason, the Belgian authorities were not informed at that stage. The presence of positive samples via the FBO's self-checking system after internal measures were taken, indicated that these measures were not adequate to eliminate the contamination. Considering that the average time from production to retail is approximately 60 days, the first reported case (disease onset on 12 December 2021) and the following cases until early January 2022 are unlikely to be explained by the contamination detected in the FBO samples in December 2021. The two months of storage before distribution suggest that the contamination in the plant occurred earlier than December 2021. Because several of the FBO samples taken from the AMF circuit tested positive, this system was considered to be the most likely point of contamination in the process. Biofilm development of *Salmonella enterica* is possible in such environments, leading to a reservoir that can potentially contaminate the production line and final food products over a long period [35]. Although the FBO implemented internal measures to eliminate the contamination those were likely insufficient to control the contamination along the production line. In these situations, it is necessary to collaborate fast and pro-actively with FSAs to correctly assess the control measures, taking into account the potential health risk.

Conclusions

This international foodborne outbreak highlights the importance of good food safety management, as required in European food safety legislation. It is necessary for FBOs to assess a potential health risk when confronted with noncompliant analytical results and to take appropriate actions to protect public health. The magnitude of this outbreak and the consequences of the extensive control measures (withdrawal of authorization and economic losses due to recalls) underscore the importance of informing food safety authorities in a timely manner on the detection of salmonella in the process and products, enabling a timely and effective risk assessment and management. Rapid sharing of microbiological and epidemiological information between international stakeholders was key in containing the outbreak. The broader implementation of routine WGS on human and nonhuman isolates will strengthen public health responses to future outbreaks.

Abbreviations

AMF	Anhydrous milk fat
AMR	Antimicrobial resistance
aOR	Adjusted Odds ratio
cgMLST	Core genome multilocus sequence typing
CI	Confidence interval
C1	Cluster 1
C2	Cluster 2
ECDC	European Centre for Disease Prevention and Control
EU	European Union
EWRS	Early Warning and Response System
FASFC	Federal Agency for the Safety of the Food Chain
FBO	Food business operator
FBOs	Food business operators
FSA	Food safety authority
HACCP	Hazard analysis and critical control point
MLVA	Multilocus variable number tandem repeat analysis
NRL	National Reference Laboratory
OR	Odds ratio
RASFF	Rapid Alert System for Food and Feed
UK	United Kingdom
WGS	Whole genome sequencing

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Authors' contributions

V.L. and D.V.C. drafted the manuscript. M.P.G. prepared the minimum spanning tree. A.H., A.V., L.L. and S.B. were responsible for the case-control study in the UK. C.S.N., P.G. and P.M.K. were responsible for the case-control study in Ireland.

C.J. and J.T. were responsible for coordination of the outbreak investigation at ECDC level. All authors, including the outbreak investigation group, contributed to the outbreak investigation and reviewed and approved the final manuscript.

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Data availability
Genomes used for the minimum spanning tree are available in EnteroBase at HC5_296366 (Cluster 1) and at HC5_298160 (Cluster 2). A line list of the cases used during the outbreak investigation and for the manuscript, is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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