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Levels and genotypes of *Salmonella* and levels of *Escherichia coli* in frozen ready-to-cook chicken and turkey products in England tested in 2020 in relation to an outbreak of *S. Enteritidis*

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ABSTRACT

Frozen reformulated (FR) breaded chicken products have previously been implicated in causing human salmonellosis. A multi-country *Salmonella enterica* serovar Enteritidis outbreak involving several strains with >400 reported human cases in the UK occurred in 2020. Initially *S.* Infantis was detected in one sample from a case home but *S.* Enteritidis was then also isolated using a *S.* Enteritidis specific PCR in combination with isolation via a Craigie-tube. This prompted a survey to examine the presence and levels of *Salmonella* and *E. coli* in ready-to-cook FR poultry products in England in 2020.

From a total of 483 samples, including two from cases' homes, *Salmonella* was detected in 42 chicken samples, these originated from six out of 53 production plants recorded. *Salmonella* detection was associated with elevated levels of generic *E. coli* (OR = 6.63). *S.* Enteritidis was detected in 17 samples, *S.* Infantis in 25, *S.* Newport in four and *S.* Java, *S.* Livingstone and *S.* Senftenberg in one each. The highest levels of *Salmonella* were 54 MPN/g for *S.* Infantis and 28 MPN/g for *S.* Enteritidis; 60% of the *Salmonella*-positive samples had <1.0 MPN/g. *S.* Enteritidis was detected together with *S.* Infantis in five samples and with *S.* Livingstone in one. Where *S.* Enteritidis was detected with other *Salmonella*, the former was present at between 2 and 100-fold lower concentrations. The *Salmonella* contamination was homogeneously distributed amongst chicken pieces from a single pack and present in both the outer coating and inner content. The *S.* Enteritidis were all outbreak strains and detected in six products that were linked to four production plants which implicated a Polish origin of contamination. Despite *S.* Infantis being most prevalent in these products, *S.* Infantis from only two contemporaneous human cases in the UK fell into the same cluster as isolates detected in one product. Except for one human case falling into the same

Abbreviations: FR, frozen reformed; SLC, single linkage cluster; SNP, single nucleotide polymorphism.

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cluster as one of the *S*. Newport strains from the chicken, no further isolates from human cases fell into clusters with any of the other serovars detected in the chicken samples.

This study found that higher *E. coli* levels indicated a higher probability of *Salmonella* contamination in FR chicken products. The results also highlight the importance of recognising co-contamination of foods with multiple *Salmonella* types and has provided essential information for detecting and understanding outbreaks where multiple strains are involved.

1. Introduction

Comminuted, reformulated, frozen chicken products including food such as chicken nuggets have been implicated in human salmonellosis outbreaks since the late 1990s in Australia, Canada and the US (Kenny et al., 1999; Morton et al., 2019; Smith et al., 2008; Trmcic et al., 2020): *Salmonella enterica* supsp. *enterica* serovars involved with these outbreaks included, *S*. Braenderup, *S*. Enteritidis, *S*. Heidelberg and *S*. Typhimurium, respectively. In 2020, public health agencies in England, Wales, Scotland and Northern Ireland instigated a national investigation into an outbreak due to *S*. Enteritidis caused by three outbreak strains each defined as within a 5-Single Nucleotide Polymorphism (5-SNP), single linkage cluster (SLC), with designations as SLCs 1.1.2.12.12.12.% (t5.12), 1.1.2.12.12.590.% (t5.590) and 1.1.2.2533.3617.4833.% (t5.4833) based on whole genome sequencing (WGS) data (Dallman et al., 2018; ECDC/EFSA, 2021a). The *S*. Enteritidis t5.12 and t5.590 SLCs fell within a wider 25-SNP cluster, where t5.12 and t5.590 contributed the majority of cases in the 25-SNP cluster. More than 400 human cases of salmonellosis infected by the *S*. Enteritidis SLCs t5.4833, t5.590 and t5.12 had been reported during 2020 in the UK. A multi-country outbreak investigation was initiated into the t5.4833 outbreak, with cases also reported in Denmark, Finland, France, Germany, Ireland, the Netherlands, Poland and Sweden (ECDC/EFSA, 2021a). Children under 16 years were disproportionately affected, and the results of the UK analytical epidemiological study provided strong evidence of an association with the consumption of frozen breaded chicken products, intended to be eaten cooked.

In the UK, reporting of human salmonellosis to the UK Health Security Agency (UKHSA, formerly Public Health England (PHE)) is a statutory requirement (Health Protection (Notification) Regulations, 2010). Surveillance of this disease also involves the voluntary submission of *Salmonella* isolates by diagnostic laboratories from clinical specimens, as well as from food and environmental samples to the UKHSA Gastrointestinal Bacteria Reference Unit (GBRU) for further characterisation. In April 2014, whole genome sequencing (WGS) was implemented by PHE for the routine characterisation of *Salmonella* isolates submitted to GBRU and has, since 2015 been the primary typing tool used for public health surveillance (Chattaway et al., 2019a).

Prior to the implementation of routine characterisation of *Salmonella* by WGS and establishment of serological types by genetic analysis (Ashton et al., 2016), Craigie tubes (Craigie, 1931) were used for phase selection of flagellin (H) types. This method consists of a hollow tube which is placed in a semi-solid agar medium inside a test tube containing antisera against specific *Salmonella* flagella antigens. The *Salmonella* culture under investigation is inoculated into the medium inside the hollow tube and, where antiserum is present against the flagella of the culture under test, motility will be inhibited. Consequently, only bacteria which do not react with the antisera will be able to move to the surface of the outer growth medium, from where they can be isolated. This approach was used here to favour isolation of *S*. Enteritidis in samples co-contaminated with other *Salmonella* serovars.

Following initial detection of one *S*. Enteritidis outbreak strain from a chicken product collected from a case's domestic freezer, a survey was instigated for similar products in England from October to December 2020. The survey aimed to identify further sources of contamination to inform the implementation of control measures. The study also sought to establish the types and levels of *Salmonella* contamination in relation to product and product origin using genome sequencing to describe phylogenetic relationships between isolates. Since *Escherichia coli* is commonly used as a hygiene indicator in food as well as a measure of process hygiene in EU microbiological criteria (European Commission (EC), 2005), the relationship between the levels of indicator *E. coli* and the presence of *Salmonella* was also investigated.

As a result of the testing of food in England, three alerts were issued through the EU Rapid Alert System for Food and Feed (RASFF) in July, August and September 2020 (RASFF 2020.3081, RASFF 2020.3237 and RASFF 2020.3868, respectively; available from https://webgate.ec.europa.eu/rasff-window/portal/?event=searchResultList&StartRow=201). Also as a result of testing described here, food alerts requiring the withdrawal of products due to contamination by *Salmonella* were promptly issued by the UK Food Standards Agency (FSA) in August, October and December 2020 and in February and March 2021 (Food Standards Agency (FSA), 2020a, 2020b, 2020c; Food Standards Agency (FSA), 2021a, 2021b, respectively).

2. Materials and method

2.1. Sample collection

Three samples of chicken products were collected by Environmental Health Practitioners in July and August 2020 from the domestic freezers of three cases as part of the *S*. Enteritidis outbreak investigations as preliminary epidemiological evidence indicated these types of products as a likely vehicle of infection (Table 1). As a result of the detection of an outbreak strain in one of these samples and further epidemiological analysis implicating these types of foods, a survey was initiated on FR, ready-to-cook chicken or turkey products with emphasis on collecting a wide range of breaded chicken products, in particular. A total of 481 survey samples were tested between October and December 2020: 95% of samples were from retail and 5% from catering establishments. Environmental Health Practitioners from 30 Local Authorities across England collected 42% samples (including all those from catering establishments), and they were transported to one of the three PHE Food Water and Environmental microbiology (FW&E) laboratories located in

Table 1
Clinical samples and chicken samples[a] collected from domestic freezers of cases' homes.

Descriptors	Case A	Case B	Case C
Clinical specimen date	July 2020	July 2020	July 2020
Salmonella detected in specimen	*S*. Enteritidis t5.12	*S*. Enteritidis t5.4833	*S*. Enteritidis t5.590
Date food collected	July 2020	August 2020	August 2020
Use by date	October 2021	May 2022	May 2022
Food product	Reformed (breaded)	Reformed (breaded)	Whole chicken breast fillets (no coating)
Production plant code	PL I	PL II	Other PL
Country of plant	Poland	Poland	Poland
Salmonella detected in food	*S*. Infantis	*S*. Infantis *S*. Enteritidis t5.4833 and t5.590	*S*. Infantis

[a] Chicken samples from cases' homes were collected as unopened packs from domestic freezers; labelling instructed cooking prior to consumption.

London, Porton Down or York in accordance with the Food Law Code of Practice and Food Law Practice Guidance (Food Standards Agency (FSA), 2021c). Due to limited local authority resources during the Covid-19 pandemic, the remaining 58% samples were collected by laboratory staff and transported to the laboratories as above. All products were collected, received and stored frozen. Samples were collected as convenience samples selecting one, to a maximum of four distinct products at random on each occasion. Products on retail sale were packaged in either cardboard boxes or in plastic bags. Product descriptors were recorded from external and internal pack labelling including product name and brand, production plant code, country of production, retailer, batch code, 'best before' and 'frozen on' dates, coating type and flavourings. Where a primary production plant had been identified to have supplied the product to the production plant displayed on the pack this was also recorded (ECDC/EFSA, 2021a). Products were categorised as non-comminuted but reformulated (typically containing at least some intact meat pieces and declaring a meat contents of around 65% to 75%) or comminuted and reformed (typically these appeared to consist of mince-like meat, were reformed and declaring lower meat contents of around 45% to 60%) based on the product descriptors. Each sample was categorised into a distinct product (named products A to Z for those where *Salmonella* was detected) based on a unique combination of name and brand. A unique batch of a product was defined from a combination of batch code and best before/frozen on date. Remaining shelf-life was calculated as the number of months from the date of collection to the best before date. Products were also categorised according to meat species (either chicken, turkey or mixed meats). Samples were collected from retail stores ranging from large national to smaller independent stores. There was sampling of further batches of products where *Salmonella* had been detected initially but on average only 1.1 samples per batch were tested with a maximum of four from a single batch for one product.

2.2. Microbiological testing

Samples were tested using internationally recognised standard methods comprising: enumeration of *E. coli* by a MPN method (either by ISO 16649-3:2015 (International Organization for Standardization (ISO), 2015) or by TEMPO (Owen et al., 2010)); and detection of *Salmonella* in a 25 g sample using buffered peptone water (BPW) followed by secondary enrichment in Rappaport Vassiliadis and Muller-Kauffmann Tetrathionate Novobiocin broths and then sub-cultured onto Brilliant Green Agar (BGA) and Xylose Lysin Deoxycholate Agar (XLD) (International Organization for Standardization (ISO), 2002). Each 25 g sample tested consisted of pieces with crumb/coating/inner meat proportions representing as sold to the consumer. Primary BPW broths were screened for *Salmonella* by a real-time polymerase chain reaction (PCR): DNA was extracted using a 16-cell DNA purification kit Maxwell 16 (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions and tested by PCR using the MicroSeq *Salmonella* detection kit (Thermo Fisher Scientific, Warrington, UK) using a ABI 7500 real-time PCR system (Thermo Fisher Scientific, CA, USA) with amplification parameters of: 95 °C for 2 min, followed by 95 °C for 15 s and 60 °C for 60 s (Jenkins et al., 2015). Where *Salmonella* was detected by PCR, enrichment broths were subjected to an additional PCR test (Thermo Fisher Scientific, RapidFinder™ Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Kit, Vantaa, Finland) to enable specific detection of *S. Enteritidis* DNA and to estimate the relative level of *Salmonella* DNA against the level of any *S. Enteritidis* DNA present based on Ct values. The RapidFinder PCR assay was performed according to the manufacturers' instructions, where 10 µl of enrichment culture and 10 µl of proteinase K were added to a lysis tube, then, following a thermal lysis, 20 µl of the upper layer was added to each PCR tube containing lyophilised reagents. For samples where *S.* Enteritidis was not initially isolated using conventional culture despite being detected by the RapidFinder PCR, 10 µl from the enrichment broth was used to inoculate a Craigie tube containing specific antisera against *S.* Infantis: 30 µl each of H factors r and 5 (PL.6242 and PL.6154 respectively; Pro-Labs Diagnostics, Wirral, UK). The Craigie tube was incubated at 37 °C overnight after which 10 µl from the top-layer outside of the inner tube was sub-cultured onto BGA and XLD agar plates that were incubated overnight at 37 °C. Suspect *Salmonella* colonies were identified as above. One or more isolates from each sample was submitted to the GBRU laboratory for further characterisation by WGS.

Enumeration of *Salmonella* in samples was conducted by a five-tube MPN method based on the *Salmonella* ISO method (International Organization for Standardization (ISO), 2002) testing 1.0 g, 0.1 g, and 0.01 g (using an initial 10 g sample) and, if sufficient material was available, repeated where a result of <0.2 MPN/g was initially obtained, with five replicates each of 10 g, 1 g and 0.1 g of the starting material. In some cases a three tube MPN was used when individual pieces from a pack were tested. Typical colonies from each plate were subjected to confirmation by PCR. The serotype specific PCR and modified Craigie method was used, where necessary, to detect and isolate *S.* Enteritidis in the presence of higher numbers of other *Salmonella*. Levels of *Salmonella* contamination were estimated using reference tables (International Organization for Standardization (ISO), 2007) and MPNs presented as total *Salmonella* and *S.* Enteritidis levels. Results for samples yielding all negative tubes are given as containing *Salmonella* at less than the nominal lower limit of detection for the range of dilutions used. One or more *Salmonella* isolates from each primary sample or from MPN dilutions was submitted to the GBRU for further characterisation.

Results for the detection of *Salmonella* and levels of *E. coli* were interpreted using European Commission (EC) Regulation 2073/2005 on microbiological criteria for foodstuffs (European Commission (EC), 2005) "Food Safety Criteria for minced meat and meat preparations made from poultry meat intended to be eaten cooked", detection of *Salmonella* in 25 g was interpreted as unsatisfactory. Levels of *E. coli* at >5000/g are unsatisfactory according to the Process Hygiene Criteria for Meat Preparations: this criterion also defines more than 2 out of 5 samples with levels between ≥500 and 5000 *E. coli*/g as unsatisfactory at the end of the manufacturing process.

2.3. Characterisation of Salmonella by analysis of genome sequencing

Salmonella isolates were sent to GBRU for DNA sequencing which was performed by the UKHSA Genome Sequencing and Development Unit using Nextera library preparation and Illumina HiSeq 2500 in fast-run mode according to the manufacturer's instructions (Illumina Inc., Albany, USA). Species confirmation, serovar, eBURST group (eBG), sequence type (ST) and multilocus sequence type (MLST) were derived as described previously (Ashton et al., 2016; Chattaway et al., 2019a). Hierarchical single linkage clustering was performed on pairwise SNP distances, allowing quantification of the genetic relatedness between isolates and identification of microbiological clusters at pre-determined (usually at ≤5) SNP thresholds of genetic relatedness (Dallman et al., 2018). Microbiological results and sample data including production plant origins as identified during the outbreak investigation were combined in a central database, the Gastro Data Warehouse (GDW).

A core SNP alignment was generated using SnapperDB, recombination removed using Gubbins and a seven-threshold SNP sequencing address generated (Croucher et al., 2015; Dallman et al., 2018). Pairwise comparisons of SNP distances were performed between the sequences from isolates: *Salmonella* sequences linked within a 5-SNP SLC were considered to be closely genetically related and having ≤5 SNPs difference with at least one other isolate within that same cluster as described previously (Chattaway et al., 2019b). This analysis was performed to compare clinical isolates between 1st January 2020 and 1st December 2021 with food isolates. A Maximum-likelihood phylogeny was derived for each sequence type using RAxML v8.2.8 under the GTRCAT model to confirm the 5-SNP clustering was monophyletic.

For *Salmonella* serovars where SNP pipelines were not developed,

core genome MLST typing (cgMLST) and hierarchal clustering analysis was performed to identify matches at the 5 allelic level (HC5) as previously described (https://enterobase.warwick.ac.uk/species/senterica/allele_st_search; Chattaway et al., 2019b; Zhou et al., 2020;). FASTQ sequences were deposited in the NCBI Short Read Archive under the BioProject PRJNA248792 (https://www.ncbi.nlm.nih.gov/bioproject/?term=248792). Refer to Supplementary Table S1 for accession numbers.

2.4. Statistical analysis

Logistic regression was used to investigate the association between detection of *Salmonella* (a yes/no variable) and the level of *E. coli* and other covariates including the production plant. The main explanatory variable of interest was *E. coli* $\log_{10}$ MPN, and a country (with four categories), as well as a production plant variable (with nine categories) and other explanatory variables such as type of meat (only chicken or other), type of preparation (either comminuted/reformed or not comminuted (but reformulated)), remaining months of shelf-life and product coating (i.e. either coated (e.g. with breadcrumbs or batter) or not coated). Of the 483 samples included in the analysis (i.e. the 481 survey samples and two of the samples collected from cases' freezers; the third sample from a cases' freezer was not included as it was a sample of plain whole chicken breast, not reformulated/coated and from a plant different from all other samples in this survey), 321 had the *E. coli* MPN censored from below and for these samples, the value used was half the lower limit of 10 or less. Statistical significance level was taken to be 0.05 and *p*-values were obtained by means of the likelihood ratio test (LRT). The form of association of *E. coli* $\log_{10}$ MPN with *Salmonella* detection on the logit scale was ascertained by starting with a quartic function and successively simplifying to the next most complicated function not fitting significantly worse, as judged by LRT. The same approach was adopted for "Remaining shelf life". Univariate analysis started with examining each covariate one at a time without consideration of any other. This was followed by a backwards stepwise multivariate analysis, where all covariates were included and started by examining the appropriate functional form for logarithm to base ten of MPN, keeping "Remaining shelf life" as linear. Once this had been determined, the appropriate functional form of "Remaining shelf life" was ascertained. Odds ratios (ORs) and 95% confidence intervals (CIs) were also obtained. Firth regression was used to obtain ORs and 95% CIs for the coating covariate due to all those detected having a coating. Analysis was done in Stata v16.1 (StataCorp LLC, Texas, USA).

3. Results

3.1. Clinical samples and chicken samples collected from cases' homes

During 2020, as part of the *S.* Enteritidis outbreaks investigations, unopened packs of chicken products were recovered from three cases' (A, B and C) domestic freezers; therefore these particular samples were not consumed by the cases (Table 1). The *S.* Enteritidis isolates detected in the clinical specimens from the cases belonged to the t5.12 SLC for Case A, the t5.4833 SLC for Case B and the t5.590 SLC for Case C (ECDC/EFSA, 2021a). From the testing of the associated chicken samples *S.* Infantis was initially isolated from all three. However, *S.* Enteritidis was then also detected in one of the samples as a result of using the serotype specific PCR combined with isolation via the modified Craigie tube; first an isolate falling into the t5.4833 SLC and then an isolate belonging to the t5.590 SLC (the latter via a MPN/Craigie-tube).

3.2. Detection of Salmonella in samples collected

Salmonella was detected in 40 of the 456 chicken samples in the survey (8.8%; 95% CI 6.3–11.8), all of which were on retail sale, and these were unsatisfactory according to the food safety criteria in the EU (European Commission (EC), 2005). The 456 pure chicken samples were from 11 different countries including Poland (33%), UK (30%), Thailand (11%), the Netherlands (9%), and Ireland (7%). The majority (79%) of the chicken samples were comminuted/reformed, 69% were coated in breadcrumbs or batter and 58% contained additional flavourings. The remaining shelf-life of the chicken samples ranged from 0 to 22 months and 62% of all samples had more than one year of shelf-life remaining. The date of production was available for 41 samples and the total shelf-lives for these were 12 months for 9 samples, 18 months for 28 and 24 months for four samples. *Salmonella* was not detected in the 25 reformed samples that were not purely chicken (turkey, chicken and turkey or chicken and beef), produced in three different countries (21 were from UK plants) and from major supermarkets, except for one sample. The prevalence of *Salmonella* in relation to country and other product characteristics for the total of 483 poultry samples (where the two reformed samples obtained from cases' freezers were included), was investigated (Table 2). Detection of *Salmonella* was confined to samples from six production plants in Poland, Ireland and the UK of a total of 53 plants recorded. *Salmonella* were detected in 25 products (49% of all products recorded) including samples that were battered, breaded or otherwise coated products and were detected more frequently in comminuted and reformed, compared to other product types. Remaining shelf-lives were from 7 to 22 months for the samples where *Salmonella* was detected and 97% of the comminuted/reformed chicken samples had between 7 and 18 months of shelf-life remaining.

None of the levels of *E. coli* exceeded the process hygiene criteria for *E. coli* (>5000 cfu/g) but four samples were between 500 and 5000 *E. coli* MPN per g. The level of *E. coli* was <10 MPN per g for 75% of the samples (Table 2).

3.3. Risk-factors for Salmonella contamination

In a univariate analysis (Table 3), the frequency of detecting *Salmonella* was significantly associated with country, production plant category, levels of *E. coli*, product state and the remaining shelf-life. In the multivariate analysis, $\log_{10}$ of *E. coli* MPN/g, production plant category and other co-variates were included as independent risk factors for detection of *Salmonella* (Table 3). This analysis found strong evidence of an association between the $\log_{10}$ MPN of *E. coli* and the odds of detecting *Salmonella* (OR = 6.63, 95%CI: 3.54–12.4; $p < 0.001$). Production plant category was independently associated with the odds of detecting *Salmonella* ($p < 0.001$). Country was not included in the multivariate model as there were too few *Salmonella* detections for the majority of countries to analyse country independently. After adjusting for other variables, we found no evidence of an association between the type of coating (e.g. products coated with breadcrumbs as compared to those with other coatings) and the detection of *Salmonella* (Table 3).

3.4. Salmonella serovars and MPN levels

Amongst the 40 survey samples from which *Salmonella* was isolated, *S.* Infantis was detected in 23 (58%), *S.* Enteritidis in 16 (40%), *S.* Newport in four (10%): *S.* Java, *S.* Livingstone and *S.* Senftenberg were detected in one sample (3%) each. Of the total of 483 samples that included the two reformulated samples collected from the cases' homes, *S.* Enteritidis was detected in 17 samples, comprising six products from 12 different batches (Table 4). In four products (six samples) co-contamination with *S.* Enteritidis and *S.* Infantis was detected while a fifth product had *S.* Enteritidis and *S.* Livingstone (Table 4). In five of the 17 samples, detection of *S.* Enteritidis was only achieved using the serotype-specific PCR and isolation via the Craigie tube, affecting four products (Table 5).

There was considerable variation in the levels of *Salmonella* detected in the products: the average level was 3.50 MPN/g, with a minimum, first, second and third quartile and maximum of <0.02, 0.10, 0.47, 1.93 and 54 MPN/g, respectively. The highest level (54 MPN/g) detected was for a sample with *S.* Infantis and the next highest (28 MPN/g) for a

Table 2
Detection of *Salmonella* in frozen ready-to-cook products in 2020, in England in relation to country, production plant and product.

Variable	Category	Number of samples	% of samples where *Salmonella* was detected
Country	Poland (PL)	154	15.7
	United Kingdom (UK)	159	6.3
	Ireland (IE)	30	26.7
	Other[a]	140	0
Production plant	PL I	78	21.8
	PL II	54	13.0
	Other PL[b]	22	0
	UK III	40	2.5
	UK V[c]	17	35.3
	UK VI[c]	8	37.5
	Other UK[d]	94	0
	IE IV	30	26.7
	Other[e]	140	0
Meat type	Chicken only	458	9.2
	Mixed and other meat	25	0
Product state	Comminuted and reformed	386	10.4
	Other reformulated	97	2.1
Remaining shelf-life (months)	13–22	301	10.0
	7–12	146	8.2
	0–6	32	0
	Not known	4	0
E. coli level (MPN/g)	<10	363	1.7
	10–99	91	23.1
	100–999	26	50.0
	1000–5000	3	66.7
Product coating	Breadcrumb	345	9.9
	Batter	116	6.9
	None	15	0
	Other or not known	7	0
Sample collected from	Catering	23	0
	Retail	460	9.1
	- of which major supermarkets[f]	379	9.8
	- of which other stores	81	6.2

[a] This included samples from Thailand ($n = 40$), The Netherlands ($n = 43$), Germany ($n = 20$), Hungary ($n = 12$), Romania ($n = 9$), Spain ($n = 6$), Brazil (n = 2), France (n = 2) and Belgium (n = 1) and five samples where no country information (or production plant) was provided on the pack.
[b] Three plants recorded; one sample stated a Polish origin but had no specific production plant code.
[c] These two plants were registered to the same company.
[d] 14 production plant codes were recorded.
[e] 30 production plant codes recorded, including Thai ($n = 10$ plants), Dutch ($n = 5$ plants), German ($n = 4$ plants), Hungarian (n = 4 plants), Romanian ($n = 3$ plants), Spanish (n = 1 plant), French (n = 1 plant), Belgian (n = 1 plant) and Brazilian (n = 1); for eight samples no plant code was available.
[f] Salmonella was detected in products from six of 10 major retailers (5–74 samples from each major retailer were tested and percentage where Salmonella was detected ranged from 5 to 26%).

Table 3
Analysis of the frequency of detection of *Salmonella* in relation to country of production, production plant, level of *E. coli*, formulation, coating, remaining shelf life and type of meat.

		Univariate		Multivariate	
Variable	Category (if appropriate)	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Country category	Poland (PL)	1.00	<0.001	Not included	NA
	UK	0.36 (0.17, 0.79)			
	Ireland (IE)	1.97 (0.79, 4.94)			
	Other	0.00 (NE[a])			
Production plant category	PL I	1.00	<0.001	1.00	<0.001
	PL II	0.53 (0.01, 0.72)		0.75 (0.21, 2.65)	
	UK III	0.09 (0.01, 1.11)		0.12 (0.01, 1.07)	
	IE IV	1.30 (0.49, 3.45)		1.66 (0.48, 5.79)	
	UK V	1.96 (0.63, 6.06)		3.01 (0.55, 16.5)	
	UK VI	2.15 (0.47, 9.93)		1.96 (0.21, 17.9)	
	Other PL	0.00 (NE)		0.00 (NE)	
	Other UK	0.00 (NE)		0.00 (NE)	
	Other[b]	0.00 (NE)		0.00 (NE)	
$\log_{10}$ MPN of *E. coli*	Continuous	6.90[c] (4.25, 11.2)	<0.001	6.63 (3.54, 12.40)	<0.001
Reformulated	No	1.00	0.003	1.00	0.07
	Yes	5.49 (1.30, 23.1)		4.38 (0.73, 26.2)	
Coated with breadcrumbs	No	1.00	0.08	1.00	0.5
	Yes	3.50 (0.21, 59.3)		0.59 (0.02, 15.4)	
Remaining shelf life (months)	Continuous	1.11 (1.00, 1.22)	0.03	1.03 (1.00, 1.22)	0.8
Meat type	Chicken only	1.00	0.03	1.00	>0.999
	Mixed/other	0.00 (NE)		0.22 (NE)	

[a] NE - Not estimable.
[b] These included plants not in the UK, Poland or Ireland.
[c] Per one $\log_{10}$ unit.

sample with *S.* Enteritidis (Table 4). In co-contaminated samples, *S.* Enteritidis was outnumbered by other salmonellas from 2 to 100-fold (Table 5) and this was associated with a higher Ct value for *S.* Enteritidis compared to *Salmonella* spp. (with average Ct values in secondary enrichment broths of 31.5 for *S.* Enteritidis and 23.7 for *Salmonella* spp.) and consistent with the need to use the Craigie tube method for isolation of *S.* Enteritidis. There was no clear association between Ct in the primary enrichment broth and *Salmonella* MPN values (results not shown). Variation in the levels of *Salmonella* in samples from the same batch of a product was observed, for example this varied by over 100-fold between samples of products A, B and C (Table 4). The variation in the levels of *Salmonella* amongst single pieces from seven packs with *S.* Enteritidis was further investigated (Supplementary Table S2). MPN analysis was performed on 6–10 pieces (e.g. single nuggets of 5–10 g each) for each pack and *Salmonella* was isolated from the highest dilution(s) where detected by PCR (Table 6). Replicate tests on individual nuggets from the same pack were all within a factor of 10 with overlapping 95% CIs. In five pieces from a single pack of one FR breaded product (B2) the outer coating (with crumb weights of 0.8 to 3.3 g per piece) was dissected from the inner material (with weights of 4.3 to 6.9 g per piece) and the levels of *Salmonella* were separately estimated (Supplementary Table S2). While not significantly different, higher levels of *Salmonella* were found in the inner product sample (mean 4.9 MPN/g, range 3.5–6.8) as compared to outer coating (mean 1.4 MPN/g, range 0.7–2.9).

Table 4
Salmonella type and MPN/g in relation to production plant and product.

Plant[a]	Product (number of samples, batches)	Number of positive samples (batches)	Sample, best before date	*Salmonella* detected	SNP SLC	*Salmonella* MPN/g (LCL, UCL)[b]
PL I	C (9, 5)	4 (1)	C1, Feb/22	*S.* Enteritidis	t5.590	0.13 (0.04, 0.37)
			C2, Feb/22	*S.* Enteritidis	t5.590	0.078 (0.02, 0.25)
			C3, Feb/22	*S.* Enteritidis	t5.590	1.70 (0.64, 4.60)
				S. Infantis	t5.1959; t5.1967	
			C4, Feb/22	*S.* Enteritidis	t5.590	2.40 (0.78, 7.40)
				S. Infantis	t5.1967	
	F (1)	1	Jan/22	*S.* Enteritidis	t5.590	1.70 (0.62, 4.40)
	G[c] (1)	1	Oct/21	*S.* Infantis	t5.1888	1.30 (0.44, 3.70)
	H (3,2)	2 (1)	H1, Feb/22	*S.* Infantis	t5.1952	24.0 (7.80, 74.0)
			H2, Feb/22	*S.* Infantis	t5.1952	3.30 (1.10, 10.0)
	K (3,3)	1	Feb/22	*S.* Infantis	t5.1957	0.78 (0.24, 2.50)
	L (2,2)	1	Apr/22	*S.* Infantis	t5.1958	<0.20[d] (NE)
	M (2, 2)	1	Mar/22	*S.* Infantis	t5.1968	54.0 (16.0, 190.0)
	P (4, 3)	2 (2)	P1, Feb/22	*S.* Infantis	t5.1957	0.13 (0.04, 0.37)
			P2, Mar/22	*S.* Infantis	t5.1973	0.23 (0.08, 0.70)
	R (2, 2)	1	Mar/22	*S.* Infantis	t5.1965	0.49 (0.15, 1.60)
	S (1)	1	Apr/22	*S.* Infantis	t5.1966	0.05 (0.01, 0.19)
	W (6, 3)	1[e]	Jan/22	*S.* Newport	2.5.14.49.155.171.198	0.02 (0.003, 0.15)
	X (1)	1	Mar/22	*S.* Newport	2.5.14.130.158.173.200	< 0.20 (NE)
PL II	B (4, 4)	4 (4)	B1[f], 25/05/22	*S.* Infantis	t5.1795	0.79 (0.25, 2.50)
				S. Enteritidis	t5.4833; t5.590	
			B2, 11/05/22	*S.* Enteritidis	t5.12	3.30 (1.10, 10.00)
			B3, 27/04/22	*S.* Infantis	t5.1936	3.30 (1.10, 10.00)
				S. Enteritidis	t5.590	
			B4, 22/06/22	*S.* Infantis	t5.1937	0.18 (0.03, 1.30)
	D (5, 5)	2 (2)	D1, 05/02/22	*S.* Enteritidis	t5.590	2.00 (0.27, 15.00)
			D2, 05/02/22	*S.* Infantis	t5.1962	4.90 (1.50, 16.00)
				S. Enteritidis	t5.590	
	Y (2, 2)	1	Jul/21	*S.* Newport	2.5.14.49.155.168.203	0.02 (0.003, 0.15)
UK III	V (15, 15)	1	Jan/22	*S.* Senftenberg	NA	< 0.02 (NE)
IE IV	J (2,1)	2	J1, Nov/21	*S.* Infantis	t5.1956	1.30 (0.44, 3.70)
			J2, Nov/21	*S.* Infantis	t5.1956	1.30 (0.44, 3.70)
	I (5, 5)	2 (2)	I1, Nov/21	*S.* Infantis	t5.1956	< 0.20 (NE)
			I2, Nov/21	*S.* Infantis	t5.1972	0.08 (0.02, 0.25)
	N (1)	1	Jul/21	*S.* Infantis	t5.1948	0.45 (0.11, 1.90)
	O (2, 2)	1	Nov/21	*S.* Infantis	t5.1871	0.45 (0.11, 1.90)
	Q (1)	1	Feb/22	*S.* Infantis	t5.1964	0.04 (0.01, 0.17)
	T (2, 2)	1	Jan/22	*S.* Infantis	t5.1963	0.13 (0.04, 0.37)
UK V	U (5, 5)	1	Oct/21	*S.* Java	1.1.1.42.55.59.67	0.05 (0.01, 0.19)
UK V (PL II)	E (2, 2)	1	Nov/21	*S.* Livingstone	NA	0.20 (0.027, 1.50)
				S. Enteritidis	t5.590	
	A (14, 10)	7 (7)	A1, Sep/21	*S.* Enteritidis	t5.4833	0.18 (0.03, 1.30)
			A4, Oct/21	*S.* Enteritidis	t5.12	28.0 (12.0, 67.0)
			A5, Oct/21	*S.* Enteritidis	t5.12	4.10 (1.80, 9.30)
			A6, Oct/21	*S.* Enteritidis	t5.12	1.70 (0.64, 4.60)
UK VI (PL II)			A2, Feb/22	*S.* Newport	2.5.14.49.155.168.201	0.05 (0.01, 0.19)
			A3, Feb/22	*S.* Infantis	t5.1961	0.20 (0.03, 1.50)
				S. Enteritidis	t5.590	
			A7, Feb/22	*S.* Enteritidis	t5.590	2.40 (0.78, 7.40)

[a] Production plant as displayed on pack (supplying plant where identified according to ECDC/EFSA, 2021a).
[b] LCL and UCL denote lower and upper 95% confidence limits of MPN.
[c] Sample from Case A freezer.
[d] Detection limit.
[e] Sole batch.
[f] Sample from Case B freezer; NE no further material examined; NA not applicable (no SNP typing).

3.5. *Salmonella* genotypes in relation to products and production plants

Maximum-likelihood phylogenetic SNP-based analysis of genome sequences generated from *S.* Enteritidis and *S.* Infantis is shown in Fig. 1 and Fig. 2, respectively. *S.* Enteritidis formed two major clusters comprising the t5.4833 cluster, and the larger t25.12 group which included the t5.12 and t5.590 clusters (Fig. 1). These three SLCs were linked to human cases as illustrated by the *S.* Enteritidis isolates from the three cases that had samples from their domestic freezers tested (ECDC/EFSA, 2021a).

While different serovars (as noted above) and different *S.* Enteritidis SLCs were detected in any one product there was strong evidence for an association between the type of *S.* Enteritidis detected and production plant (Fig. 1). Isolates belonging to *S.* Enteritidis SLCs t5.12, t5.590 and t5.4833 were detected in product A (Table 4), in six samples labelled as from plants UK V and UK VI. Isolates belonging to the same three clusters were also detected in three samples of product B from plant PL II. *S.* Enteritidis isolates in the t5.590 cluster were detected in two samples of product D from PL II and in product E from UK V. The genetic links between the *S.* Enteritidis recovered from the products from the UK and Polish plants is consistent with analysis in the Rapid Outbreak Assessment (ROA) which identified that chicken product originating from plant PL II was supplied to plants UK V and UK VI (ECDC/EFSA, 2021a). *S.* Enteritidis t5.590 isolates were also detected in products C and F from plant PL I, however these t5.590 isolates formed a separate cluster from the t5.590 isolates from PL II (Fig. 1).

For the *S.* Infantis isolates, phylogenetic analysis showed a diverse population structure with 22 different SLCs detected across 19 products

Table 5
Levels and types of *Salmonella* in samples co-contaminated with two *Salmonella* serovars.

Sample	Initial Salmonella result		Further testing[a]	
	MPN/g	*Salmonella*	*S.* Enteritidis SLC strain	*S.* Enteritidis MPN/g (CI[b])
C3	1.70	*S.* Infantis	t5.590	0.14 (0.05, 0.35)
C4	2.40	*S.* Infantis	t5.590	1.00 (0.47, 2.20)
B1	0.79	*S.* Infantis	**t5.4833, t5.590**	0.02 (0.00, 0.15)
B3	3.30	*S.* Infantis	**t5.590**	0.04 (0.01, 0.27)
D2	4.90	*S.* Infantis	**t5.12,** t5.590	2.20 (0.88, 5.60)
A3	0.20	*S.* Infantis	**t5.590**	<0.02 (NE[c])
E	0.20	*S.* Livingstone	**t5.590**	<0.02 (NE)

[a] Detection via modified Craigie tube indicated with bold font and if via MPN with underlined.
[b] Clopper–Pearson's 95% confidence interval.
[c] Not estimable (and not enough material to repeat MPN with more sample material).

Table 6
Levels of *Salmonella* and *E. coli* in replicate pieces from individual packages.

Sample	Number of pieces tested	Mean weight in g per piece (range)	Range of *Salmonella* MPN/g[a]	Range of *E. coli* MPN/g
A4	9	7.3 (6.1–10.0)	4.30–24.0	21–57
A5	5	7.6 (4.8–10.0)	0.92–7.50	Not done
B2	5	7.9 (7.0–10.0)	2.30–9.30	Not done
B3	5	8.9 (8.2–10.0)	0.45–2.30	Not done
C2	5	22.7 (22.1–23.0)	0.00–0.18	Not done
C4	5	20.9 (19.4–21.8)	1.50–13.0	31–510
G	4	15.9 (15.4–16.6)	1.50–9.30	< 10 to 59

[a] There was overlap of the 95% confidence intervals amongst all pieces tested for each of the samples.

(Fig. 2). An association with production plant was less evident than for the *S.* Enteritidis isolates although all isolates in 17 of the SLCs were from products originating from plants PL I or PL II. The *S.* Infantis detected in product N from plant IE IV was closely related to the *S.* Infantis SLCs detected in products from PL I and PL II (Fig. 2), and product N had been sourced from Poland according to the ROA (ECDC/EFSA, 2021a). Most of the *S.* Infantis isolates from plant IE IV were in clusters that did not have isolates from samples produced at PL I or PL II, although the precise origin of some of these samples was not established in the investigation (ECDC/EFSA, 2021a).

The *S.* Enteritidis contaminated products were associated with plants PL II and PL I and were produced over six months during 2020. Although most of the products containing *S.* Infantis also came from these plants and were made at a similar time (April – October 2020), there was a greater diversity amongst the *S.* Infantis isolates compared to the *S.* Enteritidis isolates (Figs. 1 and 2). *S.* Newport was detected in four products from PL I, PL II and UK VI; the isolates from products A and Y belonged to the same SLC and both samples were linked to PL II (Table 4).

S. Enteritidis isolates from samples A4, A5 and A6, with the same best before date, were contaminated with isolates belonging to the t5.12 SLC (Table 4). However, despite identical batch codes on product C samples, *Salmonella* detections differed with *S.* Enteritidis detected alongside *S.* Infantis (belonging to two SLCs), in two of the four samples. *S.* Infantis isolates from the same sample or from samples of the same batch generally fell into the same SLCs (Fig. 2 and Table 4; products M, P2, I2, Z and J1/J2 and H1/H2). Where different batches of a product were tested, the *Salmonella* detected usually differed. Of the four product B samples, with production dates spanning two months, there were different combinations of *Salmonella* serovars and/or SLCs detected (Table 4). In two batches of product D, *S.* Enteritidis was detected in both but *S.* Infantis only in one of these; in different batches of products I and P, different SLCs of *S.* Infantis were detected (Fig. 2 and Table 4).

In summary, *S.* Enteritidis t5.4833, t5.12 and a sub-cluster of t5.590 were from PL II with a different t5.590 subcluster present in samples from PL I. The majority of the *S.* Infantis SLCs and all of the S. Newport isolates were linked to production plants PL I or PL II and these observations further support the results for the *S.* Enteritidis analysis and the product tracing (ECDC/EFSA, 2021a). There was evidence of one human case (in addition to those mentioned in Table 1) co-infected with *S.* Infantis t5.1795 and *S.* Enteritidis t5.4833 and sample B1 contained isolates belonging to both these SLCs.

Analysis of the UKHSA database of genome sequence data of *S.* Infantis isolates from clinical samples identified isolates from two contemporaneous human cases in the UK occurring in the same t5.1871 SLC as isolates from product O, with two additional historical (2016 and 2019) human cases in the same SLC. No strain matches were detected for the *S.* Newport isolates falling into the t5.173 or t5.171 SLCs but one human case fell into the same t5.168 SLC (HC5_ 23260) as isolates detected in samples Y and A2, both from plant PL II. We did not find any isolates from human cases matching the *S.* Java (t5.59) isolates by SNP analysis, or the *S.* Livingstone (HC5_ 250141) or *S.* Senftenberg (HC5_ 252249) isolates using cgMLST analysis in Enterobase.

4. Discussion

We report here on the presence and levels of *Salmonella* and generic *E. coli* in a range of frozen chicken products (in particular comminuted and reformed) collected in England in 2020 as part of an international Salmonella outbreak investigation. Microbiological evidence for a vehicle of infection was obtained through testing of a FR chicken product obtained from the freezer of one case. The *S.* Enteritidis t5.4833 strain recovered was representative of that involved with the multi-country outbreak (ECDC/EFSA, 2021a). As a result of the subsequent survey of similar products, five further products were implicated with detections of further *S.* Enteritidis SLCs (t5.12, t5.590) associated with the outbreak in the UK. From one follow up sample (March 2021), an isolate belonging to a fourth *S.* Enteritidis SLC (t5.180) was detected in an eighth sample of product A (see Table S1).

Despite *S.* Infantis being the most frequent serovar detected in the chicken products, we only detected relatively limited human infection associated with *S.* Infantis (isolates from three cases feel within one of the 5-SNP SLC) although isolates from a further 28 cases fell into a wider 10 SNP SLC with the *S.* Infantis detected in products P2 and B1. Considering the diverse population structure and sampling limitations it is possible that the *S.* Infantis burden of illness associated with these products is under-ascertained. One human case was found to match one of the *S.* Newport strains while no evidence for human infections associated with the *S.* Java, *S.* Senftenberg or *S.* Livingstone isolates from the chicken samples were detected in UK human disease datasets in 2020 and 2021. Novel information is presented here on food safety and the sanitary quality of FR ready-to-cook chicken products in relation to levels of contamination by *Salmonella* and generic *E. coli*. The study highlights how results of food testing can enhance our understanding of foodborne salmonellosis outbreaks involving multiple *Salmonella* strains and provides information to support future quantitative microbial risk assessment.

Initially, *S.* Infantis were detected in chicken samples from three cases' domestic freezers but *S.* Enteritidis belonging to the t5.4833 and t5.590 SLCs were then also detected in one of these as a result of using a serovar-specific approach for detection of outbreak strains. *Salmonella* was ultimately recovered from 8.8% of the survey chicken products with over 60% having between 13 and 22 months of shelf-life remaining. *Salmonella* was detected in a range of products that included battered and non-comminuted and this has widened the scope of *Salmonella* 'risky' products where previous data had focussed on breaded reformed chicken products (Hobbs et al., 2017; Morton et al., 2019; Smith et al.,

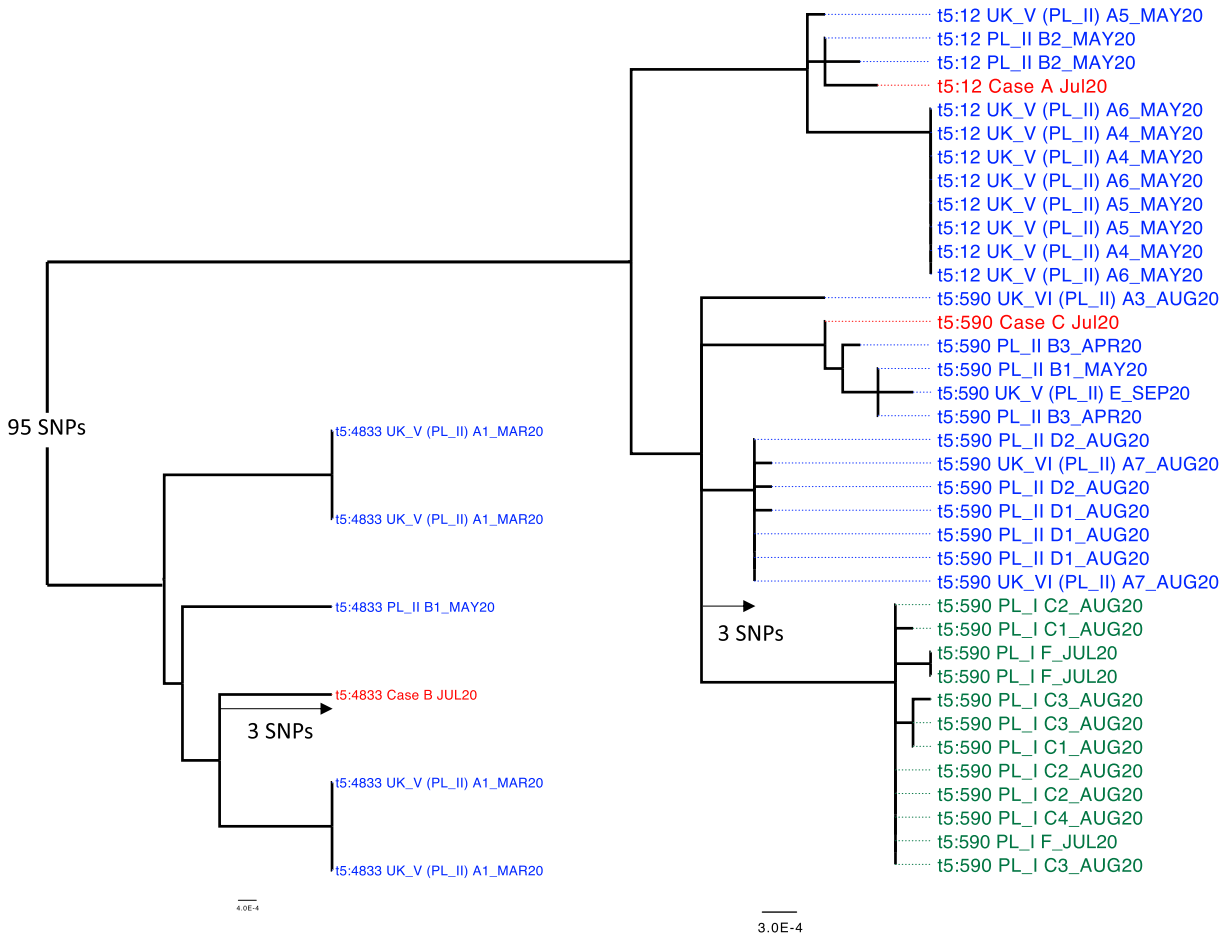

Fig. 1. Maximum-likelihood phylogenetic clustering of *S.* Enteritidis SLCs t5.12, t5.590 and t5.4833 isolated from frozen, reformulated ready-to-cook chicken products. Annotation for the food isolates: blue isolates indicate a PL II origin; green a PL I origin and comprising: designated SLC, country of production plant, plant (supplier plant), product ID and month of production. Red are clinical isolates from cases where *Salmonella* were recovered from chicken products collected from their domestic freezer followed by the month of isolation. Note the different scale for t5.4833 compared to t5.12 and t5.590. PL = Poland, UK = United Kingdom. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2008; Trmcic et al., 2020). The highest rate and levels of *Salmonella* contamination detected in this study were in breaded comminuted and reformed chicken products and therefore these may represent a greater risk than other formulations. Contamination by *Salmonella* was identified in products from only six of more than 50 different production plants and contamination rates varied amongst the samples from these plants. In Canada, contamination rates were also found to vary between plants producing similar products (Trmcic et al., 2020). The study described here was a cross-sectional survey using convenience sampling, collecting samples at random, although with some limited preferential sampling of specific products if already identified as being more likely to be contaminated with *Salmonella*. However, a very wide range of products were obtained, with both larger supermarket chains and smaller independent stores represented. Nevertheless, the *Salmonella* recovered were representative of those associated with the UK outbreak and therefore provided information for control of food safety and ultimately disease control (ECDC/EFSA, 2021a).

Levels of *Salmonella* reported here ranged from <0.02 to 54 MPN/g and were generally higher than those reported elsewhere. For example, *S.* Enteritidis in breaded chicken products associated outbreaks in Canada were detected at between 0.003 and 0.089 MPN/g (Trmcic et al., 2020) and between 0.002 and 3.000 MPN/g (Catford et al., 2017). In the contaminated products here, we found that contamination with both *Salmonella* and *E. coli* was relatively homogeneous between individual chicken pieces from a single pack, with a variation in levels of only about one $\log_{10}$. This lack of variation, together with the occurrence of multiple *Salmonella* serovars in a single sample may reflect mixing of ingredients during production including possible contributions from several chicken flocks and/or co-contaminated flocks in a single product. Where coated, lower levels of *Salmonella* were detected in the coating of individual nuggets, suggesting either a lower level of contamination of the coating/crumb ingredients and/or loss of viability during processing for example by heating or drying.

In this study, there was a strong relationship between the presence of *Salmonella* and the levels of *E. coli*. In contrast, in the Canadian study (Trmcic et al., 2020) *Salmonella* were isolated from 8/50 samples but *E. coli* was below the limit of detection (10 cfu/g) in all samples tested. It is possible that this could relate to our samples being of generally poorer microbiological quality (28/42 samples with >0.1 *Salmonella* MPN/g) and/or reflect the enumeration of *E. coli* by a different method (Petrifilm) in the Canadian study. Levels of generic *E. coli* have traditionally been used as a marker of microbiological quality, an indicator of faecal contamination, as well as evidence for poor cleaning and general factory hygiene (Mossel et al., 1995). Our results indicate that monitoring of trends in *E. coli* may be useful as part of the food safety management systems operated by food businesses to improve the microbiological quality of products even with respect to contamination by *Salmonella*. Based on our data it is possible that a review of the upper limit in EC Regulation 2073/2005 for *E.coli* for these products could be relevant.

The application of a serotype-specific PCR (in addition to the standard culture method) prompted extraordinary efforts to isolate *S.* Enteritidis from co-contaminated products when the standard method

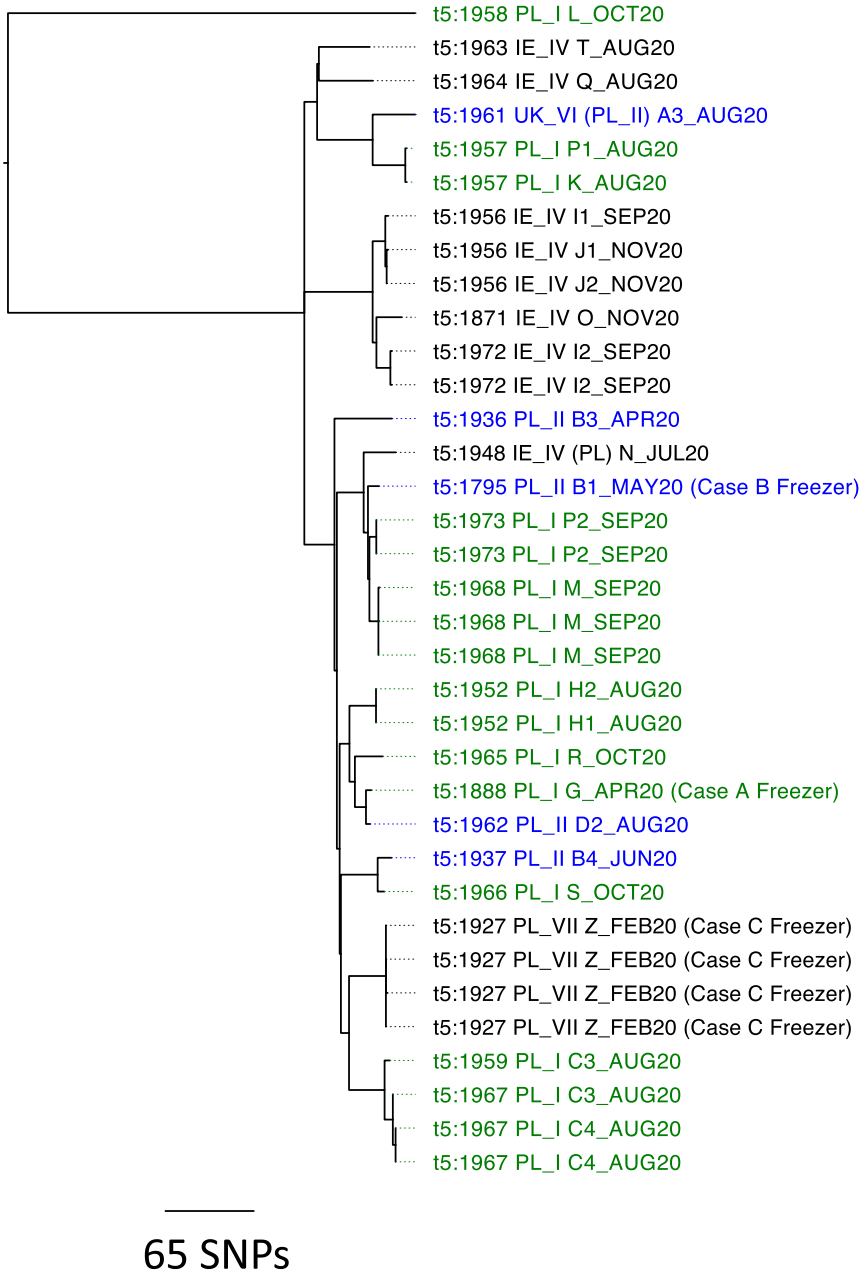

Fig. 2. Maximum-likelihood phylogenetic clustering of *S.* Infantis isolated from frozen, reformulated ready-to-cook chicken products. Annotation for the isolates: blue isolates indicate a PL II origin; green a PL I origin; black a UK IV or PL VII origin. Annotation comprised designated SLC, country of production plant, plant (supplier plant), product ID and month of production. PL = Poland, IE = Ireland, UK = United Kingdom. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

failed to detect *S.* Enteritidis. Neither serovar specific PCR, and even less so, isolation via a modified Craigie-tube, are routinely used in addition to the standard *Salmonella* culture method. Nevertheless this may partially explain a failure to detect *S.* Enteritidis from samples collected at primary production/manufacture during the investigation (ECDC/EFSA, 2021a). Of the 17 *S.* Enteritidis contaminated samples, this serovar was isolated from four products using the serotype-specific PCR/Craigie tube method, and two of the *S.* Enteritidis outbreak strains were only detected using this method from the co-contaminated samples. Notably, for another product, one *S.* Enteritidis strain was detected by MPN but not by the standard method, and this has been observed previously (Bonardi et al., 2017). In co-contaminated samples *S.* Enteritidis was approximately 2 to 100-fold less concentrated than other *Salmonella* serovars present. The antisera added to the Craigie tube targeted the flagella of *S.* Infantis (H factors 'r' and '1,5'), and it was an intriguing finding that these antisera also allowed the selective isolation of *S.* Enteritidis in a sample where *S.* Livingstone was detected, the latter has H factors of 'd' and 'l,w'. However, *S.* Infantis and *S.* Livingstone are both O6/7 type and therefore the 'H-factor' antisera may have provided sufficient cross reaction to favour isolation of *S.* Enteritidis, but this was not investigated further. This method may be helpful in future investigations where co-contaminated products are involved, although metagenomic approaches may also prove valuable (Haendiges et al., 2021). The co-contamination of chicken products highlighted the need to consider multiple hypotheses for outbreak investigation and to not exclude common food exposures between cases, even though they may be infected by different *Salmonella* strains, or, as highlighted by Waldram et al. (2018), even completely different species of foodborne pathogen. Contamination with different *Salmonella* serovars in FR breaded chicken products from the same batch or products from a single plant has been reported for products manufactured in Canada and, in common with the UK experience, described a diversity amongst both *Salmonella* recovered from the products and the *S.* Enteritidis from cases in the outbreaks (Hobbs et al., 2017; Trmcic et al., 2020). Outbreaks of human salmonellosis associated with a range of food vehicles where multiple *Salmonella* serotypes were involved have previously been

described including paprika and paprika-powdered potato chips, peanuts, sesame seed-based products, Brazil nuts, table eggs, papaya and sesame-based products (ECDC/EFSA, 2020; ECDC/EFSA, 2021b; Kirk et al., 2004; Lehmacher et al., 1995; Paine et al., 2014; Pijnacker et al., 2019; Whitney et al., 2021).

As demonstrated with this report, examination of foods is an important source of information for public health investigations, showing that more than one *Salmonella* type can be present in both specific foods and a range of foods coming from the same production plant, and where cases were infected by one of the different *Salmonella* strains recovered from foods. Co-infections by multiple *Salmonella* serovars in cases was only reported for one case in the previous studies cited above (Kirk et al., 2004) hence the importance of the results from testing foods in detecting co-contamination within individual food chains to ensure food safety. It is presently unclear exactly how multiple contamination occurred for the products in this study, it could be a result of inter- or intra-flock contamination by different strains or a result of multiple contamination events from sites within the factory, ingredients (including poultry meat), or contamination during packaging and transport both prior to, or after production. Further investigation is needed to establish the relative importance of different routes of contamination and where interventions are most effective.

Genome sequence-based analysis of the six *Salmonella* serovars provided information to understand the contamination of the FR chicken products described here. It is perhaps not surprising that with highly processed products that there can be mixtures of *Salmonella* serovars as well different strains within a serovar within single products, batches and plants. However, there was sufficient homogeneities detected amongst *S.* Enteritidis, and also amongst *S.* Infantis and *S.* Newport consistent with product tracing showing the direct or indirect origins of products from two plants in Poland (ECDC/EFSA, 2021a). Isolates in the *S.* Enteritidis in the t5.590 cluster were distributed amongst products from two Polish plants but formed distinct sub-clusters specific to each plant and it is possible that these sub-clusters have a recent common ancestor.

It is not clear why the *S.* Infantis isolates showed greater diversity when compared to the *S.* Enteritidis isolates from the products originating from a similar time frame and production plants in Poland. This could relate to independent introduction(s) of circulating *S.* Infantis strains at different times (e.g. as a result of transmission via an infected environment and/or feed) while *S.* Enteritidis may mainly transmit vertically from breeders to their progeny. It is also possible that intervention strategies focusing on *S.* Enteritidis may play a role. Further characterisation of *S.* Enteritidis and *S.* Infantis from broiler flocks and their relation to production plants in different countries may provide an understanding of these different population structures as well as the burden of illness associated with them.

For public health purposes, the testing of food described here provided further information linking the consumption of these products with *S.* Enteritidis infections amongst human cases in the UK, as well as in other countries in the EU (ECDC/EFSA, 2021a). There was no evidence to microbiologically link the *S.* Java, *S.* Senftenberg or *S.* Livingstone detected to human cases in the UK. *S.* Infantis was the most frequent serovar detected in the chicken samples, probably reflecting that *S.* Infantis is the most common serovar detected in broilers in the EU, including Poland. However, while *S.* Infantis is an important human pathogen there was evidence for only a very limited number of infections in humans with strains genetically matching the *S.* Infantis isolates detected in the food products here. There was no evidence that *S.* Enteritidis was generally present at higher MPN levels than *S.* Infantis, thus failing to support a hypothesis where the more frequent occurrence of *S.* Enteritidis cases linked to these products was related to consumption of higher doses of *S.* Enteritidis. However, we are unable to exclude the possibility of a higher prevalence and/or levels of *S.* Enteritidis in products on the market prior to this study when outbreak cases also occurred. It is also possible that clinical detection disproportionately failed to detect *S.* Infantis infection; and/or alternatively *S.* Enteritidis maybe more likely to cause infection than *S.* Infantis. There is no evidence to suggest that *S.* Infantis is more prevalent in chicken products because it survives better than *S.* Enteritidis as these serovars appear to have similar properties of survival in foods (Du et al., 2010; Nielsen and Knøchel, 2020).

Earlier detections of the *S.* Enteritidis outbreak strains suggested there was exposure to EU consumers for at least some of the clusters since 2014 (ECDC/EFSA, 2021a) and thus the total burden of the *S.* Enteritidis exposure from these types of products has yet to be determined. In 2019, *S.* Enteritidis accounted for approximately half of reported human salmonellosis cases in the EU, *S.* Typhimurium for 20% and *S.* Infantis and *S.* Newport for 2% and 1%, respectively (ECDC/EFSA, 2021c). The control of *Salmonella* in FR chicken products (including those comminuted and coated identified here as most at risk for contamination by *Salmonella*) is likely to be multifactorial and involve the whole of the food chain. EC Regulation no. 2073 requires *Salmonella* not to be detected in 25 g samples of minced meat and meat preparations made from poultry meat intended to be eaten cooked for products placed on the market during their shelf-life. This criterion should be rigorously enforced and failures to identify and recall contaminated product from the food chain should not be tolerated. However, the most effective points for interventions are at primary production and at processing. This report identified a particular problem with two production plants located in Poland. EU monitoring of poultry has identified Poland as having higher prevalence of *S.* Enteritidis in breeding flocks than most other member states (ECDC/EFSA, 2021c). *S.* Infantis was the most frequently detected *Salmonella* serovar in the chicken products described here which is consistent with *S.* Infantis being the most prevalent serovar (33% of detections) detected in EU monitoring of poultry with *S.* Enteritidis the second most common at 8% (ECDC/EFSA , 2021c). The control of *Salmonella* in poultry is a major success within the EU, and these efforts should continue in all member states as well as the UK. Multi-country sharing of epidemiological, traceback, and microbiological data is important for future disease control between third countries and the EU. This is extremely important since, when *Salmonella* contamination becomes established in widely distributed products from large companies, there is a high likelihood for outbreaks of considerable morbidity across international borders (ECDC/EFSA, 2021a). Since the *Salmonella* contamination was identified as confined to a small number of plants, as highlighted by Trmcic et al. (2020), intervention and control for a limited number of plants and their suppliers, may substantially reduce the risk to consumers. Despite the success of EU controls in reducing the burden of salmonellosis linked to poultry products, *Salmonella* contaminated products did still enter the human food chain and further interventions for control of poultry meat and poultry meat products are warranted.

During the survey, we noted that cardboard or plastic packaging did not reliably contain all of the product, particularly the breaded coatings. The plastic packaging contained holes and crumbs were observed in retailers' freezers during sample collection. Although the outer surfaces of the individual chicken products were less contaminated with *Salmonella* than the inner portions of the product, this study highlights cross-contamination risks for consumers and the retailer, during purchase and transport to home as well as within kitchens.

The samples tested in this study identified the most common shelf life for these products as 8 months or 2 years from production. The recovery of *Salmonella* from frozen products described here as well as reports from others demonstrate survival of this bacterium in these types of product during storage (Dominguez and Schaffner, 2009; Hobbs et al., 2017; Morton et al., 2019; Smith et al., 2008; Trmcic et al., 2020). *Salmonella* is therefore likely to survive in these products in domestic freezers for extended periods. Hence the need to monitor and withdraw contaminated product, ideally before distribution and retail sale, as well as to recall contaminated product which enters the retail food chain. If contaminated product does enter the food-chain, the final control point

is effective cooking by the consumer. Thorough cooking is particularly important since during manufacture of comminuted and reconstituted products exterior contamination of ingredients is introduced into the interior of the final products. These products are ready-to-cook but can appear similar to ready-to-eat products. Children and adolescents, are particularly at risk of developing salmonellosis, and age demographic related consumption and behaviours likely resulted in a notable proportion of young adults being affected. In the EU multi-country outbreak, 47% of the individuals were aged between 5 and 24 years of age (ECDC/EFSA, 2021a). Individuals within this age group may apply cooking instructions less effectively and be inexperienced in cooking and cooking processes (Catford et al., 2017; Moschonas et al., 2015; Rao et al., 2020). Clear advice is needed for consumers on cooking (Food Standards Agency (FSA), 2020d; Food Standards Agency (FSA), 2021d) but also on the prevention of cross-contamination. Rao et al. (2020) also reported that household appliances differ in their ability to inactivate *Salmonella* in these types of frozen products and specifically identified air fryers and toaster ovens as insufficient requiring revision of cooking instructions.

In conclusion we report here on the presence and levels of *Salmonella* and generic *E. coli* in FR ready-to-cook chicken products and the value of adding in serovar-specific testing. Although this study was initiated for public health reasons relating to human salmonellosis, novel information is presented here on food safety and sanitary quality which is of public health significance. *Salmonella* was detected in a range of products (comminuted, non-comminuted, breaded, battered): this widens the scope of *Salmonella* 'risky' products where previous data had predominantly identified only ready-to-cook breaded products. Contamination by *Salmonella* was exclusive to products from a small number of plants and intervention at a limited number of plants could substantially reduce the risk to consumers. The control of *Salmonella* in part-cooked chicken products is likely to be multifactorial and involve the whole of the food chain. However, when *Salmonella* contamination becomes established in widely distributed products from large companies, there is a high likelihood for outbreaks of considerable morbidity across international borders. There was a significant relationship between the presence of *Salmonella* and the levels of generic *E. coli*. This may indicate that monitoring by food business operators of trends in *E. coli* with respect to end products would be useful as part of the food safety management systems to improve the microbiological quality and safety of these products including controlling contamination with *Salmonella*. Six serovars of *Salmonella* were detected overall and *S.* Enteritidis outbreak strains were present with other serovars in the same product. This study highlights the importance of testing foods to support our understanding of foodborne salmonellosis outbreaks where multiple *Salmonella* strains are involved. Where products of the same batch or products from a single plant were tested, characterisation of *S.* Enteritidis, *S.* Infantis and *S.* Newport strongly suggested common food chains and were consistent with results from food chain tracing relating to Polish production. All the *S.* Enteritidis detected belonged to one of the SLCs associated with the outbreak but there was limited evidence for human infection associated with the other *Salmonella* serovars detected in the products. This report further demonstrates that it is crucial to combine and make available, multiple sources of data (i.e. epidemiological, microbiological (from clinical and food samples), food chain and hazard characterisation) to all those involved with control of microbiological hazards in the food chain to better understand routes of contamination and inform intervention. Food surveillance provides an important source of public health information which also facilitates assessment of the effects of interventions and in controlling contamination.

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Ethical approval

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Declaration of competing interest

We declare no conflict of interest.

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