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Research Paper

Bacteriological Survey of Fresh Minced Beef on Sale at Retail Outlets in Scotland in 2019: Three Foodborne Pathogens, Hygiene Process Indicators, and Phenotypic Antimicrobial Resistance

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ABSTRACT

The health and economic burden of foodborne illness is high, with approximately 2.4 million cases occurring annually in the United Kingdom. A survey to understand the baseline microbial quality and prevalence of food-related hazards of fresh beef mince on retail sale could inform risk assessment, management, and communication to ensure the safety of this commodity. In such a survey, a two-stage sampling design was used to reflect variations in population density and the market share of five categories of retail outlets in Scotland. From January to December 2019, 1,009 fresh minced beef samples were collected from 15 geographic areas. The microbial quality of each sample was assessed using aerobic colony count and *Escherichia coli* count. Samples were cultured for *Campylobacter* and *Salmonella,* and PCR was used to detect target genes (stx_1 all variants, stx_2 a to g, and rfb_{O157}) for Shiga toxin–producing *E. coli* (STEC). The presence of viable *E. coli* O157 and STEC in samples with a positive PCR signal was confirmed via culture and isolation. Phenotypic antimicrobial sensitivity patterns of cultured pathogens and 100 *E. coli* isolates were determined, mostly via disk diffusion. The median aerobic colony count and *E. coli* counts were 6.4×10^5 (interquartile range, 6.9×10^4 to 9.6×10^6) and <10 CFU/g (interquartile range, <10 to 10) of minced beef, respectively. The prevalence was 0.1% (95% confidence interval [CI], 0 to 0.7%) for *Campylobacter,* 0.3% (95% CI, 0 to 1%) for *Salmonella,* 22% (95% CI, 20 to 25%) for PCR-positive STEC, and 4% (95% CI, 2 to 5%) for culture-positive STEC. The evidence for phenotypic antimicrobial resistance detected did not give cause for concern, mainly occurring in a few *E. coli* isolates as single nonsusceptibilities to first-line active substances. The low prevalence of pathogens and phenotypic antimicrobial resistance is encouraging, but ongoing consumer food safety education is necessary to mitigate the residual public health risk.

HIGHLIGHTS

- The prevalence of *Campylobacter* and *Salmonella* in fresh minced beef was low.
- Almost a quarter of samples were PCR positive for Shiga toxin (ST) target genes.
- A fifth of PCR-positive ST samples were confirmed by isolation of STEC.
- The risk to consumers from bacterial hazards is mitigated but not negligible.
- The instruction to thoroughly cook fresh beef mince before eating remains relevant.

Key words: Antimicrobial resistance; Beef mince; *Campylobacter*; Prevalence, *Salmonella,* Shiga toxin–producing *Escherichia coli*

The health and economic burden of foodborne illness is high in the United Kingdom, with approximately 2.4 million cases occurring annually *(13)*. In Scotland, the top five food-associated pathogens are *Campylobacter, Salmonella, E. coli* O157, *Listeria monocytogenes,* and norovirus *(15)*. Eating raw or undercooked mince that has been contaminated with pathogens is known to cause foodborne illness.

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Although beef mince sold from retail outlets is labeled with statements that it must be fully cooked before consumption, there is still the possibility that a consumer may not fully cook the product, or that any foodborne pathogens present may cross-contaminate other foods in the kitchen. Literature suggests that the prevalence of *Campylobacter, Salmonella,* and Shiga toxin–producing *E. coli* (STEC) O157 in fresh minced beef from retail outlets in England and Europe is relatively low. Prevalence estimates reported from studies of *Campylobacter* in fresh bovine meat products range from 0 to 5.8% *(12, 17, 47)*. Estimates of the prevalence of *Salmonella* range from 0.1% in the Republic of Ireland *(14)* to 1.4% in England *(47)* and 3.4% in Denmark *(12)*. STEC organisms are a diverse group, and their presence in a range of matrices can be detected by both traditional culture and molecular techniques. Studies of cultured O157 STEC in minced beef give low prevalence estimates, from 0.1 to 2.9% *(5, 14, 48)*. Prevalence estimates for cultured STEC of all serogroups (not just O157) in fresh minced beef on retail sale range from 0 to 5.8% in European countries, with 10% reported in Chile *(12, 14, 45)*. There are no estimates available for Scotland. Furthermore, United Kingdom estimates are outdated; significant changes in agricultural practice and food hygiene legislation have occurred since their publication, which may affect the prevalence of pathogens in minced meat. Knowledge of the current baseline microbial quality and the prevalence of food-related bacterial hazards in fresh beef mince from retail outlets in Scotland would be useful. They would contribute to an improved understanding of the impact of current farming practices and regulations, as well as to risk assessment, management, and communication. To fill this evidence gap, a survey was commissioned to generate baseline data on the microbial food quality and prevalence of food-related bacterial hazards of fresh beef mince on retail sale in Scotland. Measurement of the former was based on the process hygiene indicator organisms, i.e., *E. coli* counts and aerobic colony count (ACC). The three significant food-related bacterial pathogens investigated were *Campylobacter, Salmonella,* and STEC. In addition, phenotypic antimicrobial sensitivity profiles were determined for all of the pathogens isolated and a random selection of 100 of the *E. coli* bacteria cultured. A secondary objective was to identify potential risk factors for the presence of food-related bacterial hazards, which could help to inform the development of prevention strategies.

MATERIALS AND METHODS

Sampling strategy. The minimum sample size was predetermined by Food Standards Scotland as 1,000 samples, based on advice from Biomathematics and Statistics Scotland. Due to resource constraints, this was the target number of samples for acquisition in the survey. A two-stage sampling strategy was used to facilitate the logistics of sample collection, to optimize cost-effectiveness, and to capture the requirements of the aim and objectives of the survey. The first stage was the random selection of 15 geographical areas (GAs; Supplemental Table S1). Their probability of selection was proportional to size as defined by the number of households. Due to the wide variation in population density across Scotland, neighboring local authority areas were combined into the new spatial units, the GAs, each of which included at least 41,500 households. The second stage was "retail category." All types of retail outlets were included (e.g., independent shops, butchers, supermarket chains). They were classified into five categories, each containing similar types of outlets, which were assigned numbers due to commercial confidentiality. The 1,000 samples were allocated to each retail category using the proportional distribution of market share for the types of retail outlets included in that category, according to available market data from 2017 to 2018 (*46*; Table S2).

A complete list of Scottish retail outlets actively selling fresh mince was not available. A partial list provided by Food Standards Scotland was validated and supplemented by systematic hand searches of the internet (using Google) for each of the selected GAs. These master lists of retail outlets in each retail category, by GA, were used to randomly select the premises to be visited in a sampling week. The master lists were updated as the survey progressed, using information returned by the sampling staff.

Sample collection. Sampling occurred in three rounds, each of 15 weeks, from January to December 2019. The 15 selected GAs were assigned in order of selection to a sampling week in a round (weeks 1 to 15). The same GAs were to be sampled, in the same order, in each of the three rounds. The 1,000 samples were evenly distributed across the 45 sampling weeks. Ten of these weeks were then randomly selected, in which to acquire an additional sample from retail category 4 to make up the overall total. No GA was required to provide an extra sample more than once. Detailed sampling instructions were provided to the samplers, and training was cascaded. The sampling staff were provided with a weekly list of retail premises by retail category and instructed to visit the first retailer in each category and purchase an appropriate number of samples from the product ranges of fresh beef mince that were available, one per product range, up to the required number of samples for that retail category for that week. If the total number of samples required exceeded the number of product ranges available, the sampler was to visit the next premises on the list, until the required number of samples had been purchased. Selection of the samples within the retail premises was in the order discovered. Standard operating procedures were followed for sample collection and submission to the laboratory for testing. Samples were maintained at chill temperatures (standard operating procedure aim: 3 ± 2°C) in a cool box and delivered to the testing laboratory within 12 h of collection. The cool box temperatures were monitored using either calibrated dataloggers, calibrated thermometers, or calibrated temperature probes.

Sample processing. Samples were processed by three United Kingdom Accreditation Service–accredited, European Union Official Food Control Laboratories. Processing was begun immediately on arrival at the laboratory.

The samples were examined for microbial quality using standard process hygiene indicator organisms. The ACC was based on BS ISO 4833-2:2013 *(32),* and colony counts were calculated using a weighted mean from two plates to derive the final ACC per gram of sample. The *E. coli* quantification was based on BS ISO 16649-2:2001 *(27)*. Typical *E. coli* colonies were counted from plates with fewer than 150 and 300 typical and atypical colonies, respectively. Up to 10 randomly selected colonies were biochemically confirmed as *E. coli* from each positive sample. The cut-offs used to define that these measures were below the limit of detection (BLOD) were $<4 \times 10^3$ CFU/g

TABLE 1. *The panel of antimicrobials used for phenotypic antimicrobial resistance testing of pathogens and* E. coli *of 1,009 fresh beef mince samples, purchased from retail outlets in Scotland during 2019*

Antibiotic/active substance(s)	WHO group[a]	Antibiotic class
Ampicillin 10 μg	A	Penicillins
Cefotaxime 5 μg	W	3rd generation cephalosporin
Ceftazidime 10 μg	W	3rd generation cephalosporin
Ertapenem 10 μg	W	Carbapenems
Amoxicillin + clavulanic acid 30 μg	A	β-Lactam and β-lactamase inhibitor
Piperacillin + tazobactam 30 μg	W	β-Lactam and β-lactamase inhibitor
Chloramphenicol 30 μg	A	Amphenicols
Tetracycline 10 μg	A	Tetracyclines
Gentamicin 10 μg	A	Aminoglycosides
Ciprofloxacin 5 μg	W	Fluoroquinolones
Trimethoprim 5 μg	A	Trimethoprim derivatives
Sulphamethoxazole + trimethoprim 25 μg	A	Sulphonamide and trimethoprim combinations, i.e., synergism
Colistin[b]	R	Polymyxins

[a] WHO, World Health Organization. WHO AWaRe classification: A, access; W, watch; R, reserve *(53)*.
[b] MIC established; all other active substances were sensitivity tested via disk diffusion.

for ACC and <10 CFU/g for *E. coli* counts. The pathogens *Campylobacter* and *Salmonella* were isolated based on BS EN ISO 10272-1:2017 *(33)* and BS EN ISO 6579:2002 *(29),* respectively, and were confirmed by standard methods, including biochemical and serological tests. For STEC, an enrichment broth was prepared using 25 g of mince in 225 mL of Oxoid buffered peptone water (ISO) (Thermo Fisher, Waltham, MA). Genomic DNA was extracted using SureFood Prep *E. coli* extraction kit (BioPharm, Chesham, Bucks, UK). The extracted DNA was analyzed with TaqMan based primers (Thermo Fisher) to allow the amplification of target genes. The target genes were stx_1 (ST 1 all variants), stx_2 (ST 2, a to g), and rfb_{O157} (*E. coli* O157). The method was based on ISO/TS 13136:2012 *(31)*.

All *Campylobacter* isolates, all *Salmonella* cultures, an aliquot of the enrichment broth for all samples positive for one or more *stx* gene and for all *stx*-negative and rfb_{O157}-positive samples, plus one *E. coli* isolate for each sample from which *E. coli* could be isolated, were securely packaged according to World Health Organization specifications *(51)* and sent to Scotland's Rural College at Inverness via courier. Here, further testing included antimicrobial sensitivity testing, plus isolation of *E. coli* O157 and non-O157 STEC from PCR-positive samples. Enrichment broths testing positive for *stx* and/or rfb_{O157} were cultured according to ISO 16654:2001 *(28)*. Colonies were then confirmed as *E. coli* O157 by latex agglutination (Thermo Fisher) and PCR (for *stx* and/or rfb_{O157}). Pure PCR-positive colonies were stored on microbank beads at −80°C for further testing. Evidence of phenotypic antimicrobial resistance (AMR) was determined by antimicrobial sensitivity testing, which was carried out on all *Salmonella,* STEC, O157, and a subset of 100 randomly selected *E. coli* isolates. Disk diffusion, using the panel of antimicrobials listed (Table 1), was based on ISO 20776-1:2006 *(30)*. Commercially available disks and 4-mm Mueller Hinton agar (Thermo Fisher) were used. For colistin, the Micronaut broth microdilution system (Bioconnections, Stoke-On-Trent, Staffordshire, UK) was used to establish the MIC. Cut-off values for both methods were as described by the European Committee on Antimicrobial Susceptibility Testing *(10)*.

Statistical analysis. Statistical analysis was completed in Stata software (release 13.0, StataCorp, College Station, TX). The initial data set was prepared and validated, variables described, and the apparent prevalence of the process hygiene indicators, pathogens, and antimicrobial resistance were calculated using ratio estimates to account for the clustered sampling method *(7, 16)*. A positive result for *Campylobacter* and *Salmonella* was defined by a positive enrichment culture result as per "Materials and Methods." Due to the different stages in testing for STEC and the interest in O157 status, several definitions for a positive sample were defined as follows.

For presumptive STEC, the genomic DNA extracted from sample enrichments had at least one of the genes (stx_1, stx_2) identified using real-time PCR (RT-PCR). There were two subsets to this: (i) for presumptive STEC O157, the extracted genomic DNA had at least one of the ST genes (stx_1 or stx_2) and the rfb_{O157} gene identified using RT-PCR and (ii) for presumptive non-O157 STEC, the extracted genomic DNA had at least one of the ST genes (stx_1 or stx_2) but not the rfb_{O157} gene identified using RT-PCR. Those samples confirmed STEC positive were presumptive STECs from which at least one STEC O157 and/or non-O157 STEC was isolated.

Discrete and continuous independent variables were categorized based on sample distribution or biological plausibility, except for ACC and *E. coli*. These two were categorized based on the thresholds for process hygiene indicator criteria in Regulation EC No. 2073/2005 *(9)*. If a category of an independent categorical variable had no positive samples and the original categories could logically be combined, new categories were constructed. If it was not logical to condense categories, the independent variable was excluded from the analysis. Independent variables and each pathogen were tabulated and compared using the Wald chi-squared test for each of the outcomes (sample pathogen status). Potential risk factors for the presence of a defined pathogen were investigated using mixed-effects logistic regression, with GA as a random effect. Inclusion of categorized continuous independent variables in the regression models either as a nominal, or an ordinal, variable was determined with the likelihood ratio test. Independent variables with evidence of a univariable association ($P < 0.05$) with the outcome were incorporated into a multivariable model in a forward stepwise manner. The order of inclusion was based on their impact on the crude odds ratio and Wald chi-squared statistic. Model stability was assessed based on convergence and relative differences of less than 0.01 using a quadratic check *(38)*.

TABLE 2. *Distribution of the aerobic colony counts and* E. coli *of 1,009 fresh beef mince samples, purchased from retail outlets in Scotland during 2019*

Indicators of microbiological food quality	No. of samples	Proportion of samples (95% CI)
Aerobic colony counts (CFU/g)		
BLOD $< 4 \times 10^3$	32	0.03 (0.01–0.05)
$4 \times 10^3 \leq$ CFU $< 5 \times 10^5$	447	0.44 (0.40–0.49)
$5 \times 10^5 \leq$ CFU $< 5 \times 10^6$	233	0.23 (0.21–0.26)
$\geq 5 \times 10^6$	297	0.29 (0.26–0.33)
E. coli (CFU/g)		
BLOD < 10	716	0.71 (0.66–0.75)
$10 \leq$ CFU/g < 50	174	0.17 (0.14–0.21)
$50 \leq$ CFU/g < 500	98	0.10 (0.08–0.12)
CFU/g ≥ 500	21	0.02 (0.01–0.03)

RESULTS

Sample collection. In 2019, 1,009 samples of minced beef were collected by 23 individuals from 15 Scottish GAs. In accordance with the original sampling schedule, 974 samples were collected; however, for a variety of logistical reasons, some under- and oversampling occurred. In an additional targeted sampling period between rounds 2 and 3, 13 specific samples were obtained to resolve previous challenges that had led to undersampling and to preempt issues that were anticipated to arise in round 3.

Description of the samples (Table S3). Information regarding the product range and the country of origin was not available for 31 and 33 samples, respectively. Of the remaining samples, all except three were labeled as originating within the United Kingdom and Ireland; therefore, country of origin was excluded from the statistical analysis. There were 70 loose samples. The majority of the prepacked samples (98%, $n = 920$) had a modified atmosphere. Data on fat percentage was not available for 268 samples. Where known, the distribution of fat percentage was bimodal with peaks at 5 and 20% and a range of 1 to 25%. Two samples were purchased 1 day after their use-by date, 48 (5%) were purchased on their use-by date, and the remainder had a median of 4 days until their use-by date expired.

Microbial food quality. The median ACC and *E. coli* count were 6.4×10^5 (interquartile range, 6.9×10^4 to 9.6×10^6) and <10 CFU/g (interquartile range, <10 to 10) of minced beef, respectively (Table 2).

Pathogens. *Campylobacter* was isolated from only one sample, leading to a low estimate of apparent prevalence (Table 3). Although the identity of this isolate was confirmed at this point as *Campylobacter* spp., it subsequently proved to be unrecoverable. Both ACC and *E. coli* count were BLOD for this sample. There were three *Salmonella*-positive samples (Table 3). Two were purchased on the same date in September, in the Edinburgh GA, on the same day as their use-by date—one each from retail categories 1 and 5. The cool box temperature was recorded as 2.6°C. Of these two samples, one had an ACC BLOD and an *E. coli* count of 30 CFU/g, the other had an ACC of 2.1×10^7 CFU/g and *E. coli* count of 8.4×10^3 CFU/g. The third *Salmonella*-positive sample was purchased in January from a retail category 3 outlet, in the Aberdeen GA, 5 days before the use-by date, with a cool box temperature recorded as 2°C. The sample had an ACC of 4.6×10^5 CFU/g and an *E. coli* count BLOD. Further statistical analysis to identify potential risk factors for positive samples was not conducted for either of these two pathogens due to the low number of positive samples. There were 226 presumptive STEC-positive samples (Table 3). Of these, 35 were confirmed as STEC positive. There were 20 samples in which the rfb_{O157} gene but no *stx* genes were detected by PCR, of which seven yielded isolates that possessed the rfb_{O157} but not *stx* genes. A further six *stx*-negative *E. coli* O157 (i.e., rfb_{O157}-positive) isolates were cultured from presumptive STEC-positive samples. In total, 48 STEC and/or O157 colonies were isolated from 47 samples because one presumptive STEC O157 sample yielded two different colonies: a *stx*-negative *E. coli* O157 and a STEC non-O157 colony.

Potential risk factors for presumptive STEC. With GA included as a random effect throughout, there was no evidence ($P = 0.861$) that ACC as a nominal, as opposed to ordinal, variable improved model fit, so ACC was modeled as an ordinal exposure variable. However, there was strong evidence ($P = 0.001$) that *E. coli* count should be modeled

TABLE 3. *Prevalence of pathogens in 1,009 fresh beef mince samples purchased from retail outlets in Scotland during 2019*

Pathogen	No. of positive samples	Proportion of samples (95% CI)
Campylobacter	1	0.001 (0–0.007)
Salmonella	3	0.003 (0–0.01)
Presumptive STEC (O157 and non-O157)	226	0.22 (0.20–0.25)
Presumptive non-O157 STEC	193	0.19 (0.17–0.22)
Presumptive O157 STEC	33	0.03 (0.02–0.04)
Confirmed STEC (O157 and non-O157)	35	0.035 (0.02–0.05)
Confirmed non-O157 STEC	31	0.03 (0.02–0.05)
Confirmed O157 STEC	4	0.005 (0.002–0.02)
Presumptive O157 (non-STEC)	20	0.02 (0.01–0.27)
Confirmed O157 (non-STEC)	13	0.01 (0.006–0.02)

TABLE 4. *Final multivariable mixed-effect logistic regression model for the association between* E. coli *count and presumptive STEC status based on 1,009 fresh beef mince samples purchased from retail outlets in Scotland during 2019*

Risk factor	Total	No. of presumptive STEC positive	Adjusted odds ratio (95% CI)[a]	*P* value
E. coli				
BLOD CFU < 10	716	104	Base	<0.001
10 ≤ CFU < 50	174	65	3.04 (2.06–4.49)	
50 ≤ CFU < 500	98	49	4.53 (2.80–7.33)	
CFU ≥ 500	21	8	2.70 (1.04–6.70)	
Retail category				
1	274	37	Base	<0.001
2	269	51	1.31 (0.82–2.12)	
3	180	78	3.13 (1.92–5.12)	
4	101	18	1.08 (0.56–2.06)	
5	185	42	1.64 (0.99–2.72)	
Season of sampling				
Spring	281	63	Base	0.015
Summer	234	66	1.29 (0.83–2.00)	
Autumn	289	64	0.92 (0.60–1.41)	
Winter	205	33	0.57 (0.34–0.94)	

[a] The model constant is 0.13 (95% CI: 0.08 to 0.20). The measure of between-cluster variation is 0.14 (95% CI: 0.02 to 1.15) and the within-cluster variation is 0.01 (95% CI: 0 to 0.29). The likelihood ratio test provided no evidence ($P = 0.297$) of within-cluster correlation.

as a nominal variable. There was strong evidence ($P < 0.001$, Table S4) for a univariable association between each of the process hygiene indicator organisms (ACC and *E. coli* count) and the presumptive STEC status of the sample. In addition, three other independent variables—the retail category, the season the sample was collected, and the packaging atmosphere—showed evidence of an association with presumptive STEC status. After adjusting for the effect of *E. coli* count and the retail category, there was no evidence for an association between either ACC ($P = 0.119$) or the type of packaging atmosphere ($P = 0.159$) and presumptive STEC status. However, after adjusting for retail category and season, there remained strong evidence ($P < 0.001$) that the *E. coli* count was associated with a fresh beef mince sample being presumptive STEC positive, with GA as a random effect. This was adopted as the final model (Table 4). For each category of *E. coli* count, the odds of the sample being presumptive STEC positive was increased compared with the baseline category of BLOD (Table 4).

Potential risk factors for confirmed STEC. Due to model instability, the crude odds ratio and associated values could not be reported for the variable "product range." Because none of the independent variables had evidence of an association with confirmed STEC status of a sample, a multivariable model could not be justified empirically (Table S5).

Potential risk factors for confirmation of presumptive STEC-positive samples. There was evidence that (i) retail category, (ii) categorized *E. coli* count, as an ordinal variable, and (iii) categorized ACC, as an ordinal variable, were each individually associated with confirmation of STEC status of presumptive STEC-positive samples (Table S6). After adjusting for other variables, the best-fitting model only included *E. coli* count (as an ordinal categorized variable), with GA as a random effect (Table S6). For every categorical increase in *E. coli,* the odds of a presumptive STEC-positive minced beef sample being confirmed positive roughly halved.

Phenotypic evidence of AMR. From the 1,009 fresh beef mince samples, 100 randomly chosen *E. coli* isolates, the three *Salmonella* isolates, and the 48 STEC and non-STEC O157 isolates underwent antimicrobial sensitivity testing. The *Campylobacter*-positive isolate could not be recovered on subculture, so it could not be tested. Of the 151 isolates, 12 had evidence of phenotypic resistance. Seven of the 12 profiles had evidence of phenotypic resistance to only one of the active substances in the panel; five had phenotypic patterns of resistance to three antibiotics with three different multiresistant patterns found (Table 5).

DISCUSSION

This 2019 survey has, for the first time, established a baseline measure of the microbial food quality and the prevalence of food-related bacterial hazards in fresh beef mince on retail sale in Scotland. The aim of the survey sampling design was to ensure that it was statistically robust enough to generate prevalence estimates, as indicated by the relatively narrow 95% CIs, and to give a preliminary indication of potential major risk factors. The apparent prevalence estimates of the food-related hazards reported are without any adjustment for test sensitivity and specificity. Minor deviations from the sampling plan occurred for several reasons. The main one was when there

TABLE 5. *Frequency of nonsusceptible phenotypes in 12 of the 151 isolates tested that were obtained from fresh beef mince samples purchased from retail outlets in Scotland during 2019, by bacterial group and overall prevalence by active substance*

	Bacterial group—type of isolate					Overall prevalence of nonsusceptibility for individual active substances	
No. of isolates with phenotypic pattern	*E. coli*	*Salmonella*	O157 STEC	Non-O157 STEC	Non-STEC O157	*n* for active substance ($N = 151$)	Proportion (95% CI) $N = 151$
Not susceptible to[a]:							
Ampicillin	4	0	0	0	0	4	0.03 (0.01–0.05)
Tetracycline	5	0	0	1	1	3	0.05 (0.01–0.08)
Trimethoprim	1	0	0	2	0	3	0.02 (0–0.04)
Sulphamethoxazole-trimethoprim	1	0	0	2	0	3	0.02 (0.002–0.04)
Chloramphenicol	2	0	0	2	0	4	0.03 (0.002–0.05)
Colistin[b]	0	1[b]	0	0	0	1	0.001 (0–0.02)
Multiple nonsusceptible combinations[a]							
Ampicillin, tetracycline, chloramphenicol	2	0	0	0	0		Not applicable (NA)
Ampicillin, trimethoprim, sulphamethoxazole plus trimethoprim	1	0	0	0	0		NA
Cholamphenicol trimethoprim, sulphamethoxazole plus trimethoprim	0	0	0	2	0		NA
Overall no. of[a]:							
Nonsusceptible isolates	7	1	0	3	1	12	NA
Susceptible to whole panel	93	2	4	28	12	139	NA

[a] The number of nonsusceptible phenotypes is higher than the number of nonsusceptible isolates overall because some isolates were nonsusceptible to more than one antibiotic–active substance combination. Therefore, they appear in both the not susceptible to the individual active substance rows and the multiple nonsusceptibility patterns.

[b] This result is not classed as a cause for concern. It is based on phenotype. It is a recognized phenomenon among certain *Salmonella* isolates *(43)*. The *mcr* gene was not found on sequencing of these isolates.

was either too few retail outlets in a retail category, or insufficient product types in the retail outlets, to obtain the requisite number of samples that week. The additional sampling introduced to try to remedy any shortfall is highly unlikely to have had a significant impact on the findings because of the small number of samples involved.

Microbial food quality. The category thresholds for the process hygiene indicators were based on Regulation EC No. 2073/200526 *(9)*. However, there are differences between the regulation and the sampling strategy in the survey. Although these differences mean that the survey results are not directly comparable with findings from statutory testing and the results should not be used to label individual samples as "unsatisfactory" or "satisfactory," the regulation did provide relevant limits for categorizing these variables.

Only a very small proportion of samples had ACCs BLOD of the method used, with almost a third in the highest category. ACC is a general measure of the background microbial status of meat that includes bacteria arising from the animals and the slaughterhouse and from the subsequent meat processing environment. Some of these bacteria will be responsible for spoilage, so ACC also provides an indication of the keeping quality of the meat. This is an important factor for maintenance of consumer confidence. Statutory sampling involves collation of results from several samples tested out of a batch and occurs at the end of the manufacturing process. The survey samples were from the point of retail sale. Because ACC will increase between the two points, the survey findings are unsurprising. This increase may be influenced by storage conditions, use-by dates, and packaging atmosphere, in a complex multifactorial manner that it was not possible to elucidate statistically in this survey, due to the sample size and survey design.

E. coli counts are an indicator of fecal contamination that has survived the production process. Because they can be used as a proxy for the risk of pathogens transmitted to consumers by the fecal–oral foodborne route *(18),* it is encouraging to find that more than two-thirds of the samples had *E. coli* counts BLOD. The survey results are also consistent with *E. coli* counts often being a better indication than ACC of the risk of the presence of pathogenic organisms *(18)*. After adjustment for other factors, increased *E. coli* counts were associated with presumptive STEC status, whereas ACC was not retained in the multivariable model.

Campylobacter. The baseline prevalence estimate obtained in this survey falls within, and toward the lower end of, the range reported in the European literature. Diagnostic and study methodologies vary, so, although not directly comparable, the published figures provide an indicative range (0 to 5.8%) and context within which to place the current survey estimates *(12, 17, 35, 36, 47)*. In 1982, Turnbull and Rose *(47)* reported a prevalence of 1.0% in minced beef at retail and other outlets in England based on 2,015 samples. In a more recent United Kingdom–based

study (2003 to 2005), the estimated prevalence of *Campylobacter* in red meat was 4.9% *(35)*. However, it was unclear whether minced beef was included in the sampling and how retail outlets were selected. In a survey of butchers and large supermarkets in the Republic of Ireland, using a cultural protocol that was adapted for recovery of fastidious *Campylobacter* species, a higher prevalence of 36% was obtained for minced beef *(36)*. In Scotland, chicken is thought to be the most common cause of foodborne-related human clinical *Campylobacter* cases *(21)*. The low prevalence of *Campylobacter* in this survey provides reassurance that existing controls, along the chain from farm to retail point of sale, are contributing to management of any potential risk to the consumer from fresh minced beef. Although it was disappointing that no viable organism was available for further study, it is likely that the original *Campylobacter* was lost during transport through competition with the *Ochrobactrum* that was recovered from the transport swab and medium. The genus *Ochrobactrum* is ubiquitous across ecological niches and has previously been reported from cattle at slaughter *(1)*. The Gastrointestinal Bacteria Reference Unit at Public Health England reports *Campylobacter* losses of approximately 5% due to die-off or contamination *(41)*.

***Salmonella*.** Absence of *Salmonella* in minced meat intended to be cooked is an established food safety criterion in the European Union; however, achieving a state of zero risk is not feasible. The baseline prevalence of *Salmonella* in the survey, as with *Campylobacter,* falls within the lower end of the range (0.1 to 3.4%) reported in the European literature *(12, 14, 19, 20, 35, 47)*. Again, the diagnostic and study methodologies vary, so direct comparisons are difficult. A previous study in the United Kingdom, during 2003 to 2005, assessed the prevalence of *Salmonella* in raw red meat and offal samples at point of sale *(33)*. Of the 1,563 beef samples, 1.3% were positive for *Salmonella,* with a significantly higher prevalence in offal than muscle: 6.1 compared with 1.1% *(33)*. During 2019, there were 756 isolates of human nontyphoidal *Salmonella* reported to Health Protection Scotland *(23)*. The two most commonly reported serotypes were *Salmonella* Enteritidis and *Salmonella* Typhimurium *(23)*. Because *Salmonella* isolates are reportable, further investigation of the serotypes at the Scottish *Salmonella, Shigella,* and *C. difficile* Reference Laboratory, by whole genome sequencing, identified the survey isolates as a single *Salmonella* Dublin and two *Salmonella* Mbandaka.

STEC. The baseline prevalence of presumptive STEC-positive mince samples was approximately five times the prevalence of samples in which the presence of STEC was confirmed. The majority of confirmed isolates were non-O157 STEC, with very few STEC O157 confirmed by culture. A difference between the number of enrichment broths found to be positive by PCR and the number of STEC or *E. coli* O157 isolates has been noted in other studies. The frequency of STEC PCR-positive samples found in ground beef from Australia, Asia, North and South America, and South Africa ranged from 8 to 78%, with rates for isolation of STEC by culture from the same samples between 5 and 27% *(3, 4, 25, 34, 39, 45)*. A number of factors may contribute to culture negative results from positive PCR enrichments. The PCR assays used for the initial identification and assignation of presumptive STEC and/or O157 status can detect as few as five copies of a given target. PCR demonstrates that the target nucleic acids are present, whereas culture and isolation demonstrate that viable bacteria are present. There is the possibility that viable bacteria experience (sub)lethal injury during either meat processing or the freezing and thawing of the enrichment culture. In addition, loss of the *stx* prophage may occur during subculture *(44),* plus testing used a subaliquot of the initial enrichment broth, i.e., it was not completed on identical material. Any of these factors could contribute to the observed results. It is also possible that *E. coli* pathogens may have been outcompeted, or their presence masked, by other bacteria either in the enrichment broth or on culture. This hypothesis is supported by the accepted final model for the odds of a presumptive STEC sample being confirmed positive on culture, in which, for every categorical increase in the *E. coli* count, the odds of a presumptive STEC-positive minced beef sample being confirmed positive roughly halved.

With the caveats about the influence of methods on measurement, previous published estimates of the prevalence of STEC in a range of beef products at the retail level vary. In the United Kingdom, between 1996 and 1997, only 1.1% of 2,075 samples of minced beef from 81 butchers in Yorkshire were O157 *E. coli* positive with one or more STEC genes *(6)*. This is substantially lower than the presumptive STEC prevalence estimate obtained here. However, not only has more than two decades passed between the two studies, but there is also the possible effect of the type of retail outlet and geographical representation, as well as the laboratory methodology, to consider. Old surveys of raw beef in the United Kingdom reported a STEC prevalence by "DNA probes" of 17% *(49),* and in the Republic of Ireland, 2.8% of 457 minced beef samples were PCR STEC positive *(5)*. In 2011, the European Food Safety Authority reported that the proportion of STEC-positive samples varied between the individual Member States, ranging from 0 to 14.9%, and the proportion of all samples that were O157 STEC positive varied between 0.3 and 2.3% *(8)*. Hence, the overall prevalence of confirmed STEC isolates in this Scottish survey, although low, is not exceptional.

In the United Kingdom, cattle are considered the major reservoirs of infection for human clinical cases of STEC, particularly O157 STEC. Although the role of environmental reservoirs is recognized, source attribution has not been fully elucidated *(50)*. In 2019, the survey year, 150 human clinical cases of *E. coli* O157 and 108 cases of non-O157 STEC were reported in Scotland *(42)*. However, positive samples confirmed as non-O157 STEC, rather than O157 STEC, predominate in this survey of retail mince. A wide range of herd-level prevalence estimates for different non-O157 STEC serogroups have been published recently *(26)*. A variety of PCR targets were used to identify positive non-

O157 STEC samples from frozen fecal enrichments *(26)*. These enrichments were aliquots of the 2014 to 2015 Scottish survey samples that were part of the British *E. coli* O157 in Cattle Study *(24)*; the original study in 2014 to 2015 found that between a fifth and a quarter of cattle herds were positive for STEC *E. coli* O157 on immunomagnetic separation, culture, and isolation from fecal pat samples *(24)*. This frequency had not changed significantly since an earlier comparable study in 2002 to 2004 *(40)*. This earlier study coincided with one in which 222 cattle from 34 farms were followed to 12 slaughterhouses in Scotland, in which 55% of their hides tested positive (using immunomagnetic separation and culture) for O157 STEC *(37)*. With such a high frequency of occurrence of STEC at farm and slaughterhouse level, and given the ubiquitous nature of these organisms, it is perhaps not surprising to detect so many presumptive PCR-positive samples in fresh beef mince samples. This is especially so given that mince is often an economical product, containing surface parts from multiple carcasses, and the production process can distribute organisms throughout the product. However, even these prevalence estimates in fresh mince are much lower than those obtained from the cattle field study samples. Without truly contemporaneous field, slaughterhouse, and end-product surveys, it can only be hypothesized that measures introduced during the last two decades since 2002 to 2003, such as stricter requirements for the presentation of clean cattle at slaughter, have contributed to the reduction of the risk posed for foodborne transmission. As discussed earlier, it is probable that these figures for presumptive STEC prevalence translate into a much lower frequency of viable pathogens in the end product, indicating that risk mitigation measures between the farm and final product are effective. However, the 2019 retail mince survey results do highlight the need for continued education to ensure that risk mitigation measures are understood by consumers and are taken between purchase and consumption.

AMR. The antimicrobials for the phenotypic panel were chosen to be comparable with the Scottish One Health Antimicrobial Use and Antimicrobial Resistance report *(22)*. Most of the phenotypic AMR detected in isolates from the fresh mince samples was in *E. coli*. They were primarily resistant to single, commonly used, first-line active substances that have a long history of use in ruminant populations, such as ampicillin, tetracycline, and trimethoprim. No phenotypic evidence for resistance was identified to any of the antimicrobials on the World Health Organization's Watch list *(53)* that were tested for by disk diffusion. This is in line with expectations from the Scottish slaughterhouse surveys of *E. coli* from cattle fecal samples *(22)*.

Low-level fluoroquinolone resistance is of concern in *Salmonella,* with the European Committee on Antimicrobial Susceptibility Testing clinical breakpoint reduced to 0.06 mg/L because of evidence of treatment failures in the medical field *(11)*. However, no evidence of phenotypic resistance to ciprofloxacin was observed in any of the isolates from the retail mince survey samples. This is consistent with the infrequent occurrence in the cattle slaughterhouse fecal samples (1.4% in 2017 and 0% in subsequent years *(22)*). In contrast, the percentage of human *E. coli* bacteremia isolates in Scotland that were non-susceptible to ciprofloxacin in 2018 ranged from 15.5% in the community to 26.2% in health care–associated infections *(17)*. The lack of phenotypic susceptibility to colistin identified by MIC is a recognized phenomenon among certain *Salmonella* isolates *(43)*. Subsequent analysis of the whole genome sequencing of this organism by the Scottish *Salmonella, Shigella,* and *C. difficile* Reference Laboratory found no evidence of recognized colistin resistance genes conferred by mobile genetic elements (*mcr* genes). The phenotypic finding was, therefore, not a cause for concern. AMR is one of the current global challenges *(2, 52)* affecting everyone, not just public health policy makers. The survey results, therefore, provide some reassurance that fresh beef mince on retail sale in Scotland is unlikely to be a major foodborne route for transmission of AMR to humans from cattle.

Risk factors. The investigation of potential risk factors was a secondary objective of the survey, and therefore, the findings should not be overinterpreted. Given the low estimated prevalence of the pathogens, the statistical power of the analysis to investigate variation and potential risk factors was low. Furthermore, the survey design only allows hypotheses to be raised about the associations that are detected. There is no temporal component, and a causal relationship cannot be inferred. However, the potential does exist for biologically plausible factors that have been identified to be risk factors.

The strong evidence for an association between the presumptive STEC-positive status and samples of minced beef with an *E. coli* count above the limit of detection compared with those with an *E. coli* count BLOD is biologically plausible. Lower standards of hygiene processes may lead to increased contamination with fecal pathogens if they are present. In addition, it is possible that the more *E. coli* organisms that are present, the more likely that there will be *E. coli* with *stx* genes present in the population. There was no clear dose–response relationship between *E. coli* count and presumptive STEC. However, the statistical power of the analysis was low, so it is possible that, with a larger sample size, a trend may be seen.

Retail category was associated with the odds of a sample of minced beef being presumptive STEC positive when adjusted for other variables. This was driven by the odds for a sample purchased from an outlet in one retail category (3), which was three times that of the baseline category. All retail category 3 samples were prepacked minced beef, which generally had a higher proportion of presumptive STEC-positive samples and had fewer samples with *E. coli* BLOD. The model may not fully adjust for these factors. It is also possible that additional factors within the distribution and supply chain not captured in this survey have led to clustering. Therefore, it would be inappropriate to consider this finding as a cause for concern about fresh mince sourced specifically from this retail category without additional evidence.

The evidence for an association between a minced beef sample being presumptive STEC positive and season was driven by a reduction in the odds for those samples collected in winter (January, February, and December) compared to the baseline of spring (March to May, inclusive). It might be hypothesized that this is due to environmental temperature influencing the cold chain and bacterial growth. However, all samples were transported in a cool box, and the median temperature and interquartile range of the cool box was the same in summer and winter. Again, there are many other hypotheses and factors for which season may be a proxy, some of which occur earlier in the supply chain, prepurchase. It would not be appropriate to develop risk mitigation measures based solely on the outcomes of this single study. Further investigation would be required.

In conclusion, the survey has established baseline estimates of the prevalence of the three major bacterial food-related hazards in fresh beef mince on retail sale in Scotland, and a measure of the microbiological food quality. Although the prevalence estimates observed provide reassurance that existing controls along the chain from farm to retail point of sale are contributing to managing the potential risk to the consumer, it is essential that these products continue to be labeled with clear advice on cooking and safe handling. To minimize the risk to human health, it is also essential that consumers are educated about their responsibilities.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found on line at: https://doi.org/10.4315/JFP-22-051.s1

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