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Research Paper

Assessment of the Microbiological Quality and Safety of Unpasteurized Milk Cheese for Sale in England between 2019 and 2020

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ABSTRACT

Cheese made with unpasteurized milk has been associated with outbreaks of illness. However, there are limited data on the prevalence of Shiga toxin–producing *Escherichia coli* (STEC) in these products and a lack of clarity over the significance of *E. coli* as a general indicator of hygiene in raw milk cheeses. The aim of this study was to provide further data to address both of these issues, as well as assessing the overall microbiological quality of raw milk cheeses available to consumers in England. A total of 629 samples of cheese were collected from retailers, catering premises, and manufacturers throughout England. The majority (80%) were made using cow's milk, with 14% made from sheep's milk and 5% from goat's milk. Samples were from 18 different countries of origin, with the majority originating from either the United Kingdom (40%) or France (35%). When interpreted against European Union microbiological criteria and United Kingdom guidance, 82% were considered to be of satisfactory microbiological quality, 5% were borderline, and 12% were unsatisfactory. Four samples (0.6%) were potentially injurious to health due to the isolation of STEC from one, $>10^4$ CFU/g of coagulase-positive staphylococci in two, and >100 CFU/g of *Listeria monocytogenes* in the fourth sample. Indicator *E. coli* and *Listeria* species were detected more frequently in soft compared with hard cheese. Higher levels of indicator *E. coli* were significantly associated with a greater likelihood of detecting Shiga toxin genes (stx_1 and/or stx_2).

HIGHLIGHTS

- Of 629 cheese samples examined, 82% were of satisfactory microbiological quality.
- Four samples (0.6%) contained potentially harmful levels of bacteria.
- Shiga toxin genes (stx_1 and/or stx_2) were detected in 10 samples.

Key words: Foodborne infection; Microbiological quality; Raw milk cheese; Unpasteurized milk cheese

Cheese is generally considered a safe and nutritious food. However, foodborne illnesses have been linked to cheese consumption in many countries. Donnelly *(9)* reported eight outbreaks of foodborne illness associated with raw milk cheese in the United Kingdom between 1983 and 2018 and 53 outbreaks globally. Of the United Kingdom outbreaks, six were due to *Escherichia coli* O157, one to *Salmonella,* and one to *Staphylococcus* spp. *(9)*. Verraes et al. *(31)* reviewed outbreaks in Europe, the United States, and Canada that were caused by the consumption of dairy products made from raw milk, and where cheese was the source; these included outbreaks caused by *Salmonella,* Shiga toxin–producing *E. coli* (STEC), *Listeria, Brucella, Campylobacter, Staphylococcus aureus, Streptococcus equi* and *Streptococcus zooepidemicus,* and tickborne encephalitis virus.

STEC organisms occur in bovine herds and have been associated with outbreaks linked to the consumption of raw milk cheese *(3, 7, 21)*. Contamination of cheese with these pathogens is a particular concern, as risk assessment of the hazard is difficult due to lack of available data on the prevalence of non-O157 STECs in food and on the association with cases of illness. Furthermore, there are technical challenges with the detection of STEC in food, which involves the use of PCR and microbiological culture *(16)*: the initial detection relies on amplifying Shiga toxin genes stx_1 and/or stx_2 (as well as other virulence genes) from an enrichment broth by PCR, but subsequent culturing and isolation of *E. coli* that can be shown to possess these

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virulence genes can be complex and resource intensive. The significance of the detection of the *stx* genes in the absence of culture of an associated *stx*-positive *E. coli* isolate is not yet fully understood.

Raw cow's milk cheese made in Scotland was implicated in an outbreak of STEC O157 in 2016, following which the significance of the detection of STEC organisms in other cheese products (made from sheep's milk) from the implicated producer became the subject of legal proceedings *(2)*. The environmental health department sought to remove batches of raw sheep's milk cheese from sale due to the detection of several different STEC strains in samples of cheese and raw milk from the implicated producer, as well as an *stx*-negative *E. coli* O157 strain. Consideration was given to the potential pathogenicity of the different strains on the basis of the presence of stx_1 or stx_2, absence of other virulence genes (*eae, aaiC,* and *aggR*), and absence of evidence of human cases of infection with these strains. The court ruled that the batches of cheese from which potentially pathogenic STEC (O unidentifiable:H20, stx_{2d}, sequence type 1308; O unidentifiable:H14, stx_{2b}, sequence type 7010; O8:H9, stx_{2e}, sequence type 23; and O153-O178:H7, stx_{1c}, sequence type 278) had been isolated were unsafe, but that remaining batches from the same producer could be sold.

The safety of raw milk cheese is dependent upon a range of hurdles that influence the growth, survival, and inactivation of pathogenic microorganisms. These include the microbiological quality of the raw milk, the rate and degree of acidification during production, and the water activity (a_w) of the final product. Cross-contamination from the cheesemaking environment can also affect the microbiological quality of the cheese *(9)*. In the United States, the sale of cheese made from unpasteurized milk is only allowed if it has been aged for at least 60 days at a temperature of at least 1.7°C, on the basis that the combination of low pH, low a_w, high salt, and competitive flora would reduce the numbers of any pathogens present in the cheese over time *(4)*.

Microbiological criteria for assessment of the safety and process hygiene of cheese are included in European Commission Regulation (EC) No 2073/2005 (as amended) *(10)*. These specify limits for *Salmonella, Listeria monocytogenes,* and coagulase-positive staphylococci (CPS). However, this regulation does not provide criteria for *E. coli* in unpasteurized milk cheese, despite giving limits for this organism in cheese made from pasteurized milk. The United Kingdom's Health Protection Agency (HPA) specified that all ready-to-eat foods sampled at the point of sale were considered to be of borderline quality if the *E. coli* count was greater than 20 CFU/g and unsatisfactory if greater than 100 CFU/g, but that for raw milk cheeses, investigation should be undertaken if any change in trend of *E. coli* levels is detected *(13)*. Meanwhile, the United Kingdom Specialist Cheesemakers' Association suggests a target of <100 CFU/g *E. coli* for hard cheese and <10,000 CFU/g for soft or semisoft cheese *(28)*. Therefore, there is a lack of consensus regarding the interpretation of levels of *E. coli* in these products, in terms of the ability to use this group of bacteria to indicate problems with process controls or hygiene during cheese production and postproduction handling.

The aim of this study was to gain further data on the prevalence of pathogens and bacterial indicators of poor hygiene in raw milk cheese, with the objectives of informing future risk assessments and further investigating the significance of elevated levels of indicator *E. coli* in these products.

MATERIALS AND METHODS

Sample collection. A total of 629 samples of raw milk cheese were collected by sampling officers from 76 environmental health departments in England between April 2019 and March 2020. These included 285 samples from 36 local authorities in London, East of England, and East Midlands, 166 samples from 24 local authorities in the South East, South West, and West Midlands, and 178 samples from 17 local authorities in the North East and North West of England. A cross-sectional study design was used for raw milk cheese from retailers, caterers, and the final product (i.e., packaged for sale) from manufacturers. Products known to be associated with incidents of foodborne illness or subject to public product recalls were not included in this study.

Samples (of at least 100 g) were collected and transported in accordance with the Food Standards Agency's Food Law Code of Practice *(11)* and were examined by official laboratories in England (Public Health England, Food, Water and Environmental Microbiology Laboratories at London, Porton and York). Samples were transported in cold boxes between 0 and 8°C and tested within 36 h of collection.

Information on samples and food businesses was obtained by local authority sampling officers' observation and inquiry and then recorded on a standard questionnaire. This included information on the type of food business, type of cheese collected (e.g., hard or soft, as specified by the manufacturer or retailer), what species of animal the milk originated from, country of origin, and type of packaging (prepacked or loose, i.e., unpackaged at the time of sampling).

Description of samples. Cheese samples were collected from retailers (513 samples), catering premises (49), wholesalers (11), and manufacturers (56). These included 304 samples described as hard cheese, 212 soft, 77 blue, 20 semihard, 9 semisoft, 5 fresh, and 2 not specified. The majority (502, 80%) were made by using cow's milk, with 86 (14%) made from sheep's milk, 31 (5%) from goat's, and the remainder from mixed milk types (4), buffalo (1), or not specified (5). Samples were from 18 different countries of origin, with the majority originating from either the United Kingdom (252, 40%) or France (222, 35%). In addition, 511 (81%) of 629 samples were stored at 0 to 8°C at the time of sampling, while 70 (11%) of 629 samples were stored above 8°C, and the question was not answered for 48 samples (8%). Of those stored above 8°C, 31 (44%) were described as hard cheeses, 28 (40%) were soft, and 11 (16%) were blue. In terms of sampling locations, 20 (29%) samples stored above 8°C were collected from manufacturers, 11 (16%) from catering premises, 7 (10%) from supermarkets, and 32 (46%) from other retail outlets.

Microbiological examination. A 10^{-1} homogenate of each sample was prepared by diluting 27 g of cheese in either dipotassium hydrogen phosphate buffer or buffered peptone water according to the International Organization for Standardization method, ISO 6887-1 *(17),* giving a total homogenate volume of 270 mL. A 20-mL portion of this homogenate was decanted and used to enumerate CPS, *Listeria* species (including *L. monocytogenes*), and *E. coli* by using the methods indicated in Table 1.

TABLE 1. *Test methods used for the various microbiological parameters*

Microbiological parameter	Test method
Enumeration of CPS, including *S. aureus*	BS EN ISO 6888-1 *(15)*
Detection of presumptive STEC (*stx* genes) and isolation of STEC	CEN/ISO/TS 13136 *(16)*
Isolation and enumeration of *Listeria* spp., including *L. monocytogenes*	BS EN ISO 11290-1 *(18)* and 11290 *(19)*
Isolation of *Salmonella* spp.	ISO 6579 *(20)*
Enumeration of β-glucuronidase producing *E. coli*	On the basis of BS ISO 16649-2 by using either a surface spread or a pour plate technique *(26)*

Briefly, 0.5-mL portions of the 10^{-1} homogenate were inoculated onto Baird Parker, Oxoid chromogenic *Listeria,* and tryptone bile glucuronide agar plates, respectively. These were incubated at 37°C for 48 h (CPS and *Listeria*) or 30°C for 4 h, followed by 44°C for 20 h (*E. coli*). The remaining 250 mL of cheese homogenate was incubated at 37°C for 18 h. This preenrichment broth was then subcultured to selective enrichment media, Rappaport-Vassiliadis, and Muller-Kauffmann tetrathionate novobiocin broths, which were subsequently subcultured onto brilliant green and xylose lysine desoxycholate agars to allow detection of *Salmonella* species (Table 1).

In addition, a real-time PCR technique was used to examine portions of the same buffered peptone water preenrichment broths, as described previously, for the presence of STEC on the basis of ISO/TS 13136 *(16)*. Enrichment broths that were PCR positive for *stx* genes were streaked onto MacConkey and tryptone bile glucuronide agars, and up to 50 colonies were retested by using the same PCR assay.

The presence of *Listeria* species in 25 g was also determined for all samples, using a separate 25-g aliquot of cheese, with primary enrichment in half Fraser broth, secondary enrichment in Fraser broth, and subculture to Oxford agar and Oxoid chromogenic *Listeri*a agar (Table 1). Presumptive *Listeria* isolates were further identified to species level by using miniaturized biochemical arrays (API *Listeria* tests; bioMérieux, Basingstoke, UK).

The a_w was determined for each sample, using an electric hygrometer (Novasina, Lachen, Switzerland). A portion of the core of the cheese was brought to ambient temperature and then placed within the air-tight measuring cell of the hygrometer. The atmosphere within the cell was allowed to equilibrate before recording the a_w reading.

Microbiological results were interpreted by using European Commission Regulation (EC) No 2073/2005 *(10)* and the HPA guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market *(13)* (Table 2). The detection of stx_1 or stx_2 genes and/or the intimin (*eae*) gene in the absence of the isolation of STEC organisms was interpreted as satisfactory in this analysis.

Characterization of isolates. Isolates of *L. monocytogenes* and STEC were sent to the Public Health England, Gastrointestinal Bacteria Reference Unit for confirmation and further characterization by whole genome sequencing by using the Illumina HiSeq 2500 instrument *(25, 29)*. For STEC, the sequence type, serotype, and *stx* subtype were determined as described previously *(8)*. For *Listeria,* clonal complexes were determined in accordance with the designation of the Institut Pasteur International Network multilocus sequence typing database for *L. monocytogenes* (https://bigsdb.pasteur.fr/listeria/listeria.html). All data were stored in a customized database (not publicly available; sequences are available at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA248549), and pairwise comparisons of SNP distances were performed between isolates from food and cases of human infection that occurred in the United Kingdom and were examined as part of national surveillance.

Statistical analysis. Descriptive and statistical analysis of the data was undertaken by using Excel 2010 (Microsoft Corporation, Redmond, WA). Relative proportions were compared by using the Fisher's exact test (GraphPad Software, San Diego, CA). A probability value of less than 5% was defined as significant.

RESULTS

Overall microbiology results. When interpreted by using the criteria shown in Table 2, 517 samples (82%) were considered to be of satisfactory microbiological quality, 34 (5%) were borderline, 74 (12%) were unsatisfactory, and 4 (0.6%) were unsatisfactory and potentially injurious to health. The samples interpreted as unsatisfactory and potentially injurious were due to the isolation of STEC in one, $>10^4$ CFU/g CPS in two, and >100 CFU/g *L. monocytogenes* in the fourth sample.

Of 616 samples for which an STEC test was performed, 10 (2%) gave positive results for the presence of *stx* genes (two blue, four hard, and four soft cheeses, all made from cow's milk). Of these, one was positive for both stx_1 and stx_2, four were positive for stx_2 only (two of which were of the same product type), one for stx_2 and *eae* genes, and for four samples, an overall *stx* gene assay was positive, but further testing was not undertaken to distinguish between stx_1 and stx_2 (one of these was also positive for O157 and *eae* genes, and a second was positive for *eae* but not O157). STEC was only isolated from one, a hard cheese collected from retail and where the indicator *E. coli* level was <20 CFU/g. The STEC isolate was identified as *E. coli* O181:H49 (ST 173; $stx_{1a/2a}$; *eae* negative). A second sample of the same brand of cheese, collected at the producer, gave a positive *stx* result by PCR (further testing to distinguish between stx_1 and stx_2 was not undertaken in this case), but STEC could not be isolated: the indicator *E. coli* level for this sample was 6,900 CFU/g. When the Public Health England data on human STEC cases in the United Kingdom were interrogated, one human case with the same sequence type (ST) and *stx* subtype (O181:H49, ST173, $stx_{1a/2a}$) was identified. However, this was not sufficiently closely related to indicate a common source.

Salmonella was not detected in any samples. *L. monocytogenes* was present at an unsatisfactory or potentially hazardous level of >100 CFU/g in one sample (a hard goat's cheese). This strain was identified as belonging to clonal complex type 200. *L. monocytogenes* was detected at

TABLE 2. *Criteria for the interpretation of microbiology results*

	Satisfactory	Borderline	Unsatisfactory	Unsatisfactory: potentially injurious to health
CPS (CFU/g)[a]	<20	$20–\leq10^4$	NA[b]	$>10^4$
E. coli O157 or any STEC in 25 g	Not detected	NA	NA	Isolated in pure culture[c]
L. monocytogenes (CFU/g)[a]	<10[d]	$10–\leq100$	NA	>100
Salmonella in 25 g[a]	Not detected	NA	NA	Detected
E. coli (CFU/g)[a]	<20	$20–\leq100$	>100	NA
Listeria species (not *L. monocytogenes*; CFU/g)[a]	<10	$10–<100$	>100	NA

[a] HPA *(13)*.
[b] NA, not applicable.
[c] Detection of *stx* genes by PCR in the absence of isolation of an STEC in pure culture was interpreted as satisfactory.
[d] Isolation of *L. monocytogenes* at <20 CFU/g was interpreted as satisfactory, on the basis of Regulation (EC) No 2073/2005 *(10)* and HPA *(13)*.

levels of <100 CFU/g in two other cheeses: a blue cow's milk cheese (clonal complex 489) and a blue sheep's milk cheese (clonal complex 5). Other *Listeria* species were detected in 18 (3%) of 584 samples, of which five cheeses had levels of >100 CFU/g (with counts ranging from 140 to 1.7×10^6 CFU/g).

CPS were detected at levels of >20 CFU/g in 7 (1%) of 629 samples, with two of these (two hard cow's milk cheeses, both from the same producer) giving counts of $>10^4$ CFU/g. No samples had CPS levels above 10^5 CFU/g (the upper limit, or M-value, specified in Regulation (EC) No 2073/2005) *(10)*). For the four samples classified as unsatisfactory and potentially injurious to health, as well as those where *L. monocytogenes* was detected at <100 CFU/g, no evidence for associated cases of human infection was found.

Indicator *E. coli* levels are shown in Table 3 along with the detection of pathogens (STEC, *Listeria,* and CPS). Presumptive STEC was detected by PCR more frequently in cheeses with higher levels of indicator *E. coli* (i.e., in 0.8, 4, and 7% of the samples with <20, 20 to $<10^2$, and $\geq10^2$ indicator *E. coli* per gram, respectively). This association was significant (Fisher's exact test; $P = 0.02$).

Microbiology results in relation to cheese type, species of milk, and country of origin. Results in relation to cheese type and species of milk are shown in Table 4. The proportion of cheese samples made from sheep's milk with unsatisfactory results (3 of 86, 4%) was significantly lower than for cheeses made from cow's milk (72 of 502, 14%; Fisher's exact test; $P = 0.003$). Similarly, the occurrence of *E. coli* levels of >20 CFU/g was significantly lower in sheep's milk cheeses than for cow's milk (Fisher's exact test; $P = 0.037$). No other significant differences were observed in results for cheeses made from milk of different species.

E. coli levels of >20 CFU/g were detected more frequently in cheeses described as soft, semisoft, or blue compared with those described as hard cheeses (Fisher's exact test; $P < 0.0001$, $P = 0.010$, and $P < 0.0001$, respectively). *Listeria* species (other than *L. monocytogenes*) were also detected significantly more frequently in soft compared with hard cheese (Fisher's exact test; $P = 0.001$).

Overall, unsatisfactory results, as well as the presence of *stx* genes, were detected in cheeses originating in the United Kingdom significantly more frequently than those originating from other countries (Fisher's exact test; $P <$

TABLE 3. *Comparison of indicator* E. coli *counts with detection of other bacterial pathogens and hygiene indicators and with different water activities*

E. coli counts (CFU/g)	Total no. (%) of samples	No. (%) of samples with presumptive STEC (*stx* genes) detected in 25 g	No. (%) of samples with CPS level >20 CFU/g	No. (%) of samples with *L. monocytogenes* and other *Listeria* species detected in 25 g	No. (%) of samples with a_w in specified range[a]:			
					<0.93	0.93–<0.95	0.95–<0.97	≥0.97
<20	531 (84.4)	4 (0.8)[b]	5 (0.9)	15 (2.8)	227 (97.0)	111 (90.2)	98 (68.1)	82 (73.2)
20–$<10^2$	25 (4.0)	1 (4.0)	0	0	4 (1.7)	4 (3.3)	9 (6.3)	8 (7.1)
10^2–$<10^3$	22 (3.5)	2 (9.1)	0	0	1 (0.4)	4 (3.3)	12 (8.3)	5 (4.5)
10^3–$<10^4$	30 (4.8)	1 (3.6)	1 (3.6)	3 (10.7)	2 (0.8)	2 (1.6)	16 (11.1)	8 (7.1)
10^4–$<10^5$	11 (1.7)	2 (20.0)	0	1 (10.0)	0	2 (1.6)	3 (2.1)	5 (4.5)
10^5–$<10^6$	6 (1.0)	0	0	1 (16.7)	0	0	5 (3.5)	1 (0.9)
$>10^6$	4 (0.6)	0	0	0	0	0	1 (0.7)	3 (2.3)
Total	629	10 (1.6)	6 (1.0)	20 (3.2)	234 (37.6)	123 (19.8)	144 (23.2)	112 (18.0)

[a] Only 622 samples had a_w determined.
[b] The sample from which STEC O181 was isolated is included within this category.

TABLE 4. *Proportion of different types of raw milk cheese with indicator organisms or pathogens detected*

	Total no. (%) of samples	No. (%) of samples with unsatisfactory or potentially injurious results	No. (%) of samples with *E. coli* level >20 CFU/g	No. (%) of samples with *L. monocytogenes* detected in 25 g	No. (%) of samples with *Listeria* spp. (not *L. monocytogenes*) detected in 25 g	No. (%) of samples with CPS level >20 CFU/g	No. (%) of samples with presumptive STEC (*stx* genes) detected in 25 g
Type of cheese							
Hard	304 (48.3)	13 (4.3)	12 (3.9)	1 (0.3)[a]	3 (1.0)	6 (2.0)[b]	4 (1.3)[c]
Semihard/semisoft	29 (4.6)	5 (17.2)	5 (17.2)	0	2 (6.9)	1 (3.4)	0
Soft	212 (33.7)	47 (22.2)	62 (29.2)	0	13 (6.1)	0	4 (1.9)
Blue	77 (12.2)	12 (15.6)	17 (22.1)	2 (2.6)	0	0	2 (2.6)
Fresh	5 (0.8)	0	1 (20.0)	0	0	0	0
Not specified	2 (0.3)	1 (50.0)	1 (50.0)	0	0	0	0
Milk species							
Cow	502 (79.8)	72 (14.3)	85 (16.9)	1 (0.2)	17 (3.4)	5 (1.0)[b]	10 (2.0)[c]
Sheep	86 (13.7)	3 (3.5)	7 (8.1)	1 (1.2)	0	1 (1.2)	0
Goat	31 (4.9)	2 (7.7)	4 (12.9)	1 (3.2)[a]	0	0	0
Other/not specified	10 (1.6)	1 (9.1)	2 (18.2)	0	1 (9.1)	1 (9.1)	0
Packaging							
Loose	200 (31.8)	40 (20.0)	44 (22.0)	0	6 (3.0)	0	5 (2.5)[c]
Prepacked	381 (60.6)	29 (7.6)	45 (11.8)	2 (0.5)	10 (2.6)	2 (0.5)	5 (1.3)
Not specified	48 (7.6)	9 (18.8)	9 (18.8)	1 (2.0)[a]	2 (4.2)	5 (10.4)[b]	0
Temp of storage (°C)							
0–8	511 (81.2)	44 (8.6)	61 (11.9)	2 (0.4)	11 (2.1)	2 (0.4)	7 (1.4)[c]
>8	70 (11.1)	22 (31.4)	27 (38.6)	0	2 (2.9)	0	3 (4.3)
Not specified	48 (7.6)	12 (24.0)	10 (20.8)	1 (2.0)[a]	5 (10.4)	5 (10.4)[b]	0
Total	629	78 (12.4)	98 (15.6)	3 (0.5)	18 (2.9)	7 (1.1)	10 (1.6)

[a] *L. monocytogenes* at potentially injurious level ($>10^2$ CFU/g) in one sample.
[b] CPS at potentially injurious level ($>10^4$ CFU/g) in two samples.
[c] STEC isolated from one sample.

0.0001 and $P = 0.0016$ for unsatisfactory results and *stx* detection, respectively), but no other significant differences were observed between results for different countries of origin (Table 5).

Microbiology results in relation to packaging and storage temperature. Cheeses that were described as being prepacked (381 of 629, 61%) gave significantly fewer unsatisfactory results than those that were described as "loose" at the time of sampling (200 of 629, 32%; see Table 4; Fisher's exact test; $P < 0.0001$). In addition, 511 (81%) of 629 samples were stored at 0 to 8°C at the time of sampling, while 70 (11%) of 629 samples were stored above 8°C, and the question was not answered for 48 samples (8%). Those that were stored at 0 to 8°C had significantly fewer unsatisfactory results compared with those stored above 8°C (see Table 4; Fisher's exact test; $P < 0.0001$). This difference was seen with hard cheese (5 [2.0%] of 250 unsatisfactory when stored at 0 to 8°C compared with 3 [9.7%] of 31 when stored above 8°C), soft cheese (31 [18.8%] of 165, at 0 to 8°C compared with 11 [39.3%] of 28 above 8°C), and blue cheese (4 [6.3%] of 63 at 0 to 8°C and 8 [72.7%] of 11 above 8°C).

***E. coli* levels in comparison to a_w.** The proportion of cheeses with *E. coli* results of ≥100 CFU/g was significantly higher in samples with the a_w measured as ≥0.95 (59 of 256, 23.0%) compared with a_w 0.93 to <0.95 (8 of 123, 6.5%) and a_w <0.93 (3 of 231, 1.3%; Fisher's exact test; $P = 0.01$; Table 5).

DISCUSSION

The results from this study indicate that 82% of raw milk cheeses were of satisfactory microbiological quality, with 12% being considered unsatisfactory and an additional four samples (0.6%) being described as potentially injurious to health (one due to the presence of *L. monocytogenes,* one due to STEC isolation, and two due to elevated CPS levels), when interpreted according to the HPA guidelines for assessing the microbiological quality of ready-to-eat food on the market *(13)*. However, if CPS results are interpreted according to the criteria specified in Regulation (EC) No 2073/2005 (as amended) *(10),* then none were above the upper acceptable limit (M-value) of 10^5 CFU/g. While the majority of samples were collected at retail or from catering premises, 56 samples (9%) were collected from the manufacturer. However, as these were taken at the end of production and were considered ready-to-eat, interpretation by using the same criteria for all samples was considered appropriate.

These results can be compared with a Canadian study of 595 raw milk cheeses sampled between 2012 and 2017 of

TABLE 5. *Microbiology results of raw milk cheeses in relation to country of origin*

Country of origin	Total no. (%) of samples	No. (%) of samples with unsatisfactory or potentially injurious results	No. (%) of samples with *E. coli* level >20 CFU/g	No. (%) of samples with *L. monocytogenes* detected in 25 g	No. (%) of samples with *Listeria* spp. (not *L. monocytogenes*) detected in 25 g	No. (%) of samples with CPS level >20 CFU/g	No. (%) of samples with presumptive STEC (*stx* genes) detected in 25 g
United Kingdom	252 (40.1)	50 (19.8)	57 (22.6)	2 (0.8)	5 (2.0)	4 (1.6)	9 (3.6)
France	222 (35.3)	25 (11.2)	38 (17.1)	1 (0.5)	9 (4.1)	1 (0.5)	1 (0.5)
Italy	64 (10.2)	0	0	0	2 (3.1)	0	0
Switzerland	43 (6.8)	0	0	0	0	0	0
Spain	11 (1.7)	0	0	0	0	1 (9.1)	0
Czech Republic	6 (1.0)	0	0	0	0	0	0
Romania	6 (1.0)	2 (33.3)	2 (33.3)	0	1 (16.7)	0	0
Bulgaria	3 (0.5)	0	0	0	0	0	0
Canada	3 (0.5)	0	0	0	0	0	0
The Netherlands	3 (0.5)	0	0	0	0	0	0
Poland	3 (0.5)	0	0	0	0	0	0
Other[a]	10 (1.6)	0	0	0	0	0	0
Not specified	3 (0.5)	1 (33.3)	1 (33.3)	0	1 (33.3)	1 (33.3)	0
Total	629	78 (12.4)	98 (15.6)	3 (0.5)	18 (2.9)	7 (1.1)	10 (1.6)

[a] Other countries included Cyprus (2), Hungary (1), Norway (1), Portugal (1), Sweden (2), Turkey (2), and the United States (1).

which 6% were unsatisfactory due to elevated levels of indicator *E. coli* or *S. aureus* or the detection of *L. monocytogenes* (6 samples) or *Salmonella* (2 samples) *(12)*. A further Canadian study of 2,009 raw milk cheeses collected between 2014 and 2018 found that only 4 (0.2%) were considered to be of unsatisfactory quality due to the presence of *L. monocytogenes* or elevated levels of *S. aureus (12)*. Although the Canadian report appears to indicate a better overall microbiological quality of cheeses available in Canada than in England, the differences in results may be partly explained by differing acceptance criteria; for example, indicator *E. coli* was interpreted as unsatisfactory if greater than 2,000 CFU/g (or if more than two samples from a batch had a level of greater than 500 CFU/g) for the Canadian study, rather than the threshold of 100 CFU/g used in the study described here. However, Ganz et al. *(12)* did also demonstrate poorer microbiological quality in imported compared with domestic raw milk cheeses (7 and 2%, respectively). A study of 1,606 samples of raw milk cheese by the U.S. Food and Drug Administration in 2014 and 2015 *(30)* demonstrated an overall contamination rate of less than 1% for each pathogen examined (*Salmonella* was detected in 0.2%, *L. monocytogenes* in 0.6%, STEC in 0.7%, and *E. coli* O157 was not detected in any samples). *E. coli* was considered to be present at violative levels in 5.4% of samples.

A study of 126 sheep's or goat's milk cheese samples in England and Wales found that two samples (one goat's and one sheep's milk) were contaminated with unacceptable levels of *S. aureus,* but *Salmonella, Campylobacter, E. coli* O157, and *L. monocytogenes* were not detected in any samples *(23)*. Similarly, a study of 41 raw milk cheese samples made in the United States found *S. aureus* at a level of greater than 10^4 CFU/g in one sample (a blue, cow's milk cheese) but did not detect *Salmonella, Campylobacter, E. coli* O157, or *L. monocytogenes* in any samples *(4)*.

Because numerous environmental health practitioners throughout England were asked to collect raw milk cheese samples that were available in shops, catering premises, and manufacturers in their own geographical areas, there was some (albeit limited) duplication of product types within the data set. For example, two samples in which stx_2 genes were detected were from different batches of a blue cheese from the same producer; an additional two *stx*-positive samples were detected in different batches of the same product, one collected at retail and another from the producer; and the two samples with CPS levels of greater than 10^4 CFU/g were different products from the same producer. However, although it is recognized that the study was not specifically designed to reflect market share of different product types, the samples collected are likely to be representative of the different product types commonly available throughout the country.

The presence of *stx* genes in a small proportion (2%) of samples is a concern. In particular, *E. coli* O181 was isolated from one sample of hard, cow's milk cheese. The combination of stx_{1a} and stx_{2a} genes found in this strain has been demonstrated to be associated with severe clinical outcomes when present in clinical isolates of *E. coli* O157:H7 *(5),* although note that these *E. coli* O157:H7 strains may also have possessed other virulence factors that were not present in the cheese isolate of *E. coli* O181. It has been reported previously *(32)* that the PCR method used to detect *stx* genes is a more sensitive technique for detecting presumptive STEC than traditional culture methods. One sample that was positive for *stx* genes but negative for STEC by culture in this study was from the same producer as the sample that yielded an STEC O181 by culture. Therefore, it seems likely that the PCR technique was simply more sensitive in detecting the presence of an STEC in this sample compared with the culture procedure. This is consistent with the relatively high level of indicator *E. coli*

in this sample (6,900 CFU/g), making it more difficult to find the *stx*-positive *E. coli* among the larger number of *stx*-negative types on a culture plate following enrichment. One concern regarding the interpretation of PCR results relates to the ability of PCR to detect genes derived from dead cells, as well as viable bacteria. However, this is unlikely to be a factor in results described here, as the method uses an enrichment stage prior to DNA extraction and PCR testing. Because dead cells would not multiply during the enrichment stage, any DNA derived from these cells would be rendered undetectable by the dilution effect of the enrichment broth, unless they were present at exceptionally high numbers. The presence of *stx* genes in free bacteriophage particles *(24)* or in other bacterial hosts *(22)* has been reported, and these may potentially be propagated during the enrichment stage of the STEC detection method. Therefore, in samples giving a positive PCR result for *stx* genes, but which are negative for STEC by culture, the possibility of *stx* genes being present in free bacteriophage or in bacteria other than *E. coli* cannot be excluded. For this reason, the detection of *stx* genes by PCR without subsequent culture of *E. coli* is not reported as an unsatisfactory result, although it should still be taken as an indication of potential STEC presence and investigated appropriately.

Verraes et al. *(31)* undertook a literature review of frequency of occurrence of pathogens in products made from raw milk in Europe. Of the reports on raw milk cheese that they considered in the review, frequency of detection of the *stx* gene ranged from 0 to 41%, while the frequency of isolation of STEC organisms ranged from 0 to 9%. The higher frequencies related to studies in France (31% of 180 samples), Italy (30% of 112 samples), and Switzerland (41% of 39 samples). A study of raw milk cheeses in Scotland did not detect *E. coli* O157 in any of 739 samples *(6),* but other STECs were not included in this study. Boyd et al. *(3)* described an outbreak of STEC O121 associated with raw milk Gouda-like cheese in Canada and concluded that thermization of milk prior to use in Gouda and Gouda-like cheese production would reduce the risk of microbial contamination.

Overall, cow's milk cheeses were of poorer microbiological quality than those made from milk of other species. This is in agreement with a previous study of raw milk *(32)* that found that raw cow's milk was of poorer microbiological quality than other species. Cheeses originating in the United Kingdom were significantly more likely to be unsatisfactory and to have *stx* genes detected than cheeses made in France, despite the French cheeses including a higher proportion of those types that tend to be considered higher risk (i.e., 51% of the French cheeses were soft and 24% were blue) and therefore potentially being expected to have poorer results than the United Kingdom cheeses (of which 31% were soft and 10% blue). Of the cheeses from the United Kingdom, 84% were made from cow's milk, 7% from goat's, and 8% from sheep's milk, compared with cheeses from France, of which 70% were cow's, 4% goat's, and 26% sheep's milk cheese. This difference may partly explain the poorer results for United Kingdom cheeses, because cow's milk has been shown to more commonly carry pathogens, such as STEC, than sheep's and goat's milk *(32)*. Poorer results were seen in loose, unpackaged cheeses compared with prepacked products. This is likely to be, at least partly, due to cross-contamination from the postproduction, catering, or retail environment, although it may also represent smaller-scale, less automated production processes that do not involve a routine packing stage.

The United Kingdom's Food Safety and Hygiene (England) Regulations 2013 *(1)* require that food that is likely to support the growth of pathogenic microorganisms must be stored below 8°C, unless it can be demonstrated that storage at ambient temperature for the duration of the shelf life will not create a risk to health. An exemption is made for foods that must be ripened or matured at ambient temperature but not when the process of ripening or maturation is complete. Although hard cheeses may fall into the category of not supporting growth of pathogens during shelf life, it is likely that the majority of soft cheeses would require storage below 8°C, according to these requirements. Of samples collected for this study, 44% of hard cheeses, 41% of soft, and 16% of blue cheeses were stored above 8°C at the time of sampling, suggesting a failure to comply with food hygiene requirements in a significant number of samples. A greater proportion of unsatisfactory results were seen in all cheese types when stored above 8°C. However, the increase in unsatisfactory results when stored at higher temperatures was greater for soft and blue cheeses compared with hard cheeses. These results indicate that the higher storage temperatures could have contributed to the survival and growth of pathogens and hygiene indicator bacteria. However, it is not possible to rule out confounding factors such as a poorer general level of hygiene in premises that do not store products at the correct temperature.

Interpretation of indicator *E. coli* levels in raw milk cheese is not well understood, with criteria suggested by the Specialist Cheesemakers Association *(28)* differing from those in the HPA guidelines *(13),* and no criteria provided in European legislation for this product type. Guidance for raw milk cheese enforcement from the Scottish Food Enforcement Liaison Committee recommended that a target level of $<$100 CFU/g is achievable for some cheese types, and where this is exceeded, further evidence should be provided to verify food safety *(27)*. Similarly, criteria published by the Institute of Food Science and Technology also suggest 100 CFU/g as an upper limit for *E. coli* in unpasteurized milk soft cheese, while no limit is specified for hard cheese *(14)*. Levels of *E. coli* below 100 CFU/g were achieved in 88% of cheeses tested in this study; therefore, 12% would be considered unsatisfactory according to this criterion (including 3% of hard cheeses, 17% of semisoft and semihard, 16% of blue, and 20% of soft cheeses). A review of routine testing of raw milk cheeses in England *(25)* found that 192 (24%) of 787 samples had *E. coli* levels above 100 CFU/g. While 82% of samples in the study reported here were collected at retail, 85% of those described by McLauchlin et al. *(25)* were collected from the producer where levels of *E. coli* are likely to be higher than those at retail, due to die-off of these bacteria during the maturation and shelf life of the cheese *(9)*. Therefore, an *E. coli* level of

<100 CFU/g appears to be a target that can be met for the majority of cheese products at all stages of shelf life, albeit with a higher proportion of soft cheeses exceeding this limit compared with hard cheese. Moreover, data from this study indicate an rising risk of the presence of *stx* genes (and therefore presumptive STEC) with increasing indicator *E. coli* levels. McLauchlin et al. *(25)* demonstrated an association between increased *E. coli* levels and unsatisfactory levels of CPS, although no such association was demonstrated in the current study. Given the evidence from both McLauchlin et al. *(25)* and from the current study that higher *E. coli* levels are associated with an increased risk of potential pathogens and/or toxins being present, a level of 100 CFU/g appears to be a useful threshold for further investigation in both soft and hard cheeses.

Overall, the results of this study indicate that the majority of raw milk cheese available at retail and catering premises in the United Kingdom is of a good microbiological quality. Although the presence of pathogenic bacteria is rare, it is important to understand how these risks can be monitored and controlled.

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