doi: 10.1111/j.1471-0307.2010.00596.x

ORIGINAL RESEARCH

Microbiological characterisation of artisanal farmhouse cheeses manufactured in Scotland

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Twenty-eight Scottish artisanal farmhouse cheeses were examined in respect of 16 microbial groups of significance for food safety and cheese character development. Microbial populations were diverse and although Escherichia coli O157 and Salmonella spp. were not detected the occurrence of potential foodborne pathogens was confirmed in 86% of the samples analysed. Mycobacterium avium subsp. paratuberculosis was detected in 25% of the cheeses tested and some Staphylococcus aureus and the Bacillus cereus isolates were enterotoxigenic. Resistance to methicillin and vancomycin and other clinically important antibiotics was detected in some S. aureus and Enterococcus strains. The inappropriate labelling of some raw milk cheeses and the consequences of the complexity of the microbial population on isolation media specificity is discussed.

Keywords Raw milk cheese, Foodborne pathogens, Antibiotic resistance, *Mycobacterium avium* subsp. *paratuberculosis*.

INTRODUCTION

Cheese manufacture is increasingly becoming more industrial in scale in modern automated plant that has been designed to apply recent technological developments to ensure improved product uniformity and consistency. The increased mechanisation and control during production is perceived by some consumers to have resulted in less variety and choice and in consequence there has been renewed interest in artisanal cheeses produced using more traditional methods that nevertheless comply with current regulatory standards. Cheeses made by small scale specialist producers offer individuality and variety to the consumer and are important to the rural economy in some European countries (Cogan and Rea 1996, Beuvier and Buchin 2005).

In Scotland cheese production is dominated by a small number of large modern creameries that manufacture 2000–2500 tonnes of cheddar per month (Harbutt 2008). There are, however, approximately 25 artisanal cheesemakers located in Scotland who each produce only 0.3–3 tonnes/month, and although their combined output is only 1–2% of the total Scottish cheese production it comprises some 70–80 different farmhouse cheese types, of which approximately a third are made from unpasteurised milk. Currently just over half of the artisanal producers make cheese with pasteurised milk whilst 10 only use raw milk in their cheesemaking processes; the other artisanal producers include both unpasteurised and pasteurised milk cheeses in their product range.

Approximately 10% of the 7×10^6 tons of cheese produced annually in Europe and Switzerland is made from unpasteurised milk (Beuvier and Buchin 2005). The use of unpasteurised milk, unlike in the UK, is not restricted to specialist varieties and production can be large scale and economically significant. The bacterial population diversity is greater in raw milk cheeses and this is associated with accelerated ripening and a more complex developed flavour (Beuvier and Buchin 2005). Although, the flavour may be enhanced and more intense the attribute profile can be variable between different batches, but it is these particular characteristics that are attractive to food connoisseurs. However, the potential for the survival of pathogenic bacteria in unpasteurised milk cheeses may compromise their safety (Institute of Food Science and Technology 2000). The involvement of cheese as the food vector in foodborne disease outbreaks has been reviewed comprehensively (Johnson *et al.* 1990; Donnelly 2001, 2005) and the indications are that cheese has only been infrequently implicated in outbreaks of human illness, whilst Beuvier and Buchin (2005) claim that it has not been conclusively established that raw milk cheeses are intrinsically less safe than those made from pasteurised milk.

The four pathogens that constitute the principal threat to cheese safety are *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp. and pathogenic *Escherichia coli*. There is also concern that *Mycobacterium avium* subsp. *paratuberculosis* (MAP) could be present in cheese, especially those

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SDT International Journal of Dairy Technology

made from contaminated raw milk. The organism is the causative agent of Johne's disease in ruminants and may have an association with Crohn's disease in humans (Hermon-Taylor *et al.* 2000). Milk is a recognised food vehicle for the transmission of MAP (Grant 2006) and the organism has been shown to persist during the manufacture and ripening of some Swiss varieties, Hispanic style and Cheddar cheese (Sung and Collins 2000; Spahr and Schafroth 2001; Donaghy *et al.* 2004). The organism has also been detected in retail samples of cheese from the USA, Switzerland, Greece and the Czech Republic (Ikonomopoulos *et al.* 2005; Clark *et al.* 2006; Stephan *et al.* 2007), but to date there has only been a preliminary report confirming the occurrence of MAP in retail cheese manufactured in Britain (Williams and Withers 2008).

Studies commissioned at the instigation of the EU and on behalf of the Food Standards Agency in the UK on the microbiological quality of retail cheese in Britain focus mainly on key pathogen groups and also include dominant cheese varieties on sale, but not necessarily manufactured in the UK (Cree *et al.* 2008; Little *et al.* 2008). Consequently, little is known about the microbiology of artisanal cheeses, and hence this study was undertaken to investigate the microbial diversity of speciality farmhouse cheeses that are manufactured from unpasteurised milk in Scotland. Emphasis was placed both on monitoring the principal microbial groups that are involved in cheese attribute formation, as well as investigating the occurrence of not only recognised foodborne pathogens but also the incidence of the potentially pathogenic *M. avium paratuberculosis* in the samples examined.

MATERIALS AND METHODS

Cheese studied

The artisanal cheeses examined were all produced on-farm by small scale producers (0.3–3 tonnes/month) at island locations and throughout mainland Scotland. Cheese was either purchased directly from the manufacturer at farm shop outlets or farmers markets, or was obtained from specialist cheesemongers, delicatessens, supermarkets and on-line gourmet food suppliers in Scotland. The range of varieties studied varied from semi-soft to hard in texture. Internally and surface ripened varieties, with and without added mould cultures, were represented. The cheese had been manufactured from bovine, ovine or caprine milk, and some varieties were made with milk that had been produced organically. The ripening period for the varieties included in the survey ranged from a few days to approximately 12 months. The details of the types of cheese examined are summarised in Table 2.

Detection and enumeration of targeted micro-organisms

The occurrence, in the cheese samples analysed of 16 microbial groups associated with either cheese attribute development or food safety, was monitored by cultivation on commercially available selective and differential growth media. Two different media were usually used for each population; the microbial groups investigated, the media used and the suppliers are summarised in Table 1. The media and supplements used were purchased from Oxoid Ltd (Basingstoke, UK), Becton Dickinson UK Ltd (Oxford, UK), VWR Merck Ltd (Lutterworth, UK) and M-Tech Diagnostics Ltd (Warrington, UK). However, unless stated otherwise growth media and microbiological reagents were purchased from Oxoid Ltd. All methods used were fully evaluated in preliminary experiments to confirm their effectiveness for bacterial recovery and detection using type cultures. The colonial morphology and growth characteristics of the test organisms on the specific media were also predetermined using type cultures. The Type strains used were obtained either from the departmental culture collection or were purchased as Culti-Loop® cultures from Oxoid Ltd. Cheese varieties to be examined were purchased as single pieces 250–400 g in weight. Any rind and exposed surfaces were removed to a depth of approximately 0.5 cm using a sterile knife in the microbiologically clean environment of a class II recirculating Microbiological safety cabinet (Labcaire, Clevedon, UK). Samples (25 g) removed randomly from the prepared cheese were homogenised using a Seward Lab Blender 400 Stomacher (Seward, London, UK) for 5 min in 225 mL of prewarmed (to 45°C) Maximum Recovery Diluent (MRD; Oxoid Ltd) containing 2% (w/v) trisodium citrate. A dilution series, prepared from the cheese homogenate in MRD, was used to inoculate duplicate plates of each test medium using a WASP Spiral Plater (Don Whitley Scientific Ltd, Shipley, UK). The incubation conditions for the different growth media used are summarised in Table 1.

The screening for the presence of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157 was undertaken after pre-enrichment. Cheese (25 g) was homogenised in 225 mL of prewarmed (45°C) buffered peptone water (BPW) or Oxoid Novel Enrichment (ONE) broth-Listeria containing the specified NOVEL enrichment Listeria selective supplement and incubated at 37°C for 24 h. Serial dilutions, in MRD, of the enrichment cultures were used to inoculate selective media listed, and incubated under the conditions detailed, in Table 1. The remainder of the BPW and ONE broth-Listeria enrichment cultures were used for the immunomagnetic recovery of *Salmonella* spp. and *Listeria*

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Table 1 Target micro-organisms and culture media used

Target micro-organism	*Culture media*	*Incubation conditions*
Bacillus cereus	*Bacillus cereus* selective agar (PEMBA), *Bacillus cereus* chromogenic agar	30°C, 48 h
Coliforms	Violet red bile lactose agar, *E. coli*/coliform chromogenic agar	37°C, 24 h
Escherichia coli	Tryptone bile X-glucuronide (TBX agar), *E. coli*/coliform chromogenic agar	37°C, 24 h
Escherichia coli O157	*E. coli* O157 Chromagar[a,c]	37°C, 24 h
Enterobacteria	Violet red bile glucose agar	37°C, 24 h
Enterococci	Kanamycin aesculin azide agar, *Enterococcus* Chromocult agar	43°C, 24 h; 37°C, 48 h
Lactic acid bacteria	De Man Rogosa Sharpe (MRS) agar	30°C, 96 h
Lactobacilli	*Lactobacillus* selective agar[a]	30°C, 96 h
Lactococci	M17 agar	30°C, 24 h
Listeria spp.	Oxford *Listeria* selective agar, ALOA[b] (Agar Listeria Ottaviani & Agosti), Oxoid chromogenic *Listeria* agar (OCLA)	37°C, 48 h
Mycobacterium avium subsp. *paratuberculosis*	Herrolds egg yolk medium[a] containing VAN antibiotic mix and mycobactin J; Middlebrook 7H10[a] containing PANTA antibiotic mix, OADC enrichment and mycobactin J (Donaghy *et al.* 2003)	37°C, 3–6 m
Salmonella spp.	Xylose lysine desoxycholate (XLD) agar, *Salmonella* chromogenic agar	37°C, 24 h
Staphylococcus aureus	Baird-Parker agar containing rabbit plasma fibrinogen, *Staphylococcus aureus* chromogenic agar[c]	37°C, 48 h
MRSA	Oxacillin resistance screening agar base (ORSAB), Methicillin resistant *Staphylococcus aureus* (MRSA) chromogenic agar	37°C, 48 h
Total viable count	Milk agar	30°C, 48 h
Yeast and moulds	Rose Bengal chloramphenicol agar, Oxytetracycline glucose yeast extract agar[b]	25°C, 7 days

Unless otherwise stated growth media, broths and media supplements used were purchased from Oxoid Ltd (Basingstoke, UK).
Other suppliers used were: [a]Becton Dickinson UK Ltd (Oxford, UK); [b]VWR Merck Ltd (Lutterworth, UK); [c]M-Tech Diagnostics Ltd (Warrington, UK).
Media were incubated aerobically for the time and at the temperature indicated.

spp. respectively using the Pathatrix rapid purification system (Matrix MicroScience Ltd, Newmarket, UK). The enrichment broths were maintained at 37°C and were re-circulated for 30 min in the presence of antibody coated paramagnetic particles (50 μL) to selectively bind the appropriate target organism. The captured paramagnetic beads were recovered using the protocol described by the manufacturer and were washed once with, and resuspended in, MRD. The washed beads (200 μL) were used to inoculate duplicate plates of two different selective media that were incubated at 37°C for either 24 h or 48 h for *Salmonella* spp. and *Listeria* spp. respectively.

Additional confirmatory tests and isolate identification

Further tests were undertaken to confirm the identity of isolates recovered on the various differential and selective media. Isolates were maintained on tryptone soya agar (TSA), brain heart infusion agar (BHIA) or nutrient agar (NA); for longer term storage broth cultures were stored at −20°C in the presence of 30% (v/v) glycerol.

The identity of organisms exhibiting the colony characteristics and morphological features of the target organisms on the selective recovery media was confirmed using preliminary discriminatory tests and by phenotypic species level assignment. Gram negative rods isolated on *E. coli* O157 chromogenic agar were examined for reactivity with the Dryspot *E. coli* O157 latex agglutination test (Oxoid Ltd) to establish if they belonged to the O157 serogroup. Gram positive rods exhibiting the colonial morphology of *Listeria* spp. on two *Listeria*-specific differential media were screened for reactivity in the *Listeria* rapid latex agglutination test (Oxoid Ltd; Microgen Bioproducts, Camberley, UK). The presence of coagulase activity in presumptive *S. aureus* strains was confirmed using the Staphaurex® latex agglutination test (Oxoid Ltd). All tests were performed according to the instructions provided by the manufacturers with the kits. Genus and species level assignment of the isolates was achieved phenotypically on the basis of biochemical profiles established using the appropriate API® identification system and database in accordance with the instructions provided by the manufacturer (bioMérieux UK Ltd, Basingstoke, UK).

Detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

Mycobacterium avium subsp. *paratuberculosis* was recovered from cheese samples using immunomagnetic capture with the Pathatrix system (Matrix MicroScience Ltd, Newmarket, UK). The use of this system in the recovery of MAP from Swiss raw milk cheeses was reported by Stephan *et al.* (2007) and the techniques involved were described in detail and in the operating instructions

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provided by Matrix MicroScience with the equipment. Cheese samples (25 g) were homogenised using a Stomacher 400 laboratory homogeniser for 5 min in prewarmed (45°C) MRD (225 mL) containing 2% trisodium citrate. The homogenate was maintained at 37°C and was re-circulated for 30 min in the presence of anti-MAP antibody coated paramagnetic particles (50 μL). The captured paramagnetic beads were transferred to a stoppered tube and were washed once with, and re-suspended in, MRD. The washed beads (200 μL) were used to inoculate duplicate slopes of isolation media. The two media used were Herrolds egg yolk medium (HEYM) containing VAN antibiotic mix and mycobactin J and Middlebrook 7H10 containing PANTA antibiotic mix, OADC enrichment and mycobactin J (Donaghy *et al.* 2003). Mycobactin J was purchased from Synbiotics (Lyon, France). A separate 25 g cheese sample was processed for each medium. The slopes were incubated at 37°C for at least 3 months. Washed immunomagnetic beads obtained from the treatment of a third 25 g cheese sample were resuspended in 500 μL lysis buffer L1 (from Adiagene, Adiavet® ParaTB kit, AES Chemunex, Ker Lann, France) for DNA extraction of MAP recovered directly from the cheese.

After incubation bacterial growth was washed from the slope with MRD, the washings from the duplicate slopes were combined and the bacteria sedimented by centrifugation (10 000 *g* for 10 min). The bacterial pellet was washed by re-suspension in MRD and recovered by centrifugation. The presence of MAP in the washed slope populations was assessed using the commercially available Adiagene Adiavet® ParaTB kit for DNA extraction and PCR detection of MAP (AES Chemunex, Ker Lann, Bruz Cedex, France). The washed bacterial pellet was resuspended in lysis buffer L1 (500 μL) and transferred to a screw capped microtube containing 300 mg Q-BIOgene lysing matrix B glass beads (MP Biomedicals, Cambridge, UK). The cells were mechanically disrupted (4 m/s, 3 × 45 s) using a FastPrep™ FP120 (Bio 101 Inc., San Diego, CA, USA) and then incubated in the presence of proteinase K (50 μL) for 30 min at 37°C, 70°C and 95°C. Immunomagnetic beads used for the direct recovery of MAP from cheese were also, after resuspension in lysis buffer, treated in this manner to effect DNA release from any bound cells present. The released DNA was purified using a QIAmp® DNA mini kit (QIAGEN Ltd, Crawley, UK). PCR amplification of the IS900 insert was performed using the reagents provided, and heating cycles detailed in, the protocol from the Adiagene Adiavet® ParaTB kit. Amplified products were detected by electrophoresis on 1.3% agarose gels in Tris Borate EDTA (TBE) buffer; loading buffer and internal size markers were provided in the kit. A negative control and a positive control using DNA prepared from *M. avium* subsp. *paratuberculosis* NCTC 8578 were included with every amplification undertaken.

Toxin formation by cheese isolates of *B. cereus* and *S. aureus*

Duplicate cultures (10 mL) in 25 mL Erlenmeyer flasks were incubated with shaking (200 rev/min) for 48 h. *Bacillus cereus* isolates were grown in BHI broth at 30°C while *S. aureus* isolates were cultivated at 37°C in TS broth. After incubation the cells were removed from a 1.5-mL aliquot of the culture by centrifugation (10 000 *g*, 10 min) and the supernatant was passed through a 0.45-μm pore size filter. The presence of *B. cereus* diarrhoeal enterotoxin and *S. aureus* enterotoxins A, B, C and D in the respective cell-free culture supernatants was tested for by reversed passive latex agglutination assays using Oxoid BCET-RPLA and SET-RPLA toxin detection kits (Oxoid Ltd) according to the protocols detailed by the manufacturer.

Antibiotic susceptibility screening

Antimicrobial susceptibility was tested using antibiotic impregnated discs (Oxoid Ltd) and AB Biodisk Etest® gradient strips (bioMerieux UK) using the protocols described by the British Society for Antimicrobial Chemotherapy (2008). Colonies selected from an overnight culture of the test organism were resuspended in physiological saline to produce an inoculum density equivalent to a 0.5 McFarland standard. The cell suspension was diluted tenfold and plates of Oxoid Iso-Sensitest agar were spread inoculated with a cotton wool swab and the antibiotic impregnated strip applied. The effects of the antibiotics were determined after incubation at 35°C for 18 h or after 24 h to evaluate the effects of teicoplanin and vancomycin on enterococci. The susceptibility of *S. aureus* to oxacillin and methicillin (5 μg discs) was evaluated on Columbia agar containing 2% NaCl after incubation for 24 h at 30°C. The antibiotics tested are listed in Table 4.

Isolates of *S. aureus* were tested for the presence of penicillin binding protein (PBP2′) using a specific latex agglutination test (Oxoid Ltd) using the protocol provided with the test kit.

RESULTS

The study represents the first comprehensive survey undertaken to establish the microbiological characteristics of artisanal farmhouses cheeses produced within Scotland. Of the 28 cheeses fully characterised 25 had been made from raw milk and three from pasteurised milk by 15 different producers. Amongst the 16 different microbial groups monitored six were considered to be potential

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pathogens. The principal findings are presented in the following sections.

Microbial populations of artisanal cheese

The artisanal cheeses examined possessed a complex and diverse microbial population. Viable bacteria numbers on milk agar ranged from approximately 10^5–10^{10} cfu/g, with yeast and mycelial fungal (mould) numbers ranging up to 10^9 and 10^7 cfu/g respectively (Table 2). Either fungi or yeast were not detected (i.e., $<\log_{10}$ 1.7 cfu/g), on either the RBCA or OGYE isolation media in samples of 10 of the 28 cheeses analysed. The identity of the dominant yeasts and moulds isolated was not established. Microbial numbers were affected by the method of production (cheese type), the ripening period and in some instances the type of milk used and its method of production (organic/conventional).

Enterobacteriaceae and coliforms were detected in cheeses made with pasteurised and raw milk and were present at up to10^7 cfu/g (Table 2), although in some cheeses coliforms were only detectable after enrichment at 37°C for 24 h in BPW. The number of presumptive coliforms detected was influenced by the growth medium. Coliform numbers were lower in cheeses that had been ripened for >3 months than in those varieties that had been matured for shorter periods.

Among the bacterial groups that are associated with cheese flavour and character development the lactic acid bacteria (LAB) and the enterococci were monitored in this survey. Lactic acid bacteria were dominant in the cheese viable bacterial population and amongst these the nonstarter lactobacilli were predominant with numbers in some cheese varieties in excess of 10^9 cfu/g (Table 2). Nonstarter LAB numbers were higher in the cheeses made from raw milk than in the pasteurised milk varieties. *Lactobacillus paracasei* was the most frequent isolate recovered with 53 of 77 isolates from the 15 cheese LAB populations tested being identified as this species. It was present in 13 of these LAB populations and was the only species of Lactobacillus detected in nine of the cheeses examined. *Lactobacillus plantarum* was detected in three cheeses and was the only species recovered from one cheese. *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus fermentum* and *Lactobacillus rhamnosus* were each present in a single cheese. No information was gathered on the biochemical properties of the isolates.

Enterococci have been implicated in the formation of specific flavour attributes particularly associated with cheese manufactured from unpasteurised milk. The genus was detected in 22 of the 25 raw milk cheeses and two of the three varieties made from pasteurised milk. Enterococcal populations that exceeded 10^6 cfu/g were not uncommon and tended to be higher in cheeses made from raw milk (Table 2). Enterococci were not recovered ($<\log_{10}$ 1.7 cfu/g) from four raw milk cheeses produced by three different manufacturers and one unripened cheese made from pasteurised milk from which *Aerococcus viridans* was recovered. All of the four unpasteurised cheeses were made from cows' milk and had been ripened for approximately 3 months with the exception of one that had been matured for more than 12 months. Among the 136 isolates assigned phenotypically to the species level *Enterococcus faecalis* (53%), *Enterococcus faecium* (31%) and *Enterococcus durans* (16%) were predominant. These three dominant species were detected in 22, 9 and 8 of the 25 raw milk cheeses respectively. *Enterococcus faecalis* and *E. durans* were also recovered from a Brie-type cheese made from pasteurised milk whilst an unpressed, unripened traditional cheese made from pasteurised milk contained *E. faecium*. *Enterococcus avium* was isolated from one cheese while the occurrence of *E. casseliflavus* in some of the cheeses tested was indicated but not confirmed.

Occurrence of potentially pathogenic bacterial groups

At least one of the targeted groups of potentially pathogenic organisms (*Salmonella* spp., *E. coli* O157, *B. cereus*, *Listeria* spp., *S. aureus*, *M. paratuberculosis*) was detected in 21 (84%) of the 25 raw milk cheeses tested whilst all three cheeses made from pasteurised milk contained a potential pathogen (either *S. aureus* or *M. paratuberculosis*). A proportion of the raw milk cheeses (28%) contained at least two different pathogens.

Although presumptive colonies of *E. coli* O157 and *Salmonella* spp. were recovered from some cheese samples (i.e. colonies exhibited specific morphology on the respective media) they were subsequently eliminated by confirmatory tests. *Escherichia coli* was not detected in cheeses made from pasteurised milk but presumptive O157 isolates were detected in 24% of the raw milk cheeses on media selective for *E. coli* O157; the recovery rate of *E. coli* on TBX agar was lower (8% positive). Subsequent tests confirmed that the presumptive *E. coli* isolates were not the O157 serogroup. Isolates from three cheeses that had the colonial characteristics of *E. coli* O157 were identified as strains of *Hafnia alvei*, *Enterobacter aerogenes* and *Proteus* spp.

Isolates with the appropriate characteristics were recovered from 11 of the 28 cheeses tested using the *Salmonella*-selective media. They were subsequently shown to be strains of *Hafnia alvei* and *Proteus* spp., including *Proteus vulgaris*. The detection of such false positive growth for *E. coli* O157 and *Salmonella* spp. was media dependent

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Table 2 Populations [mean (range $\log_{10}$ cfu/g)] of various microbial groups in different cheese types

Population	*P/UNP*	*Cow milk (3/20)*[a]	*Sheep milk (0/4)*	*Goat milk (0/1)*	*Organic (1/12)*	*Nonorganic (2/13)*	*<3 months maturation (3/11)*	*>3 months maturation (0/14)*
Total viable bacteria	P	8.17 (4.95–9.38)	No sample	No sample	8.69 (8.00–9.38)	7.64 (7.07–8.26)	8.17 (7.04–9.38)	No sample
	UNP	7.86 (4.95–9.76)	7.69 (7.11–9.00)	7.48	7.56 (4.95–9.15)	8.05 (6.62–9.75)	8.29 (7.04–9.41)	7.44 (4.95–9.76)
Moulds (Mycelial Fungi) (OGYE)	P	2.71 (1.70–3.48)	No sample	No sample	3.48	2.33 (1.70–2.96)	2.71 (1.70–3.48)	No sample
	UNP	4.74 (nd–7.30)	4.88 (nd–6.99)	2.60	4.56 (nd–6.97)	4.72 (nd–7.30)	5.80 (nd–7.30)	3.50 (nd–4.30)
Moulds (Mycelial Fungi) (RBCA)	P	2.90 (1.70–3.52)	No sample	No sample	3.48	2.61 (1.70–3.52)	2.90 (1.70–3.52)	No sample
	UNP	4.81 (nd–7.48)	5.00 (nd–7.00)	2.60	4.52 (nd–6.93)	4.89 (nd–7.48)	5.81 (nd–7.48)	3.74 (nd–5.48)
Yeast (OGYE)	P	5.13 (3.85–6.26)	No sample	No sample	3.85	5.77 (5.28–6.26)	5.13 (3.85–6.26)	No sample
	UNP	6.12 (nd–8.76)	3.97 (nd–5.94)	3.56	5.39 (nd–7.58)	5.99 (nd–8.76)	6.30 (nd–8.04)	5.17 (nd–8.76)
Yeast (RBCA)	P	5.70 (4.61–6.58)	No sample	No sample	4.61	6.24 (5.90–6.58)	5.70 (4.61–5.90)	No sample
	UNP	6.18 (nd–8.85)	4.11 (nd–5.78)	3.41	5.56 (nd–7.51)	5.73 (nd–8.85)	6.03 (nd–7.93)	5.37 (nd–8.85)
Lactic acid bacteria	P	6.99 (5.70–8.40)	No sample	No sample	8.40	6.29 (5.70–6.88)	6.99 (5.70–8.40)	No sample
	UNP	7.91 (4.88–9.66)	7.51 (6.36–8.80)	8.18	7.66 (4.88–8.15)	8.03 (6.36–9.20)	8.29 (7.56–9.20)	7.54 (4.88–9.66)
Lactococcus	P	7.51 (7.19–8.00)	No sample	No sample	7.34	7.59 (7.19–8.00)	7.51 (7.19–8.00)	No sample
	UNP	6.44 (3.00–9.08)	5.56 (4.48–6.48)	4.63	6.53 (3.00–9.08)	5.97 (4.48–8.11)	7.18 (5.71–8.72)	5.53 (3.00–9.08)
Lactobacillus	P	5.55 (4.20–7.66)	No sample	No sample	6.30 (4.94–7.66)	5.74 (4.20–7.63)	5.55 (4.20–7.66)	No sample
	UNP	7.19 (5.15–9.70)	7.64 (6.52–8.75)	8.04	7.18 (4.86–8.84)	7.40 (5.15–9.70)	7.39 (5.15–8.84)	7.22 (4.86–9.70)
Enterobacteriaceae	P	5.99 (5.52–6.41)	No sample	No sample	5.52	6.23 (6.04–6.41)	5.99 (5.52–6.41)	No sample
	UNP	4.13 (nd–7.15)	0.81 (nd–3.23)	nd	3.77 (nd–7.15)	4.32 (nd–6.78)	4.51 (2.00–7.51)	2.84 (nd–3.51)
Coliforms (VRBLA)	P	5.65 (4.72–6.51)	No sample	No sample	4.72	6.11 (5.71–6.51)	5.65 (4.72–6.51)	No sample
	UNP	4.05 (nd–7.00)	0.78 (nd–3.11)	nd	3.55 (nd–6.30)	4.12 (nd–7.00)	4.51 (2.34–7.00)	2.85 (nd–4.30)
Coliforms (Chromogenic)	P	6.32 (5.04–7.34)	No sample	No sample	5.04	6.96 (6.58–7.34)	6.32 (5.04–7.34)	No sample
	UNP	4.67 (nd–6.95)	5.30 (nd–6.04)	3.85	4.61 (2.30–6.51)	4.79 (nd–6.95)	4.91 (nd–6.95)	4.25 (nd–6.04)
Enterococcus (KAAA)	P	2.58 (nd–4.23)	No sample	No sample	4.23	1.75 (nd–3.51)	2.58 (nd–4.23)	No sample
	UNP	4.27 (nd–6.75)	5.01 (4.46–6.34)	5.11	4.50 (nd–6.61)	4.39 (nd–6.75)	4.22 (nd–6.75)	4.63 (nd–6.34)
Enterococcus (Chromogenic)	P	4.70 (4.26–5.28)	No sample	No sample	4.57	4.77 (4.26–5.28)	4.70 (4.26–5.28)	No sample
	UNP	5.13 (nd–7.34)	6.09 (5.15–6.99)	6.08	5.41 (2.70–8.15)	4.99 (nd–6.93)	4.82 (nd–7.34)	5.59 (nd–8.15)

[a]Number of samples made from pasteurised/unpasteurised milk in the category that were tested.

P, pasteurised milk variety; UNP, variety made from raw milk; nd, not detected (<$\log_{10}$ 1.70 cfu/g).

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Table 3 Prevalence (%) of potential pathogens in different cheese categories

Population	P/UNP	Cow (3/20)[a]	Sheep (0/4)	Goat (0/1)	Organic (1/12)	Nonorganic (2/13)	<3 months maturation (3/11)	>3 months maturation (0/14)
Bacillus cereus	P	nd	ns	ns	nd	nd	nd	ns
	UNP	30.0	50.0	nd	16.7	38.5	45.5	21.4
Listeria monocytogenes	P	nd	ns	ns	nd	nd	nd	ns
	UNP	5.0	nd	nd	8.3	nd	nd	7.1
Listeria spp.	P	nd	ns	ns	nd	nd	nd	ns
	UNP	25.0	50.0	nd	25.0	26.7	18.2	35.7
Staphylococcus aureus	P	33.3	ns	ns	nd	50.0	33.3	ns
	UNP	40.0	25.0	nd	58.3	15.4	36.4	35.7
Mycobacterium avium	P	66.6	ns	ns	100.0	33.3	66.6	ns
paratuberculosis	UNP	10.0	75.0	nd	16.7	23.1	nd	35.7

[a]Number of samples made from pasteurised/unpasteurised milk in the category that were tested.

P, pasteurised milk variety; UNP, variety made from raw milk; nd, not detected; ns, no sample.

but ranged from 24% to 33% of the cheese samples tested.

The occurrence of *B. cereus* was confirmed in eight (32%) of the raw milk cheese varieties; it was not detected in the three cheeses tested that had been made from pasteurised milk (Table 3). The populations ranged from 10^2 to 4×10^4 cfu/g. All 20 isolates formed enterotoxin after *in vitro* cultivation for 48 h at 30°C in BHI broth. Other potential enterotoxigenic *Bacillus* spp. recovered from the cheese samples included *B. mycoides*, *B. licheniformis* and *B. pumilus*.

Listeria spp. were detected in 25% of the raw milk cheese samples but were not recovered from any of the varieties made from pasteurised milk, even after enrichment for 24 h at 37°C in ONE broth (Table 3). Six of the (seven) positive cheese samples yielded at least two different *Listeria* spp. The organisms were isolated more frequently from cheeses that had been ripened for >3 months and had a higher prevalence in varieties made from sheep milk than those produced from cows' milk (Table 3). The presence of *L. monocytogenes* was confirmed in only one of the cheeses tested, a variety ripened for >3 months that had been made from organic cows' milk. Other *Listeria* spp. recovered from two different cheeses varieties were *Listeria ivanovii* and *Listeria innocua*; the presence of *Listeria seeligeri* and *Listeria welshimerii* in another five cheeses was indicated but was not confirmed unequivocally. The growth of *Bacillus* spp. and enterococci on the media used resulted in the development of 'false-positive' colonies that had the morphological characteristics of *Listeria* spp. from 56% of the samples after cultivation on Oxford Listeria selective agar and >40% of the samples tested on the two chromogenic selection media used.

Staphylococcus aureus was potentially a serious microbiological problem associated with the artisanal cheeses as it was recovered from 40% of the raw milk varieties and 33.3% of the pasteurised milk cheeses examined in this survey (Table 3). The incidence of the micro-organism in varieties made from organic milk was considerably higher than in those types made with nonorganic milk (58% vs 15% respectively) but was not affected by the duration of the ripening period (Table 3). The population size ranged from 10^2 to $>10^5$ cfu/g and was $>10^4$ cfu/g in 50% of the positive samples analysed. *Staphylococcus aureus* contamination was an apparent recurrent problem for one of the varieties examined as all three samples taken over a 20-month period tested positive for the micro-organism. Of 18 representative isolates examined seven strains from two different cheeses were able to form enterotoxin C after cultivation *in vitro* for 48 h at 37°C in Tryptone-Soya Broth.

Growth indicative of the presence of methicillin-resistant *S. aureus* occurred on Oxacillin resistance screening agar base (ORSAB) and Methicillin resistant *Staphylococcus aureus* (MRSA) chromogenic agar with 12 and 17 of the cheese samples tested respectively. Although these media are selective for the recovery of MRSA none of the isolates recovered on ORSAB were confirmed as *S. aureus*. Isolates recovered on MRSA chromogenic agar from two cheeses were subsequently identified as *S. aureus*, but the value of these media with samples that have a complex background microbial flora is questionable given the high number of false positive detections from the cheese samples tested.

Among the other *Staphylococcus* spp recovered *Staphylococcus xylosus*, *Staphylococcus sciuri* and *Staphylococcus simulans* were isolated from 13, 5 and 3 of the cheeses respectively. Five other species were each isolated once although several of the presumptive staphylococcal isolates could not be reliably assigned at the species level using the bioMerieux API identification protocol.

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The presence of the potentially pathogenic *M. avium* subsp. *paratuberculosis* (MAP) in Scottish farmhouse cheeses made from both pasteurised and raw milk has been confirmed. The presence of the organism in two samples was indicated by PCR detection of the IS900 insert after direct immunomagnetic recovery from cheese homogenates with MAP-specific paramagnetic beads. Recovered paramagnetic beads were also used to inoculate HEYM and Middlebrook slopes and the insert was detected in the subsequent growth that had occurred after 3 m at 37°C from four HEYM and two Middlebrook cultures (Figure 1). This observation was indicative of the presence of viable MAP cells in the six original cheese homogenates. *M. paratuberculosis* was detected in 20% of the cheeses made from raw milk and 67% of those made with pasteurised milk; the overall prevalence of MAP in the farmhouse cheeses was 25% (Table 3). The incidence was apparently greater after an extended ripening period and in some of the ovine milk cheese varieties (Table 3).

The detection pattern was indicative of MAP being randomly distributed at low levels in the cheese. Its occurrence may be intermittent as the micro-organism was not detected during subsequent re-examination of different batches of cheese that had initially tested positive. However, in view of the technical difficulties associated with the recovery and *in vitro* cultivation the possibility exists that its occurrence may have been underestimated in this study.

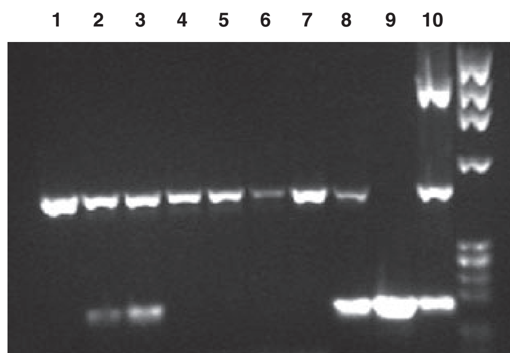

Figure 1 Detection of *Mycobacterium avium* subsp. *paratuberculosis* after immunomagnetic separation from the cheese sample and cultivation on supplemented Herrolds egg yolk medium (HEYM) and Middlebrook media. The samples applied to the lanes after PCR were as follows: (1) Cheese A, HEYM; (2) Cheese A replicate culture HEYM; (3) Cheese B HEYM cultures combined; (4) Cheese B combined Middlebrook cultures; (5) Cheese C combined HEYM cultures; (6) Cheese C combined Middlebrook cultures; (7) negative control; (8) *M. avium* subsp. *paratuberculosis* NCTC 8578; (9) *M. avium* subsp. *paratuberculosis* 743PSS; (10) internal marker provided with the Adiagene PCR Adiavet® ParaTB kit.

Antibiotic resistance among isolates of *S. aureus* and *Enterococcus* spp.

Of 70 *S. aureus* isolates tested >90% were able to grow on cefoxitin-containing MRSA chromogenic agar and >70% grew on liquid and solid oxacillin-media for the isolation of presumptive MRSA. There was also indicative evidence in the latex agglutination assay for production of penicillin-binding protein PBP2′ by almost 40% of the 51 isolates tested.

Antibiotic screening using impregnated discs and E-test gradient strips confirmed that methicillin and oxacillin resistance was widespread amongst the 25 test isolates, although all were susceptible to cefoxitin (Table 4). This study demonstrates for the first time that *S. aureus* strains exhibiting resistance to methicillin are present in dairy products manufactured in Scotland. However, unlike the clinical MRSA isolate *S. aureus* ATCC 44330 the methicillin resistant cheese isolates had a more restricted resistance profile as they were susceptible to chloramphenicol, erythromycin, gentamycin and tobramycin. Resistance to ampicillin, clindamycin and kanamycin was, however, widespread and there was resistance in a limited number of strains to tetracycline and fusidic acid, while a single strain exhibited resistance to trimethoprim (Table 4).

In view of the clinical significance of enterococci antibiotic resistance was monitored in strains recovered from farmhouse cheese produced in Scotland (Table 4). Many strains of the three species tested were resistant to ampicillin, kanamycin, streptomycin and tetracycline, and whilst some of the *E. faecalis* isolates were resistant to gentamycin two strains of *E. faecium* were resistant to ciprofloxacin. One of these *E. faecium* isolates that was recovered from an unpasteurised cows' milk, long-matured cheese was also resistant to the glycopeptide antibiotics vancomycin and teicoplanin (Table 4).

DISCUSSION

This study represents the first survey undertaken to investigate microbiological characteristics of farmhouse cheeses manufactured by small-scale artisanal producers in Scotland. The majority (25) of the 28 cheeses examined were produced from unpasteurised milk and comprised representative examples of the varieties produced by all manufacturers that utilise unpasteurised milk. The study was comprehensive as of the unpasteurised milk varieties known to the authors only two, of those that were still in production during the survey period, were unavailable for analysis. Ripened cheese samples were examined and no attempt was made to investigate population dynamics during the

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Table 4 Antibiotic susceptibility of *Staphylococcus aureus* and *Enterococcus* spp isolates

	Proportion of resistant isolates (%)[b]			
Antibiotic	*Staphylococcus aureus (23)*[a]	*Enterococcus faecalis (25)*	*Enterococcus faecium (15)*	*Enterococcus durans (9)*
Ampicillin	100	95.7	100	88.9
Cefoxitin	0	nt	nt	nt
Chloramphenicol	0	0	0	0
Ciprofloxacin	0	0	13.3	0
Clindamycin	100	nt	nt	nt
Erythromycin	0	13.1	6.7	0
Fusidic acid	8	nt	nt	nt
Gentamycin	0	13.1	0	0
Kanamycin	72	82.6	66.7	44.4
Levofloxacin	0	nt	nt	nt
Methicillin	60	nt	nt	nt
Mupirocin	0	nt	nt	nt
Oxacillin	72	nt	nt	nt
Rifampicin	0	nt	nt	nt
Streptomycin	nt	82.6	20.0	33.3
Teicoplanin	nt	0	6.7	0
Tetracycline	8	82.6	40.0	33.3
Tobramycin	0	nt	nt	nt
Trimethoprim	4	nt	nt	nt
Vancomycin	0	0	6.7	0

[a]Number of isolates screened.

[b]Based on minimum inhibitory concentration data from the British Society for Antimicrobial Chemotherapy (2007) and Franz *et al.* (2001). nt, not tested.

ripening period. The micro-organisms present may have been added deliberately by the cheesemaker as starter or adjunct cultures or alternatively entered the cheese as adventitious contaminants during the manufacturing process.

The artisanal cheeses examined possessed a complex and diverse microbial population that was affected by the manufacturing conditions pertaining. The survey was restricted to certain key microbial groups involved in cheese attribute development and safety. No attempt was made to fully characterise the microbial populations and consequently no information was obtained either on the species profile of the yeast, mould, coliform and LAB populations present, or the occurence of other bacterial groups that proliferate in other cheese types (Beresford and Williams 2005). The nonstarter lactobacilli present were similar to those reported for other European artisanal cheeses (Cogan *et al.* 1997; Beresford and Williams 2005) and represent species that are most able to withstand the specific nutritional and physiological conditions that define the cheese ecosystem. The size and species profile of the enterococcal populations present in the Scottish cheeses were typical of those previously observed in a wide variety of European artisanal cheeses (Beresford and Williams 2005; Moreno *et al.* 2006). Although the enterococci can contribute positively in cheese attribute development some species are considered to be nosocomial pathogens (Franz *et al.* 1999; Giraffa 2002) and may have a role in colon cancer development as their activities can increase the susceptibility of colon cells to DNA damage (Allen *et al.* 2008). The isolates from the Scottish cheeses, in common with the enterococci present in European cheeses (Franz *et al.* 2001) exhibited a strain-dependent resistance to a range of antibiotics, and although detected the incidence of vancomycin resistance was low as has been reported for other cheese enterococci (Teuber *et al.* 1999; Franz *et al.* 2001). Although some of the enterococci isolated were haemolytic the occurrence of other virulence determinants in the isolates was not determined and hence the potential food safety risk of the strains cannot be determined. It has been demonstrated, however, that cheese enterococci are less pathogenic than clinical isolates (Mannu *et al.* 2003). Nevertheless, the presence of a reservoir of antibiotic resistance in a food source is undesirable as cheese enterococci are able to survive intestinal transit (Gelsomino *et al.* 2003) and there is thus potential for antibiotic resistance transfer to take place, as has been demonstrated to occur between food and clinical isolates and during cheese fermentation (Eaton and Gasson 2001; Cocconcelli *et al.* 2003).

Although *E. coli* O157 and *Salmonella* spp. were not present one or more of the other four targeted potential pathogens enterotoxigenic *B.*

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cereus, *Listeria* spp., *S. aureus* and MAP were detected in 86% of the cheese samples analysed. This study has for the first time confirmed the presence of *Mycobacterium avium paratuberculosis*, methicillin resistant *S. aureus* and vancomycin resistant enterococci in the dairy food chain in Scotland. Although an analysis of international foodborne outbreak data (Greig and Ravel 2009) indicated a potential problem associated with *Salmonella* spp. in dairy products recent surveys in the UK and Scotland (Cree *et al.* 2008; Little *et al.* 2008), also, as observed in this study, did not detect *Salmonella* spp. in any of the cheese and dairy product samples tested. Similarly *E. coli* O157 was not detected in two surveys of some raw and pasteurised milk cheeses available in Scotland (Coia *et al.* 2001; Cree *et al.* 2008), although cheese can be a source of verocytotoxin-producing *Escherichia coli* (Baylis 2009).

Dairy products were the vehicle for only 4% of *B. cereus* mediated foodborne outbreaks reported internationally between 1988 and 2007 (Greig and Ravel 2009), whereas in the survey reported here *B. cereus* was present in one in three of the raw milk cheeses. The diarrhoeal disease is associated with proteinaceous foods such as dairy products (Arnesen *et al.* 2008) and all of the isolates formed enterotoxin in *in vitro* culture. Additionally other potentially toxigenic *Bacillus* spp. (Beattie 1997; From *et al.* 2005) were also present in the raw milk cheeses. Although physiological and nutritional conditions in the cheese environment may not be conducive to enterotoxin formation (Beattie and Williams 2002) the potential risk from *Bacillus cereus* may be higher in cheese produced in the late summer and early autumn when the prevalence of psychrotrophic *Bacillus* spp. in the farm environment is at its highest (McKinnon and Pettipher 1983; Beattie 1997). Six of the eight positive cheeses were manufactured during this period.

Listeria monocytogenes survives during cheese manufacture and ripening and occurs in a wide range of dairy products (Farber and Peterkin 1991) and in consequence they have been the food vehicle for over 40% of the outbreaks reported in the 10-year period to 2007 (Greig and Ravel 2009). However, *L. monocytogenes* was detected in only one of the Scottish raw milk cheeses surveyed and its prevalence was similarly low (1%) in the raw milk cheeses included in the UK retail survey conducted by Little *et al.* (2008). Furthermore, the organism was also detected in an artisanal semi-hard, rind washed, raw bovine milk cheese that had been purchased from a Scottish cheesemonger, but because it had been made in England the data were excluded from the survey. The micro-organism is thus likely to be encountered in a small proportion of raw milk cheeses where it may constitute a potential health risk. Among the other *Listeria* spp. detected during the survey the presence of *L. innocua* may be potentially significant as it is able grow more quickly than *L. monocytogenes* during the enrichment stage of the isolation procedure (MacDonald and Sutherland 1994; Oravcova *et al.* 2008) with the resultant failure to recover *L. monocytogenes* and an underestimation of its actual prevalence. A temperature of 37°C was therefore used in the enrichment protocol to facilitate *L. monocytogenes* recovery. The effectiveness of this strategy cannot be fully assessed as a concurrent comparative enrichment recovery at 30°C from the cheese samples was not performed. The detection of MAP in Scottish farmhouse cheese extends the limited range of cheese varieties in which this organism has thus far been detected worldwide (Ikonomopoulos *et al.* 2005; Clark *et al.* 2006; Stephan *et al.* 2007) although it is probable that reports of its occurrence in dairy products will increase as further monitoring for its presence is undertaken as *in vitro* experiments have confirmed that the micro-organism is able to survive the manufacturing and ripening conditions that exist during cheesemaking (Sung and Collins 2000; Spahr and Schafroth 2001; Donaghy *et al.* 2004). *M. avium* subsp. *paratuberculosis* was detected in cheese varieties made from raw and pasteurised milk and this observation is not unsurprising in view of the known heat tolerance of the micro-organism and its survival during pasteurisation treatments (Grant *et al.* 2005).

The recovery of MAP was erratic even though no decontamination stage was included in the isolation protocol. The organism was not recovered directly from the cheese and on both recovery media after incubation, suggesting that as separate pieces of cheese were used for each that the bacterial numbers in the cheese may have been low and its distribution was heterogeneous. Its occurrence may also be spasmodic as when different batches of initially MAP-positive varieties were analysed some months later recurrence of the micro-organism could not be confirmed. However, the recognised problems associated with the isolation and cultivation of the micro-organism (Donaghy *et al.* 2008) may lead to an under-reporting of its occurrence. The significance of the confirmation of its presence in artisanal cheese is ultimately dependent on the definitive establishment of a role in the aetiology of Crohn's disease.

The most frequently detected foodborne pathogen in the survey of the farmhouse cheeses was *S. aureus*. It was observed in 40% of the cheese samples analysed but was particularly problematic in cheeses made from organic milk where it was detected in seven of the 12 unpasteurised organic cheeses tested. The *S. aureus* numbers present in 50% of the cheeses that contained the organism exceeded the 10^4 cfu/g European approved limit

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for dairy products (quoted in Little *et al.* 2008). The prevalence of *S. aureus* in raw milk cheeses examined by Little *et al.* (2008) in their survey of retail cheeses in the UK was similar at 30%. These authors also reported that, as observed in the study reported here, the populations frequently exceeded the EU Recommendations (Little *et al.* 2008) and that the isolates were enterotoxigenic. Characterisation of the toxigenic profile is restricted by the detection limits of the commercially available assay kits. However, a preliminary assessment of six isolates using the Identibac® MRSA DNA microarray indicated the presence of marker genes for nine different toxins, as well as other virulence factors, in the strains tested (M. Woodward and A.G. Williams, unpublished data). Although the presence of enterotoxigenic *S. aureus* in raw milk cheeses represents a potential health risk (Little *et al.* 2008) there continue to be reports in the literature of the occurrence of enterotoxigenic strains in European artisanal cheeses (Beresford and Williams 2005; Cremonesi *et al.* 2007; Poli *et al.* 2007; Akineden *et al.* 2008).

It was also evident that the presence of *S. aureus* in some of the cheeses was a recurrent, rather than intermittent, problem and further work is required over an extended time period to address issues of organism persistence and seasonality in relationship to the incidence of mastitis. It remains to be resolved whether the *S. aureus* contamination source is animal or human in origin. However, pulsed field gel electrophoresis typing of 10 isolates at the Scottish MRSA Reference Laboratory confirmed that five of these with the PF108na profile belonged to a broad lineage that occasionally is associated with human infections (G.F.S. Edwards and A.G. Williams, unpublished data).

An assessment of the antibiotic susceptibilities of representative *S. aureus* isolates indicated that resistance to clinically significant antibiotics was present among the cheese isolates. Among the 25 *S. aureus* tested 10 exhibited resistance to methicillin and oxacillin and although their resistance profile to other antibiotics was not as extensive as observed in clinical MRSA isolates, the presence of methicillin resistant strains in the dairy food chain is undesirable given the importance of this antibiotic in the treatment of antibiotic-resistant staphylococcal infections. Normanno *et al.* (2007) concluded that antimicrobial resistant strains of *S. aureus* have become widespread in foods. However, there are only a limited number of reports of the presence of methicillin and oxacillin resistance in *S. aureus* from various foods including cheese (Corrente *et al.* 2007; Pereira *et al.* 2009). An examination of some of the most methicillin resistant strains isolated in the survey confirmed that *mec*A the gene that is involved in methicillin resistance was not present (Edwards and Williams, unpublished data). This gene is generally regarded as being present in all MRSA strains, and thus the cheese isolates exhibiting resistance to methicillin are considered to be borderline MRSA although the mechanism of that resistance remains to be established.

The identity of all the other staphylococci present in the artisanal cheeses was not established unequivocally, although where identities were established it would appear that the species present were similar to those that have been described in artisanal cheeses produced in Europe (Beresford and Williams 2005). Some of these coagulase-negative species have been shown to be haemolytic and capable of forming enterotoxins (Zell *et al.* 2008), and amongst these *S. xylosus* was regularly recovered. There is thus a need to more fully characterise the coagulase-negative staphylococci that occur regularly and persist during the maturation of artisanal cheeses.

The protocol adopted in the survey was, where possible, to utilise both conventional and chromogenic media for the detection and recovery of target species. It is proposed that the inclusion of chromogenic substrates allows greater specificity in microbial targeting especially for diagnostic clinical microbiology (Perry and Freydiere 2007). In this study the population sizes detected tended to be similar or slightly higher with some of the chromogenic media. However, the specificity of certain chromogenic media was disappointing and it was evident that colonial morphology on chromogenic agar and other differential media cannot be relied upon to provide unequivocal evidence for the presence of a target organism in foods, such as unpasteurised milk cheese, that contain a diverse natural population. The selective media used for the detection and isolation of *Listeria* spp., *Salmonella* spp., *E. coli* O157, *S. aureus*, MRSA, *B. cereus*, and MAP, with the exception of *Bacillus cereus* selective agar (PEMBA) and Baird Parker medium supplemented with rabbit plasma fibrinogen factor, produced an unacceptably high number of false positive colonies that had the morphological characteristics of the target species. In many instances, when the identity of the nonspecific contaminants was established, the organism was shown to be an *Enterococcus* spp. Thus, reliance on colonial morphological characteristics alone will lead to the inaccurate estimation of the level of the target species and care must be exercised in the interpretation of screening undertaken to assess product safety. Confirmatory secondary tests must be performed to establish that key characteristics of the target organism are detected before the identity of the culture is unambiguously established by polyphasic identification procedures.

Little *et al.* (2008) raised the issue of the inadequacy of the clarity of labelling of cheeses made

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from unpasteurised milk. It is a legal requirement in the EU that all cheeses made from unpasteurised milk must be labelled accordingly to inform consumer choice. It was our experience over the course of this study that although some producers of raw milk cheese clearly label their products with appropriate information, this is not the situation with all manufacturers. The use of phrases such as 'made by traditional methods' is inadequate and causes confusion as it is applied to both raw and pasteurised milk cheeses by producers. There was also an issue in gaining accurate information from some cheese sellers (cheesemonger, delicatessens and supermarkets) as to whether the product was produced from raw or pasteurised milk. Reliance on websites is also not advocated as sometimes misleading and contradictory information could be gained for a single product from different websites. The only reliable information came from the limited number of producer sites or through information obtained directly from the manufacturer. This is an issue that needs to be addressed by the appropriate regulatory bodies, so that at risk groups in the general public are not left confused or misled by inaccurate or inappropriate labelling.

The high incidence of potential foodborne pathogens and presence of resistance to clinically significant antibiotics in bacteria present in Scottish artisanal cheeses is undesirable. It is, however, unlikely that this problem will be restricted to dairy products made in Scotland and antibiotic resistance is likely to be an issue with farmhouse cheese production more widely throughout the UK and Europe. The use of unpasteurised milk for cheese manufacture remains a contentious issue. As the importance of the diversity of the population that develops without pasteurisation and the essentiality of its role in defining the characteristics of speciality artisanal cheese is largely unknown an alternative approach that warrants consideration is the use during cheese manufacture of adjunct cultures of strains possessing distinctive metabolic traits that impart the perceived superior characteristics of raw milk cheeses into products made with pasteurised milk. Raw milk cheeses themselves represent a potential source of such cultures.

CONCLUSIONS

The study represents the first comprehensive survey undertaken to establish the microbiological characteristics of artisanal farmhouses cheeses produced in Scotland. The 28 artisanal cheeses examined possessed a complex and diverse microbial population. The microbiological quality of the cheeses studied was disappointing in that although *E. coli* O157 and *Salmonella* spp. were not detected one or more of the other four targeted potential pathogens and MAP were detected in 86% of the cheese samples analysed. This study has for the first time confirmed the presence of *Mycobacterium avium paratuberculosis*, methicil lin resistant *S. aureus* and vancomycin resistant enterococci in Scottish dairy produce.

ACKNOWLEDGEMENTS

PFGE typing of *S. aureus* strains and *mecA* analyses were performed under the direction of Dr G. F. S. Edwards at the Scottish MRSA Reference Laboratory, Stobhill Hospital, Glasgow. Microarray analyses using the Identibac® MRSA method were co-ordinated by Prof M. Woodward, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB. Financial support for this research project was made under the auspices of a Transitional Funding Grant from the Scottish Government, Environment & Rural Affairs Department.

REFERENCES

Akineden E, Hassan A A, Schneider E and Usleber E (2008) Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. *International Journal of Food Microbiology* **124** 211–216.

Allen T D, Moore D R, Wang X, Casu V, May R, Lerner M R, Houchen C, Brackett D J and Huycke M M (2008) Dichotomous metabolism in *Enterococcus faecalis* induced by haematin starvation modulates colonic gene expression. *Journal of Medical Microbiology* **57** 1193–1204.

Arnesen L P S, Fagerlund A and Granum P E (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Reviews* **32** 579–606.

Baylis C L (2009) Raw milk and raw milk cheeses as vehicles for infection by verocytotoxin-producing *Escherichia coli*. *International Journal of Dairy Technology* **62** 293–307.

Beattie S H (1997) *Incidence and importance of Bacillus species in raw milk and in the dairy environment*. PhD Thesis University of Glasgow, Glasgow, UK.

Beattie S H and Williams A G (2002) Growth and diarrhoeagenic enterotoxin formation by *Bacillus cereus in vitro* in controlled fermentation and *in situ* in food products and a model food system. *Food Microbiology* **19** 329–340.

Beresford T M and Williams A G (2005) The microbiology of cheese ripening. In *Cheese: Chemistry, Physics and Microbiology 3rd Edition Volume 1 General Aspects*, pp 287–317. Fox P F, McSweeney P L H, Cogan T M, Guinee T P, eds. London: Elsevier Academic Press.

Beuvier E and Buchin S (2005) Raw milk cheeses. In *Cheese: Chemistry, Physics and Microbiology 3rd Edition Volume 1 General Aspects*, pp 319–345. Fox P F, McSweeney P L H, Cogan T M, Guinee T P, eds. London: Elsevier Academic Press.

British Society for Antimicrobial Chemotherapy (2008) Methods for antimicrobial susceptibility testing. [Internet document], pp 1–67, URL http://www.bsac.org.uk/_db/_documents/version_7.pdf. Accessed January 2009.

Clark D L, Anderson J L, Koziczkowski J J and Ellingson J L E (2006) Detection of *Mycobacterium avium* subspecies

paratuberculosis genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. *Molecular and Cellular Probes* **20** 197–202.

Cocconcelli P S, Cattivelli D and Gazzola S (2003) Gene transfer of vancomycin and tetracycline resistance among *Enterococcus faecalis* during cheese and sausage fermentations. *International Journal of Food Microbiology* **88** 315–323.

Cogan T M and Rea M C (1996) Artisanal European cheese. In European Commission Report EUR16788. European Commission, ed. Brussels: European Commission Directorate-General X11.

Cogan T M, Barbosa M, Beuvier E, Bianchi-Salvadori B, Cocconcelli P S, Fernandes I, Gomez J, Kalantzopoulos G, Ledda A and Medina M (1997) Characterization of the lactic acid bacteria in artisanal dairy products. *Journal of Dairy Research* **64** 409–421.

Coia J E, Johnston Y, Steers N J and Hanson M F (2001) A survey of the prevalence of *Escherichia coli* O157 in raw meats, raw cow's milk and raw-milk cheeses in south-east Scotland. *International Journal of Food Microbiology* **66** 63–69.

Corrente M, Normanno G, Martella V, Bellacicco A L, Quaglia N C, Dambrosio A, Buonavoglia M, D'Abramo M and Buonavoglia C (2007) Comparison of the methods for the detection of methicillin resistance in *Staphylococcus aureus* isolates from food products. *Letters in Applied Microbiology* **45** 535–539.

Cree L, Reid M, Robertson C and Dryburgh L (2008) The UK food surveillance system (FSS UK) – 2007 Scotland data. *HPS Weekly Report* **42** 400–402.

Cremonesi P, Perez G, Pisoni G, Moroni P, Morandi S, Luzzana M, Brasca M and Castiglioni B (2007) Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese. *Letters in Applied Microbiology* **45** 586–591.

Donaghy J A, Totton N L and Rowe M T (2003) Evaluation of culture media for the recovery of *Mycobacterium avium* subsp. *paratuberculosis* from cheddar cheese. *Letters in Applied Microbiology* **37** 285–291.

Donaghy J A, Totton N L and Rowe M T (2004) Persistence of *Mycobacterium avium* subsp. *paratuberculosis* during ripening and manufacture of cheddar cheese. *Applied and Environmental Microbiology* **70** 4899–4905.

Donaghy J A, Rowe M T, Rademaker J L W, Hammer P, Herman L, De Jonghe V, Blanchard B, Duhem K and Vindel E (2008) An inter-laboratory ring trial for the detection of *Mycobacterium avium* subsp. *paratuberculosis* from raw milk artificially contaminated with naturally infected faeces. *Food Microbiology* **25** 128–135.

Donnelly C W (2001) Factors associated with hygienic control and quality of cheeses prepared from raw milk: a review. *Bulletin of the International Dairy Federation* **369** 16–27.

Donnelly C W (2005) Growth and survival of microbial pathogens in cheese. In *Cheese: Chemistry, Physics and Microbiology 3rd Edition Volume 1 General Aspects*, pp 541–559. Fox P F, McSweeney P L H, Cogan T M, Guinee T P, eds. London: Elsevier Academic Press.

Eaton T J and Gasson M J (2001) Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Applied and Environmental Microbiology* **67** 1628–1635.

Farber J M and Peterkin I (1991) *Listeria monocytogenes* a food-borne pathogen. *Microbiological Reviews* **55** 476–511.

Franz C M A P, Holzapfel W H and Stiles M E (1999) Enterococci at the crossroads of food safety? *International Journal of Food Microbiology* **47** 1–24.

Franz C M A P, Muscholl-Silberhorn A B, Yousif N M K, Vancanneyt M, Swings J and Holzapfel W H (2001) Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. *Applied and Environmental Microbiology* **67** 4385–4389.

From C, Pukall R, Schumann P, Hormazabal V and Granum P E (2005) Toxin-producing ability among *Bacillus* spp. outside the *Bacillus cereus* group. *Applied and Environmental Microbiology* **71** 1178–1183.

Gelsomino R, Vancanneyt M, Cogan T M and Swings J (2003) Effect of raw-milk cheese consumption on the enterococcal flora of human feces. *Applied and Environmental Microbiology* **69** 312–319.

Giraffa G (2002) Enterococci from foods. *FEMS Microbiology Reviews* **26** 163–171.

Grant I R (2006) *Mycobacterium avium* ssp. *paratuberculosis* in foods: current evidence and potential consequences. *International Journal of Dairy Technology* **59** 112–117.

Grant I R, Williams A G, Rowe M T and Muir D D (2005) Efficacy of various pasteurization time-temperature conditions in combination with homogenization on inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Applied and Environmental Microbiology* **71** 2853–2861.

Greig J D and Ravel A (2009) Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology* **130** 77–87.

Harbutt J (2008) *British Cheese Directory*, pp 17–96. Churchill, Oxfordshire: Juliet Harbutt.

Hermon-Taylor J, Bull T J, Sheridan J M, Cheng J, Stellakis M L and Sumar N (2000) Causation of Crohn's disease by *Mycobacterium avium* subspecies *paratuberculosis*. *Canadian Journal of Gastroenterology* **14** 521–539.

Ikonomopoulos J, Pavlik I, Bartos M, Svastova P, Ayele W Y, Roubal P, Lukas J, Cook N and Gazouli M (2005) Detection of *Mycobacterium avium* subsp. *paratuberculosis* in retail cheeses from Greece and the Czech Republic. *Applied and Environmental Microbiology* **71** 8934–8936.

Institute of Food Science and Technology (2000) Position statement on food safety and cheese. [Internet document] URL http://www.ifst.org/hottop15.htm. Accessed January 2009.

Johnson E A, Nelson J H and Johnson M (1990) Microbiological safety of cheese made from heat-treated milk. Part II Microbiology. *Journal of Food Protection* **53** 519–540.

Little C L, Rhoades J R, Sagoo S K, Harris J, Greenwood M, Mithani V, Grant K and McLauchlin J (2008) Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. *Food Microbiology* **25** 304–312.

MacDonald F and Sutherland A D (1994) Important differences between the generation times of *Listeria monocytogenes* and *List. innocua* in two *Listeria* enrichment broths. *Journal of Dairy Research* **61** 433–436.

Mannu L, Paba A, Daga E, Comunian R, Zanetti S, Dupre I and Sechi L A (2003) Comparison of the incidence of

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virulence determinants and antibiotic resistance between *Enterococcus faecium* strains of dairy, animal and clinical origin. *International Journal of Food Microbiology* **88** 291–304.

McKinnon C H and Pettipher G L (1983) A survey of sources of heat-resistant bacteria in milk with particular reference to psychrotrophic spore-forming bacteria. *Journal of Dairy Research* **50** 163–170.

Moreno M R F, Sarantinopoulos P, Tsakalidou E and De Vuyst L (2006) The role and application of enterococci in food and health. *International Journal of Food Microbiology* **106** 1–24.

Normanno G, La Salandra G, Dambrosio A, Quaglia N C, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E and Celano G V (2007) Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology* **115** 290–296.

Oravcova K, Trncikova T, Kuchta T and Kaclikova E (2008) Limitation in the detection of *Listeria monocytogenes* in food in the presence of competing *Listeria innocua*. *Journal of Applied Microbiology* **104** 429–437.

Pereira V, Lopes C, Castro A, Silva J, Gibbs P and Teixeira P (2009) Characterization for enterotoxin production, virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiology* **26** 278–282.

Perry J D and Freydiere A M (2007) The application of chromogenic media in clinical microbiology. *Journal of Applied Microbiology* **103** 2046–2055.

Poli A, Guglielmini E, Sembeni S, Spiazzi M, Dellaglio F, Rossi F and Torriani S (2007) Detection of *Staphylococcus aureus* and enterotoxin genotype diversity in Monte Veronese, a Protected Designation of Origin Italian cheese. *Letters in Applied Microbiology* **45** 529–534.

Spahr U and Schafroth K (2001) Fate of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss hard and semi-hard cheese manufactured from raw milk. *Applied and Environmental Microbiology* **67** 4199–4205.

Stephan R, Schumacher S, Tasara T and Grant I R (2007) Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss raw milk cheeses collected at the retail level. *Journal of Dairy Science* **90** 3590–3593.

Sung N and Collins M T (2000) Effect of three factors in cheese production (pH, salt, and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. *Applied and Environmental Microbiology* **66** 1334–1339.

Teuber M, Meile L and Schwarz F (1999) Acquired antibiotic resistance in lactic acid bacteria from food. *Antonie Van Leeuwenhoek* **76** 115–137.

Williams A G and Withers S E (2008) Microbiology of some artisanal 'farmhouse' cheeses manufactured in Scotland from unpasteurised milk. In Proceedings of the 21st International ICFMH Symposium Evolving Microbial Food Quality and Safety (Food Micro 2008), Aberdeen, Scotland, 1–4 September, Poster Presentation EE4, pp 447.

Zell C, Resch M, Rosenstein R, Albrect T, Hertel C and Gotz F (2008) Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. *International Journal of Food Microbiology* **127** 246–251.

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