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ORIGINAL ARTICLE

Sources of psychrophilic and psychrotolerant clostridia causing spoilage of vacuum-packed chilled meats, as determined by PCR amplification procedure

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Abstract

Aims: To determine possible preslaughter and processing sources of psychrophilic and psychrotolerant clostridia causing spoilage of vacuum-packed chilled meats.

Methods and Results: Molecular methods based on the polymerase chain reaction (PCR) amplification of specific 16S rDNA fragments were used to detect the presence of *Clostridium gasigenes*, *Clostridium estertheticum*, *Clostridium algidicarnis* and *Clostridium putrefaciens* in a total of 357 samples collected from ten slaughter stock supply farms, slaughter stock, two lamb-processing plants, their environments, dressed carcasses and final vacuum-packed meat stored at –0·5°C for 5½ weeks. *Clostridium gasigenes*, *C. estertheticum* and *C. algidicarnis/C. putrefaciens* were commonly detected in farm, faeces, fleece and processing environmental samples collected at the slaughter floor operations prior to fleece removal, but all these micro-organisms were detected in only 4 out of 26 cooling floor and chiller environmental samples. One out of 42 boning room environmental samples tested positive for the presence of *C. gasigenes* and *C. estertheticum*, but 25 out of 42 of these samples were positive for *C. algidicarnis/C. putrefaciens*. Nearly all of the 31 faecal samples tested positive for the presence of *C. gasigenes* and *C. estertheticum*; however, only two of these samples were positive for *C. algidicarnis* and/or *C. putrefaciens*. Clostridial species that were subject to this investigation were frequently detected on chilled dressed carcasses.

Conclusions: The major qualitative and quantitative differences between the results of PCR detection obtained with the primers specific for 'blown pack' -causing clostridia (*C. gasigenes* and *C. estertheticum*) and those obtained with primers specific for *C. algidicarnis* and C. *putrefaciens* suggest that the control of meat spoilage caused by different groups of meat clostridia is best approached individually for each group.

Significance and Impact of the Study: This paper provides information significant for controlling meat spoilage-causing clostridia in the meat-processing plants.

Introduction

Until 1989, all except three species of the genus *Clostridium* (*Clostridium arcticum*, *Clostridium putrefaciens* and some nonproteolytic *Clostridium botulinum*) were considered to be mesophilic and, consequently, were thought to play only a minor role in the spoilage of chilled meat products (Roberts and Mead 1986). However, during the

last two decades more than ten new species of psychrophilic and psychrotolerant clostridia were isolated and described, and three of these newly described species, *Clostridium estertheticum*, *Clostridium gasigenes* and *Clostridium algidicarnis*, were later confirmed as causative agents of spoilage of vacuum-packed chilled meats. Of these, psychrophiles, *C. estertheticum* and *C. gasigenes* have been recognized as the causative agents of 'blown pack' spoilage of vacuum-packed beef, lamb and venison (Dainty *et al.* 1989; Kalchayanand *et al.* 1989; Broda *et al.* 1996a,b, 2000), while psychrotolerant bacterium *C. algidicarnis* has been associated with stifle joint taint of vacuum-packed chilled lamb (Broda *et al.* 1996a,b) and production of offensive odours in vacuum-packed pork (Lawson *et al.* 1994). The latter species was subsequently found to exhibit high 16S rDNA sequence similarity to *C. putrefaciens* (Broda *et al.* 2000), a psychrotolerant anaerobe causing deep-seated spoilage of hams (Ross 1965) and vacuum-packed chilled lamb (unpublished data).

Spoilage of vacuum-packed chilled meats by psychrophilic and psychrotolerant *Clostridium* spp. typically occurs in the absence of temperature abuse or packaging failure and, consequently, is of major concern to meat industries worldwide. Sudden and rapid nature of the spoilage often suggests to meat producers that a contamination of the meat-manufacturing process with clostridia has occurred and that the source(s) of these micro-organisms are harboured at some points of a processing chain. It is, then, thought that to control clostridial spoilage the source(s) of meat spoilage-causing clostridia must be eliminated from the meat-processing environment by means of extensive cleaning and sanitizing.

Little is known about immediate and intermediate sources of meat carcass contamination with psychrophilic and psychrotolerant meat spoilage clostridia. Preliminary research investigating the sources of *C. estertheticum* and *C. gasigenes* in a venison processing plant suggested that the majority of 'blown pack' spoilage clostridia are being introduced into the plant on pelts and in faeces of slaughter animals, and are only rarely found in plant environmental samples collected at postpelting (Boerema *et al.* 2003). This scenario indicates that carcass contamination with these two clostridial species is likely to occur during dressing and that measures to control meat spoilage caused by these micro-organisms may be most effective if conducted on farm, at preslaughter and/or at postslaughter but prior to dressing, rather than following pelt removal. Little is known, however, about farm and preslaughter sources of meat spoilage-causing psychrophilic and psychrotolerant clostridia. In addition, it is not known whether *C. estertheticum*, *C. gasigenes*, *C. algidicarnis* and *C. putrefaciens* prevail at the same points of a meat-processing chain and, consequently, whether the control of all these species should be approached in the similar manner.

The aim of the present study was to determine sources of psychrophilic and psychrotolerant clostridia causing spoilage of vacuum-packed chilled meats. While culture-based detection of these micro-organisms is extremely laborious and time-consuming, previous study (Boerema *et al.* 2003) demonstrated good agreement of culture- and polymerase chain reaction (PCR)-based approaches for detection of *C. gasigenes* and *C. estertheticum*. Consequently, in the present study *C. estertheticum*, *C. gasigenes*, *C. algidicarnis* and *C. putrefaciens* were detected using 16S rDNA-based methodologies (Boerema *et al.* 2002; Broda *et al.* 2003). It was thought that the determination of sources of meat spoilage-causing clostridia may enable meat processors to select processing chain points most likely to contribute to clostridial carcass contamination, to focus sporicidal control measures on these specific points and, in turn, to achieve greater efficacy in controlling meat spoilage conditions caused by psychrophilic and psychrotolerant clostridia.

Materials and methods

Sampling rationale

Clostridium estertheticum, *C. gasigenes*, *C. algidicarnis* and *C. putrefaciens* were detected in samples collected from lamb supply farms, slaughter animals, environments of a meat plant-processing ovine species and the final vacuum-packaged product. Soil, mud, vegetation and creek water from farm environments were tested to establish whether these could provide a natural habitat for psychrophilic and psychrotolerant clostridia. Faeces and fleece samples from slaughter stock were tested to determine if they could be a source of carcass contamination with these micro-organisms. Samples collected on the slaughter floor, in carcass chillers and in the boning room of two lamb-processing plants were tested to establish whether a source of contamination could be harboured within the meat plant environment.

Sample collection and handling

Sampling was conducted at two South Island lamb-processing plants that had previously experienced incidents of product spoilage by psychrophilic and psychrotolerant clostridia. A total of 309 samples were collected from ten farms supplying the plants, slaughter stock, carcasses and plant environments. In addition, 50 meat samples (25 from each plant) were collected for the detection of psychrophilic and psychrotolerant clostridia.

Following sampling, all, except meat, samples were placed into anaerobic storage, packed in ice and transported overnight to the laboratory. Samples were stored anaerobically at 2°C pending testing, which was initiated within 10 days of placement into chilled storage. For each plant, lamb cuts from 25 animals from the five supply farms (five from each of the five supply farms) were collected following carcass dressing and overnight chilling. Lamb flaps were collected from Plant 1, which has previously experienced the incidents of 'blown pack' spoilage, and lamb legs were collected from Plant 2, which has previously experienced the incident of stifle joint taint of vacuum-packed lamb. The cuts were vacuum-packed, kept on ice and transported on the same day to the laboratory, where they were placed into chilled storage at *c.* –0·5°C for 5½ weeks prior to detection of clostridia.

Preparation of sample enrichments

All sample enrichments were prepared inside an anaerobic chamber (Forma Scientific, Marietta, OH, USA) operating at 15°C. With soil, a loopful of the soil was added to 15 ml of prereduced single-strength peptone yeast extract glucose starch (PYGS; Lund *et al.* 1990) broth. After gentle mixing, the suspension was incubated anaerobically at 7°C for 3 weeks. Following incubation, the enrichments were placed into frozen storage at –18°C pending DNA extraction.

With faecal samples, a single sterile swab stick was pushed into the faecal mass, retracted and placed it into a sterile universal bottle. An aliquot (15 ml) of prereduced single-strength PYGS broth was then added to the universal bottle and the bottle swirled gently to release the micro-organisms attached to the swab. Bottles containing faecal enrichments were incubated anaerobically at 7°C for 3 weeks and then placed into frozen storage at –18°C pending DNA extraction.

With fleece samples, a volume (75 ml) of prereduced single-strength PYGS was added to a stomacher bag containing *c.* 10 g of fleece. The contents were mixed and incubated anaerobically at 7°C for 3 weeks. Following incubation, the enrichments were placed into frozen storage at –18°C pending DNA extraction.

With water samples, a volume (10 ml) of water was added to 10 ml of prereduced double-strength PYGS in a sterile universal bottle. The contents of each bottle were mixed and incubated anaerobically at 7°C for 3 weeks. Following incubation, the enrichments were placed into frozen storage at –18°C pending DNA extraction.

With swab samples collected in the meat-processing environment, an appropriate volume (15 or 50 ml) of prereduced single-strength PYGS was added to each universal bottle or stomacher bag containing swab sticks or gauze swabs, respectively. Each sample was then gently mixed and incubated anaerobically at 7°C for 3 weeks. Following incubation, the enrichments were placed into frozen storage at –18°C pending DNA extraction.

Lamb cuts were stored for 5½ weeks at *c.* –0·5°C. Following this chilled storage, 48 (50 minus two leakers) meat samples were enriched in a volume (50 ml) of prereduced double-strength PYGS broth and were incubated anaerobically at 7°C for 3 weeks. After this additional enrichment step, PYGS broths were placed into frozen storage at –18°C pending DNA extraction.

DNA isolation

Total bacterial DNA was isolated from the swab suspensions or thawed enrichments using a High Pure PCR Template Preparation kit (Roche Diagnostics, Auckland, New Zealand). A 1 ml aliquot of each suspension was subjected to centrifugation at 3000***g*** for 5 min and the supernatant was discarded. To improve lysis of Gram-positive bacteria, the centrifuged pellet was suspended in 200 μl lysozyme (10 mg ml^{-1}) and incubated at 37°C for 60 min. From this step, the protocol recommended by the kit's manufacturer, consisting of, briefly, proteinase K lysis, guanidine-HCl binding, isopropanol precipitation and ethanol washes, was followed. Eluted DNA was stored at –20°C pending PCR amplification.

PCR amplification

Genomic DNA isolated from the suspensions or enrichments was used as the PCR template. DNA extracted from reference cultures of *C. estertheticum* (DSM 8809), *C. gasigenes* (DSM 12272) and *C. algidicarnis* (NCFB 2931) were included in PCR amplification as positive controls. In addition, each PCR run included a reagent only negative control.

To detect the presence of 16S rDNA gene fragments of *C. estertheticum* and *C. gasigenes* primer pairs 16SEF and 16SER, and 16SDBSF and 16SDBSR, respectively, were used as described by Broda *et al.* (2003). To detect the presence of 16S rDNA gene fragments of *C. algidicarnis* and *C. putrefaciens*, primers CAF and CAR that detect the presence of, but do not distinguish between, these two species, were used (Boerema *et al.* 2002). The PCR was prepared according to the manufacturer's (Roche Diagnostics) recommendation and contained: PCR buffer 10 × (10 μl), 0·2 mmol l^{-1} of each deoxynucleotide triphosphate, 0·5 μ mol l^{-1} of each primer, 2·5 U of *Taq* polymerase and 10 μl of template DNA in a total volume of 100 μl. Amplifications were performed in a heated lid thermal cycler (Techne Genius, John Morris Scientific, Auckland, New Zealand). After initial denaturation for

3 min at 93°C, target DNA was amplified in 30 cycles. Each cycle consisted of denaturation for 1 min at 92°C, annealing for 1 min at 55°C and extension for 2 min at 72°C. The final extension was for 3 min at 72°C. The PCR reaction tubes were held at 4°C until further analysis.

Following amplification, an 18 μl aliquot of the PCR mix was subjected to electrophoresis in a 1·5% w/v agarose gel at 150 V for 1·75 h. DNA molecular weight marker VI (Roche Diagnostics) was used to determine the size of the PCR products. Bands of amplified PCR products were visualized with ethidium bromide by ultraviolet (UV) transillumination and photographed.

Identification of amplified PCR products by DNA sequencing

To confirm identity of the PCR amplicons, a number of DNA fragments were purified and sequenced. The PCR products were purified with the Concert nucleic acid purification kit (Gibco BRL, Auckland, New Zealand) according to the manufacturer's instructions. DNA sequencing was performed by the Waikato DNA Sequencing Facility, University of Waikato, Hamilton, New Zealand. The obtained sequences were aligned against each other and the appropriate reference strain using Sequence Navigator (Perkin Elmer, Applied Biosystems, Foster City, CA, USA). Sequence similarities were calculated using *c.* 700 nucleotide sites of the aligned sequences. Sequence similarity of >99% was considered sufficient to confirm identity of the PCR products.

Results

Results of PCR detection of *C. gasigenes*, *C. estertheticum* and *C. algidicarnis/C. putrefaciens* in samples collected from Plants 1 and 2 are summarized in Tables 1 and 2, respectively. PCR products of the expected size (920, 775 and 750 nucleotides) were amplified from the positive controls (*C. gasigenes*, *C. estertheticum* and *C. algidicarnis*, respectively) included in each PCR run. No PCR products were amplified from any of the reagent only blanks (negative controls). All sequenced PCR products showed greater than 99% sequence similarity with the 16S rRNA gene sequences of *C. gasigenes*, *C. estertheticum* and *C. algidicarnis* deposited in GenBank under accession numbers AF092548, X68181 and AF127023, respectively.

With *C. gasigenes*- and *C. estertheticum*-specific primers, PCR products of the expected size were amplified from 42 and 37, respectively, of 52 supply farm samples, including soil, mud, vegetation and creek water. In contrast, only 9 out of 52 farm samples tested positive for the presence of *C. algidicarnis* and/or *C. putrefaciens*. Similarly, nearly all slaughter animal faecal samples were positive for the presence of *C. gasigenes* and *C. estertheticum*, while only 2 out of the 31 faecal samples produced positive PCR amplicons with *C. algidicarnis*-/*C. putrefaciens*-specific primers. The majority of the fleece

Table 1 Detection of meat spoilage-causing psychrophilic and psychrotolerant clostridia in farms supplying ovine slaughter stock to Plant 1, environs of Plant 1, and slaughter stock, dressed ovine carcasses and peptone yeast extract glucose starch (PYGS)-enriched final product processed at this plant

Sample type	No. of positive samples (% of samples tested in each category)		
	Clostridium gasigenes	*Clostridium estertheticum*	*Clostridium algidicarnis/ Clostridium putrefaciens*
Slaughter stock supply farms (*n* = 25)	16 (64·0)	14 (56·0)	4 (16·0)
Mud and water (*n* = 11)	8 (72·7)	7 (63·6)	4 (36·4)
Soil, vegetation and feed (*n* = 14)	8 (57·1)	7 (50·0)	0 (0·0)
Ovine slaughter stock (*n* = 30)	28 (93·3)	25 (83·3)	12 (40·0)
Faeces (*n* = 15)	14 (93·3)	14 (93·3)	2 (13·3)
Fleece (*n* = 15)	14 (93·3)	11 (73·3)	10 (66·7)
Environs of lamb processing plant (*n* = 74)	19 (25·7)	10 (13·5)	20 (27·0)
Slaughter animals yards (*n* = 10)	8 (80·0)	5 (50·0)	3 (30·0)
Slaughter floor environmental swabs (*n* = 26)	8 (30·8)	4 (15·4)	5 (19·2)
Ventilation system (*n* = 2)	2 (100·0)	1 (50·0)	0 (0·0)
Carcass chillers environmental swabs (*n* = 14)	1 (7·1)	0 (0·0)	0 (0·0)
Boning room environmental swabs (*n* = 22)	0 (0·0)	0 (0·0)	12 (54·6)
Dressed ovine carcasses (*n* = 26)	10 (38·5)	1 (3·8)	6 (23·1)
PYGS-enriched lamb flaps (*n* = 24)	14 (58·3)	7 (29·2)	2 (8·3)
Total positive (*n* = 179)	87 (48·6)	57 (31·8)	44 (24·6)

Table 2 Detection of meat spoilage-causing psychrophilic and psychrotolerant clostridia in farms supplying ovine slaughter stock to Plant 2, environs of Plant 2, and slaughter stock, dressed ovine carcasses and peptone yeast extract glucose starch (PYGS)-enriched final product processed at this plant

	No. of positive samples (% of samples tested in each category)		
Sample type	*Clostridium gasigenes*	*Clostridium estertheticum*	*Clostridium algidicarnis/ Clostridium putrefaciens*
Slaughter stock supply farms (*n* = 27)	26 (96·3)	23 (85·2)	5 (18·5)
Mud and water (*n* = 8)	8 (100·0)	6 (75·0)	4 (50·0)
Soil, vegetation and feed (*n* = 19)	18 (94·7)	17 (89·5)	1 (5·3)
Ovine slaughter stock (*n* = 34)	34 (100·0)	33 (97·1)	17 (50·0)
Faeces (*n* = 16)	16 (100·0)	15 (93·8)	0 (0·0)
Fleece (*n* = 18)	18 (100·0)	18 (100·0)	17 (94·4)
Environs of lamb-processing plant (*n* = 66)	25 (37·8)	18 (27·3)	39 (59·1)
Slaughter animals yards (*n* = 8)	8 (100·0)	6 (75·0)	8 (100·0)
Slaughter floor environmental swabs (*n* = 23)	14 (60·9)	10 (43·5)	13 (56·5)
Ventilation system (*n* = 3)	2 (66·7)	1 (33·3)	3 (100·0)
Carcass chillers environmental swabs (*n* = 12)	1 (8·3)	0 (0·0)	2 (16·7)
Boning room environmental swabs (*n* = 20)	0 (0·0)	1 (5·0)	13 (65·0)
Dressed ovine carcasses (*n* = 27)	5 (18·5)	4 (14·8)	8 (29·6)
PYGS-enriched lamb stifle joints (*n* = 24)	0 (0·0)	1 (4·2)	0 (0·0)
Total positive (*n* = 178)	90 (50·6)	79 (44·4)	69 (38·8)

samples, however, tested positive for the presence of all clostridial species that were subjected to the PCR detection.

PCR products specific to *C. gasigenes*, *C. estertheticum* and *C. algidicarnis/C. putrefaciens* were commonly amplified from animal yards and environmental swabs collected in the slaughter floor of the meat-processing plant, especially those taken prior to, or at, fleece removal. However, only 4 out of 26 cooling floor and chiller environmental samples was positive for the presence of these micro-organisms. Interestingly, only 1 out of the 42 boning room environmental samples tested positive for the presence of *C. gasigenes* and *C. estertheticum*, but 25 out of 42 of these samples were positive for *C. algidicarnis/C. putrefaciens*.

Fifteen and fourteen out of fifty-three carcass swabs were positive for *C. gasigenes* and *C. algidicarnis/C. putrefaciens*, respectively. With *C. estertheticum*-specific primers, PCR product of the expected size was amplified from only 5 out of 53 carcass swabs.

Following the incubation of chill-stored meat samples in PYGS, 14, 8 and 2 out of 48 samples yielded PCR products with primers specific for *C. gasigenes, C. estertheticum* and *C. algidicarnis/C. putrefaciens*, respectively (Tables 1 and 2). Twenty-three positives were detected in samples obtained at Plant 1, and only one positive was obtained from Plant 2 samples. With Plant 1, *C. gasigenes* was detected in two out of five samples from farm one, five out of five samples from both farms two and three, and one out of four samples from farm five. *Clostridium gasigenes* was not detected in any of the PYGS-enriched meat samples from farm four. With the same enriched samples, *C. estertheticum* was detected in two out of five samples from farm one, five out of five samples from farm two and one sample from farm three. However, this micro-organism was not detected in any of the samples from farms four and five. *Clostridium algidicarnis/ C. putrefaciens*-specific PCR amplicons were detected in two out of five DNA of PYGS-enriched meat samples from farm one.

Discussion

In the present study, *C. gasigenes*, *C. estertheticum* and *C. algidicarnis/C. putrefaciens* were detected in farm samples, faeces and on fleeces of slaughter animals, in animal yards, in environmental samples collected at the slaughter floor operations prior to fleece removal and on dressed carcasses prior to chilling. Clostridial species subject to the present investigation were occasionally detected on chilled dressed carcasses, and rarely in cooling floor and chiller environmental swabs. In addition, the presence of these micro-organisms was detected in PYGS enriched vacuum-packed lamb samples collected at Plant 1.

Specific 16S rDNA gene fragments of *C. gasigenes* and *C. estertheticum* were amplified from over 50% samples from farms supplying Plant 1 and nearly all farm samples from farms supplying Plant 2. These micro-organisms were commonly detected in soil and mud collected from areas adjacent to the farm creeks, as well as in creek water

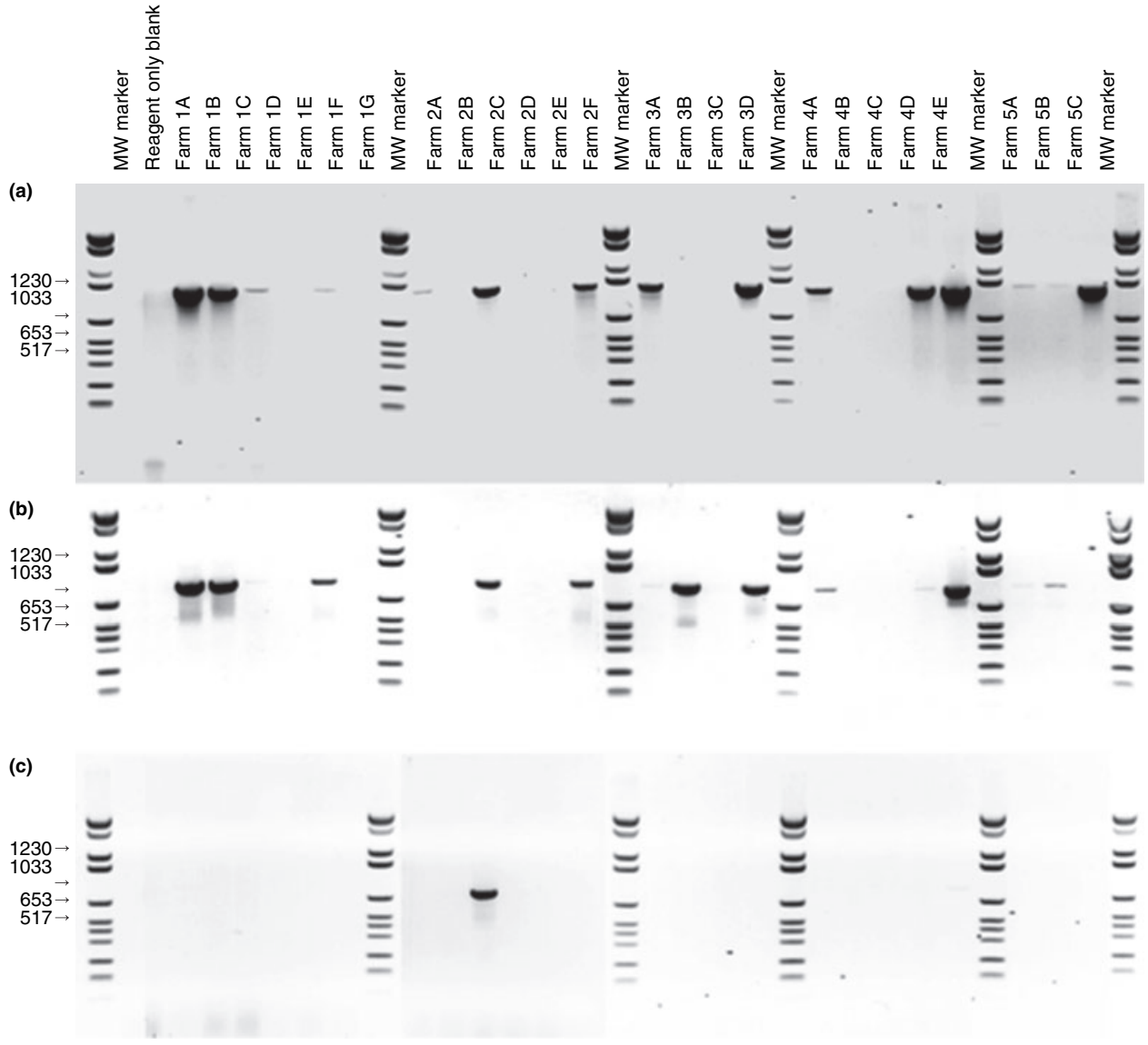

Figure 1 Detection of 16S rDNA fragments in samples from five farms supplying Plant 1, using primers specific for: (a) *Clostridium gasigenes*, (b) *Clostridium estertheticum* and (c) *Clostridium algidicarnis/Clostridium putrefaciens*. Please note that because the results of the study were obtained from reading directly from the gels, some weak positive bands are not visible on the photographs.

and in samples of aerial surfaces of farm vegetation (Tables 1 and 2). Soil and mud are known to provide the anoxic, moist conditions conductive to the growth of many mesophilic clostridia (Cato *et al.* 1986). Spores of these micro-organisms are also known to persist on leaves of horticultural plants (Lund 1986; Ercolani 1997). Strict psychrophiles were traditionally thought to be restricted to permanently cold habitats (Gounot 1991) and, consequently, the majority of psychrophilic *Clostridium* spp. known to date has been obtained from various Antarctic ecosystems (Mountfort *et al.* 1997; Spring *et al.* 2003; Alam *et al.* 2006). The results of the present study indicate that, in addition to extreme natural habitats, psychrophilic clostridia may prevail in seasonally cold habitats of varying anaerobiosis.

Clostridium gasigenes and *C. estertheticum* were detected in nearly all faecal and fleece samples, indicating that the major source(s) of these micro-organisms in a meat plant are slaughter animals themselves. Because in modern processing meat contamination from ruptured viscera is relatively uncommon, it is likely that the main route for carcass contamination with 'blown pack'-causing clostridia (*C. gasigenes* and *C. estertheticum*) is *via* the first carcass opening cuts during dressing. With the exception of

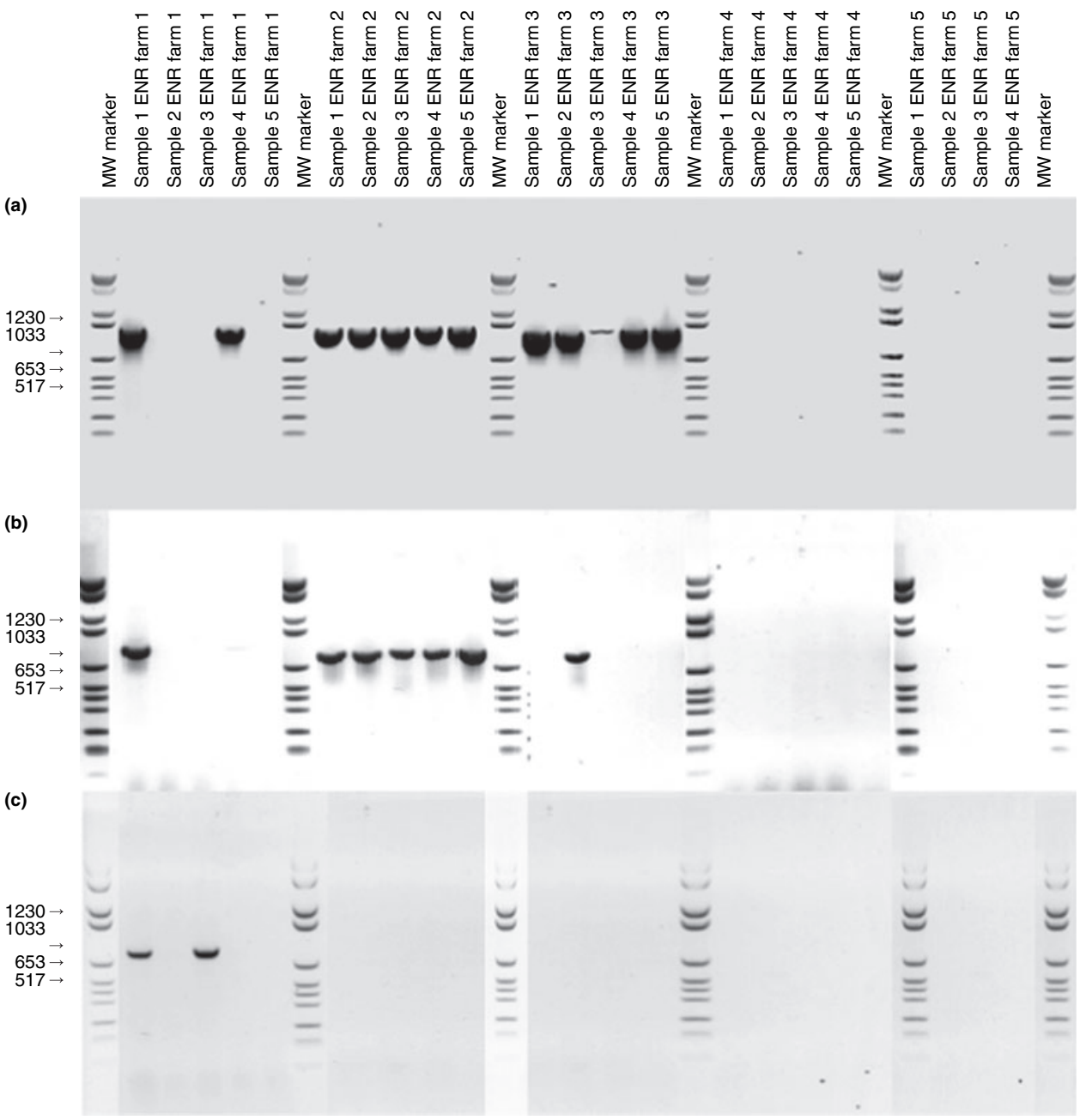

Figure 2 Detection of 16S rDNA fragments in peptone yeast extract glucose starch-enriched lamb samples using primers specific for: (a) *Clostridium gasigenes*, (b) *Clostridium estertheticum* and (c) *Clostridium algidicarnis/Clostridium putrefaciens*. Meat samples were obtained from five farms supplying slaughter stock to Plant 1.

the ventilation system, 'blown pack'-causing clostridia were not detected in this study in meat plant environmental samples collected in processing areas postfleece removal. This finding indicates that while ventilation system may serve as a secondary source for carcass contamination with *C. gasigenes* and *C. estertheticum*, other processing sources are unlikely to be harboured in cooling floor, chiller and/or boning room environments. It appears that preslaughter and preskinning sporicidal interventions, along with strict dressing hygiene, are most likely to succeed in controlling meat spoilage owing to the growth of 'blown pack'-causing clostridia.

In the present study, *C. gasigenes*- and *C. estertheticum*-specific PCR products were obtained in amplification with DNA representing ten lamb supply farms, with at least one sample testing positive for each farm, but no

farm showing positive amplification for all its samples (see Fig. 1 for Plant 1 supply farm samples). Interestingly, when these results were collated with the results of amplification for the final vacuum-packed product that originated from Plant 1 supply farms, it became apparent that meat from some farms showed more positive PCR products than those from other farms (Fig. 2). Despite that *C. gasigenes* and *C. estertheticum* were detected in three and two, respectively, out of six samples from farm two, these micro-organisms were detected in all five PYGS-enriched meat samples from this farm. In contrast, *C. gasigenes* and *C. estertheticum* were detected in a number of samples from farms four and five, but none of the vacuum-packed PYGS-enriched meat samples from these farms tested positive for the presence of these clostridia. These results indicate that some farms may be more likely to supply slaughter animals that later yield meat contaminated with *C. gasigenes* and *C. estertheticum* than other farms. It is possible that the differences in average temperatures and rainfall, and the resulting differences in animal presentation for slaughter may contribute to an increased transfer of clostridia from the fleeces of affected slaughter stock and could influence the prevalence of 'blown pack'-causing clostridia in the final product. Consequently, depending on the origin of the slaughter stock, some vacuum-packed meat may be at higher risk of spoiling prematurely than other product.

With farm, faecal and boning room swab samples, there were major differences between the numbers of positive PCR products obtained with primers specific for 'blown pack'-causing clostridia (*C. gasigenes* and *C. estertheticum*) and those obtained with *C. algidicarnis/C. putrefaciens*-specific primers. Nearly all of 31 faecal samples tested positive for the presence of *C. gasigenes* and *C. estertheticum*, however, only two of these samples were positive for *C. algidicarnis* and/or *C. putrefaciens*. It is thought that the differences in the maximum growth temperatures of these meat spoilage-causing clostridia may explain this phenomenon. The former two species are considered to be strictly psychrophilic and cannot grow at body temperature (Collins *et al.* 1992; Broda *et al.* 2000a,b); consequently, their spores are likely to pass through the animal's gastrointestinal tract without initiating germination and outgrowth. The latter micro-organisms belong to psychrotolerant clostridia and can grow at body temperature (Kalchayanand *et al.* 1989, unpublished data). More research is needed to explain in a definite manner the apparent disparity in the prevalence rates of 'blown pack'-causing clostridia and *C. algidicarnis/C. putrefaciens* in faecal samples.

Only one of 42 boning room swabs tested positive for the presence of *C. gasigenes* or *C. estertheticum*. Interestingly, *C. algidicarnis-/C. putrefaciens*-specific PCR products were found in 25 out of 42 of these samples. In addition, the majority of farm samples were positive for 'blown pack'-causing clostridia, but only 9 out of 52 of these samples tested positive for the presence of *C. algidicarnis* and/or *C. putrefaciens*. Although *C. algidicarnis* is not known to cause 'blown pack' spoilage, it has been previously isolated from spoiled vacuum packaged pork (Kalchayanand *et al.* 1989) and is known to cause stifle joint odour in chilled vacuum-packaged lamb legs (Broda *et al.* 1996a,b). The results of the present study indicate that this organism is likely to prevail in animals where, similarly to other intrinsic clostridia (Gill 1979; Eisgruber and Stolle 1996), it may proliferate in deep tissues or lymphatic system. In this scenario, organisms carried in the deep tissues of animals may be released into the boning room at carcass breakdown and may establish the boning room environment as a secondary source of contamination with *C. algidicarnis/C. putrefaciens*.

In this study, meat spoilage-causing clostridia were detected on up to 40% of chilled dressed carcasses, but none of the stored vacuum-packed samples showed any symptoms of the spoilage. These findings indicate that at the time when the study was conducted carcass contamination with meat spoilage clostridia was minimal, with only low numbers of spores surviving the processing to persist in the final product. Fourteen and eight out of forty-eight enriched meat samples tested positive for the presence of *C. gasigenes* and *C. estertheticum*, but only two of these samples were positive for *C. algidicarnis* and/or *C. putrefaciens*, probably as a result of using enrichment procedures that employed temperatures likely to favour the growth of psychrophilic, rather than psychrotolerant, micro-organisms.

The results of this study suggest that, similar to the majority of meat spoilage micro-organisms (Newton *et al.* 1978; Patterson and Gibbs 1978), psychrophilic 'blown pack'-causing clostridia are likely to be extrinsic contaminants of external dressed carcass surfaces, while *C. algidicarnis/C. putrefaciens* can be considered deep tissue contaminants. This finding indicates that the control of meat spoilage caused by these two distinct groups of meat clostridia is best approached separately, perhaps following individual screening of a meat-processing plant to indicate sources of meat spoilage-causing clostridia specific to the processing plant. Such screening may enable meat processors to select processing chain points most likely to contribute to their specific spoilage condition, and, therefore, to achieve greater efficacy in controlling meat spoilage caused by psychrophilic and psychrotolerant clostridia.

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