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PCR assay for the detection of *Campylobacter* in marinated and non-marinated poultry products

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ABSTRACT

During a period of 9 months, 194 marinated and non-marinated poultry products were collected from retail shops in a defined area in Western Finland and tested for *Campylobacter* spp. using a conventional enrichment culture and Polymerase Chain Reaction (PCR) method. For marinated poultry products, the study involved modification of a commercial DNA isolation method. Using either a conventional culture or PCR method, a total of 25 (12.9%) of all investigated samples were *Campylobacter* positive. In marinated poultry products, *Campylobacter* was detected at a prevalence of 21.1% and 9.5% in turkey and chicken products, respectively. In August, there was a peak with 28.9% positive *Campylobacter* samples. *Campylobacter* inoculation tests were carried out to test the detection limit of both methods. The PCR method used is faster than microbiological analyses. However, enrichment of the samples is necessary due to the low occurrence of *Campylobacter* spp. in retail Finnish poultry products.

1. Introduction

Campylobacter is the most common cause of bacterial enteritis worldwide (Rautelin and Hänninen, 2000; Samuel et al., 2004; Schönberg-Norio et al., 2004, 2006). In Finland, according to the National Infectious Disease Registry, the incidence of campylobacteriosis has increased steadily reaching 4003 and 3439 infections in 2005 and 2006, respectively, with 65 human cases/100,000 inhabitants in 2006 (Anonymous, 2005, 2006a). These high figures make *Campylobacter* nowadays the leading cause of bacterial enteric infections in Finland. Epidemiological studies have underlined handling and consumption of undercooked poultry meat as one of the most important sources of human campylobacteriosis (Evans et al., 2003; Potter et al., 2003; Luber and Bartelt, 2007). Limited studies have been published on the prevalence of *Campylobacter* in poultry meat at the Finnish retail level. Hänninen et al. (2000) studied the prevalence of *Campylobacter* in poultry products in the Helsinki area between June and September from 1996 to 1998 and found 12–21% *Campylobacter* positive samples in each year studied. In summer 2004, the percentages of *Campylobacter* positive fresh broiler and turkey meat at the Finnish retail level were 20.2% and 19.2%, respectively (Anonymous, 2006a–c). In contrast, studies from other countries report a high occurrence of *Campylobacter* at the retail level, for example, 71.3% in the UK (Philipps, 1998), 79.4% in Spain (Mateo et al., 2005) and 64.7% in Japan (Sallam, 2007).

There is a wide variety of poultry products available on the Finnish retail market including fresh and modified-atmosphere-packaged (MAP) products with or without spices. Approximately 80% of these products are sold marinated (Björkroth, 2005). Marinating, in this context, means salting and mixing the meat with water–oil-based, spiced sauces. High NaCl concentration, low pH and the addition of different spices to the marinade prevent the growth of spoilage bacteria, thus increasing the shelf-life of the meat products. However, marinating poultry meat does not decrease pathogenic bacteria such as *Campylobacter* (Evans et al., 1998; Perko-Mäkelä et al., 2000).

Since the popularity and the variety of marinated poultry products in Finland is increasing rapidly every year, reliable methods for the detection of *Campylobacter* in these products are of interest to laboratories in routine work and research. Traditional conventional culture methods include enrichment and plating steps followed by isolation of the bacterium and biochemical identification of the isolate. These methods are laborious, time consuming and costly. Recently designed polymerase chain reaction (PCR) methods have been found to be faster, more specific and sensitive for the detection of *Campylobacter* in naturally contaminated retail samples (Denis et al., 2001; Wong et al., 2004; Mateo et al., 2005; Sallam, 2007). However, several substances in foods can be inhibitory for the PCR. Lilja and

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Hänninen (2001) reported problems in the preparation of marinated chicken samples prior to PCR analysis. Thus, it is important to neutralize such substances by using effective DNA purification protocols or PCR facilitators.

The aim of the present study was to determine the occurrence of *Campylobacter* in naturally contaminated poultry products at the retail level in Western Finland. Special attention was paid to the wide variety of marinated products available on the market. For the detection of *Campylobacter*, a PCR assay has been compared with the conventional culturing method. The original protocol for the DNA isolation using a commercial kit was modified.

2. Materials and methods

2.1. Sampling

A total of 194 raw chilled poultry products (136 chicken samples, 56 turkey samples and two samples including both chicken and turkey meat) were randomly selected between January and September 2006 from different local retail shops in a defined area in Western Finland. All products were packed in Finland, but in 11, nine and two samples, the meat originated from Denmark, Brazil and France, respectively. All samples were transported immediately to the laboratory and kept at $4\pm3\,^{\circ}C$ until being analyzed within 24 h of purchase. Between January and June, ten samples were analyzed once a month and from July to September, 15 samples were analyzed three times a month.

2.2. Sample description

The product types and the numbers of samples in each group are presented in Table 2. According to the manufacturers, the meat concentration in all samples varied from 66% to 100% and the salt concentration from 0.6% to 1.6%. The term natural product in this study refers to a non-marinated product of 100% meat without any added substances. Lightly salted products are those with a meat content of 66–80%, a salt concentration of 1% and the addition of water, glucose and food additives. The term "marinated" includes all products with an oil- and/or water-based marinade and a blend of spices with 0.9–1.6% salt. In 47 of all 136 marinated products, honey was the most popular flavor in the marinade. Other common flavors were citrus fruits, peppers, herbs, garlic and barbecue spice. The term "spiced" refers to products with blended spices, salt (0.8–1.4%) and often other ingredients such as flour and breadcrumb rubbed onto the meat surface. The meat content in the marinated and spiced products varies from 66% to 97% and several food additives like stabilizers, antioxidants, acidity balancing agents, preservatives, thickening and flavoring agents are added. These product types also contain maltodextrin, yeast extract and other flavor enhancers. In addition, modified starch, barley or wheat may be added. All the products were packed under a modified atmosphere consisting of carbon dioxide and nitrogen in different proportions and had a shelf life of up to 10 days.

2.3. Culture method for the detection of Campylobacter in poultry samples

Microbiological analyses of the samples were based on a modified method of the National Committee of Food Analyses (2007).

Each sample was aseptically removed from the package and placed in a Stomacher bag (Seward BA6041, Worthing, UK). Equal amounts of a weighed sample and Buffered Peptone Water (BPW) (LAB46, LabM, Lancashire, UK) were mixed with a minimal amount of 300 g of meat in 300 ml of BPW (LabM). The bag was shaken manually for 3 min. For the enrichment, 25 ml of the suspension was re-suspended in 225 ml of Bolton broth (LAB135, LabM) with 5% lysed horse blood and selective supplement (LX132, LabM) and incubated in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2), generated by CampyGen^{TM} (Oxoid CN0035) at 42 °C for 24 h. A loopful of the enrichment culture (10 μl) was streaked on an modified charcoal cefoperazone deoxycholate agar plate (mCCDA) (CM 739, Oxoid, Basingstoke, UK) supplemented with SR 155 (Oxoid) and incubated microaerobically at 42 °C for 48 h. Presumptive *Campylobacter* colonies on mCCDA plates were further identified according to the method of the National Committee of Food Analyses (2007). To test their ability to grow aerobically, they were subcultured onto blood agar plates (CASO agar, Casein-Peptone Soymeal-Peptone, Merck, Darmstadt, Germany with 5% bovine blood) and incubated aerobically at 37 °C for 24 h. Strains were stored at −80 °C in Brucella broth (Scharlau Chemie, Barcelona, Spain) containing 15% glycerol.

2.4. PCR method for the detection of Campylobacter in poultry samples

For the PCR sample, 1.5 ml of the rinsing fluid was centrifuged at 1000 rpm for 8 min at 4 °C. The middle aqueous layer was removed carefully to avoid any fat and placed into an unused Eppendorf tube. After centrifugation at 13,000 rpm for 8 min at 4 °C, the supernatant was removed carefully and the pellet was frozen at −70 °C.

For the PCR of the enriched sample, 1 ml of enrichment culture was collected after 24 h incubation. The subsample was centrifuged at 13,000 rpm for 8 min at room temperature. The supernatant was removed carefully and the pellet was frozen at −70 °C.

2.4.1. DNA isolation

DNA isolation from the frozen pellet was carried out using a DNA isolation kit MagneSil® KF (MD1460, Genomic System, Promega, Madison, WI, USA) with a Dynal MPC®-S magnetic stand (Dynal Biotech, Oslo, Norway). The instructions of the supplier were modified and optimized for DNA isolation by hand using a magnetic stand. A 200 μl lysis buffer and 75 μl magnetic beads were added to an Eppendorf tube containing the pellet. The mixture was vortexed vigorously four times during a 5 min period at room temperature before placing the tube in a magnetic stand with the magnet for 30 s. The magnet was taken out after the liquid was removed from the tube. The particles were washed twice with 185 μl of salt washing buffer and twice with 200 μl of ethanol washing buffer. The tube was then placed in a 72 °C heat block for 5 min with an open lid for ethanol dehydration. The particles were re-suspended in 100 μl of sterile water and replaced in a 72 °C heat block for another 5 min with the lid closed. The tube was mixed with vortex and placed in the magnetic stand for 30 s. The liquid was removed from the tube to be frozen at −20 °C.

2.4.2. PCR assay

The detection of *Campylobacter* in the samples was based on amplification of the 16S rRNA gene (Linton et al., 1996) using two sets of oligonucleotide primers. The first set was C412F 5′-GGA TGA CAC TTT TCG GAG C-3′ (Linton et al., 1996) and 16S rRNA-campR2 5′-GGC TTC ATG CTC TCG AGT T-3′ as described by Lund et al. (2004). The second set was MD16S1, 5′-ATC TAA TGG CTT AAC CAT TAA AC-3′ and MD16S2, 5′-GGA CGG TAA CTA GTT TAG TAT T-3′ as described by Denis et al. (1999). For detection of the internal control the primers Yers F8 5′-CGA GGA GGA AGG GTT

Table 1
List of strains used for validation of specificity of the 412F-16S rRNA-campR2 primer set

Species	Strain	Species	Strain
Campylobacter jejuni	CCUG 11284	*Campylobacter*	CCUG 18267
Campylobacter jejuni	CCUG 24567	*Campylobacter lari*	CCUG 15035
Campylobacter jejuni	CCUG 10940	*Campylobacter lari*	CCUG 12774
Campylobacter jejuni	CCUG 12778	*Campylobacter lari*	CCUG 18294
Campylobacter jejuni	DCC[a] 42	*Campylobacter lari*	DCC 50
Campylobacter jejuni	DCC 43	*Campylobacter lari*	DCC 29
Campylobacter jejuni	DCC 44	*Campylobacter lari*	DCC 33
Campylobacter jejuni	DCC 45	*Campylobacter helveticus*	CCUG 30682
Campylobacter jejuni	DCC 47	*Campylobacter helveticus*	CCUG 30683
Campylobacter jejuni	DCC 48	*Campylobacter helveticus*	CCUG 30563
Campylobacter jejuni	DCC 49	*Campylobacter helveticus*	CCUG 30564
Campylobacter jejuni	DCC 52	*Campylobacter helveticus*	CCUG 30565
Campylobacter jejuni	DCC 22	*Campylobacter helveticus*	CCUG 30566
Campylobacter jejuni	DCC 27	*Campylobacter helveticus*	CCUG 34016
Campylobacter jejuni	DCC 34	*Campylobacter hyointestinalis*	CCUG 14169
Campylobacter jejuni	DCC 40	*Campylobacter hyointestinalis*	CCUG 34538
Campylobacter jejuni	DCC 41	*Campylobacter sputorum*	CCUG 37579
Campylobacter coli	CCUG 11283	*Campylobacter concisus*	CCUG 13144
Campylobacter coli	CCUG 33450	*Campylobacter curvus*	CCUG 13146
Campylobacter coli	DCC 36	*Campylobacter mucosalis*	CCUG 6822
Campylobacter coli	DCC 37	*Campylobacter fetus*	CCUG 6825A
Campylobacter coli	DCC 38	*Arcobacter cryaerophilis*	CCUG 17801
Campylobacter coli	DCC 39	*Arcobacter skirrowii*	CCUG 10374
Campylobacter coli	DCC 46	*Arcobacter butzleri*	CCUG 30485
Campylobacter coli	DCC 51	*Helicobacter pylori*	DCC 35
Campylobacter coli	DCC 28	*Helicobacter pullorum*	DCC 53
Campylobacter coli	DCC 18	*Enteroccocus faecalis*	CCUG 19916
Campylobacter upsaliensis	CCUG 23626	*Escherichia coli*	CCUG 17620
Campylobacter upsaliensis	CCUG 14913	*Streptococcus aureus*	CCUG 17621
Campylobacter upsaliensis	CCUG 24571	*Staphyloccus bovis*	CCUG 17828
Campylobacter upsaliensis	CCUG 24803	*Salmonellatyphimurium*	DVI-Å[b] 19
Campylobacter upsaliensis	CCUG 23017	*Salmonella enteritidis*	DVI-Å20
Campylobacter upsaliensis	CCUG 20818	*Proteus mirabilis*	CCUG 34293
Campylobacter lari	CCUG 23947	*Bordetella bronchiseptica*	DVI-Å50
Campylobacter lari	CCUG 20575	*Citrobacter freundii*	DVI-Å22

[a] DVI culture collection.
[b] DVI-Å: In house reference strain.

AAG TG-3′ and Yers R10 5′-AAG GCA CCA AGG CAT CTC TG-3′ according to Gibello et al. (1999) slightly modified were used. All the primers were synthesized by Oligomer Oy (Helsinki, Finland).

2.4.3. PCR amplification

The PCR conditions used in the present study are described by Lund et al. (2003) with a few modifications. Briefly, the PCR amplification was performed in 50 μl volumes containing 5 μl of the DNA, 25 μl of a PCR master mix (Promega, Madison, WI, USA), 1 μl of a 25 mM $MgCl_2$ solution, 0.5 μl of a 10 mg ml^{-1} BSA solution (New England Biolabs, Ipswich, MA, USA), 20 pmol of each of the *Campylobacter* primers and 5 pmol of each of the internal control primers.

The PCR was performed in a Peltier Thermal Cycler (PTC-200; MJ Research Inc., Watertown, MA, USA) and the conditions were one cycle of 95 °C for 2 min, 58 °C for 1 min, 72 °C for 1 min, followed by 34 cycles of 95 °C for 15 s, 58 °C for 40 s and 72 °C for 40 s. The last elongation step lasted 5 min. The PCR product was loaded onto a 2% agarose gel (1.35% SeaKem® LE Agarose and 0.65% NuSieve® GTG Agarose, Cambrex Bio Science, Rockland, ME, USA) containing 0.1 μg ml^{-1} ethidium bromide. A DNA molecular weight marker 100 bp low ladder (P1473, Sigma-Aldrich, Saint Louis, MO, USA) was included in each gel. The gel was photographed under UV light (Alpha DigiDoc, Alpha Innotech, San Leandro, CA, USA). The PCR reaction for each sample was performed 1–3 times with each primer set and considered positive if both primer sets gave a distinct band of the right size (857 bp) or at least one primer set gave a positive reaction twice. Samples with no internal control band were run again using a tenfold dilution of DNA.

2.4.4. Control strains

For PCR, *Campylobacter jejuni* EELA 49 strain (isolated from a Finnish broiler carcass) was used as a positive control. As negative controls, sterile water in the PCR method and Bolton broth (LabM) in the culture method were used. An internal control was added to the mastermix. Briefly, DNA from the bacterium *Yersinia ruckeri* (DVI-Å83) was isolated using the MagneSil KF (MD1460, Genomic System, Promega, Madison, WI, USA) as described above. Approximately 25 pg *Y. ruckeri* DNA was added to the mastermix before aliquoting into tubes (Lund et al., 2004; Lund and Madsen, 2006).

The strains used for validation of specificity of the C412F-16S rRNA-campR2 primer set and their sources are listed in Table 1. Strains were stored at −80 °C in brain heart infusion broth (Difco, Detroit, MI, USA) containing 20% glycerol. For testing the specificity of the primers used in the assay, DNA was isolated directly from the storage medium by centrifugation of 0.1 ml of the medium at 15,870 rpm for 7 min and then the pellet was subjected to DNA extraction as described before. Approximately 1 ng of DNA was used per PCR.

2.4.5. Comparison of the detection limit between the culture and PCR method

A tenfold dilution series of a *C. jejuni* broth culture was used to determine the detection limit of the culture and the PCR method.

Table 2
Types of Finnish retail poultry products and *Campylobacter* positive samples

Product type	No. of samples positive by culture/PCR	No. of samples positive[a]/No. of samples tested			
		Slices and barbecue sticks	Breast fillet and fillet steaks	Breasts, legs drumsticks and wings incl. bones and skin	All
Natural		0/3	2/4	2/8	4/15
Lightly salted		0/1	0/0	0/0	0/1
Spiced		0/1	0/6	7/18	7/25
Marinated		3/51	1/24	5/20	9/95
Total chicken samples		3/56	3/34	14/46	20/136
Natural		0/8	0/7	0/0	0/15
Lightly salted		0/0	0/1	0/0	0/1
Spiced		0/0	0/1	0/0	0/1
Marinated		4/19	0/20	0/0	4/39
Total turkey samples		4/27	0/29	0/0	4/56
Marinated		1/2	0/0	0/0	1/2
Total mixed chicken and turkey samples		1/2	0/0	0/0	1/2
Total samples	18/24	8/85	3/63	14/46	25/194

[a] No. of samples tested positive by microbiological method and/or PCR method.

100 μl of each dilution from 10^{-1} to 10^{-7} was plated out for counting the colony forming units (cfu) of the stock solution. Seven samples of 100 g poultry meat slices and 42 g of plain marinade were placed in a Stomacher bag (Seward BA6041). One ml of each dilution of *C. jejuni* broth culture was mixed with 100 ml of BPW (LabM) and this mixture was added to the samples. All samples were subjected to the cultural detection and PCR procedures as described above. This procedure was repeated once.

2.5. Data management and calculations

For data management and calculations, Microsoft® Excel 97 SR 2 and SAS® Systems vers. 8 (Cary, NC, USA) were used. The level of agreement according to precision was expressed as the kappa statistic, defined as the proportion of potential agreement beyond chance exhibited by two tests. Diagnostic specificity was calculated as: $d/(b+d)$ where d is the number of samples negative both by PCR and by culture and b is the number of samples positive by PCR, but negative by culture. The level of agreement between two tests was calculated as: $(a+d)/n$, where a is the number of samples positive both by PCR and by culture, d is the number of samples negative by both methods and n is the total number of samples under examination (Smith, 1995; Martin et al., 1997).

3. Results

3.1. Prevalence of Campylobacter in poultry products

Using either a conventional culture or PCR method, a total of 25 (12.9%) of 194 investigated samples were *Campylobacter* positive (Table 3). The isolation rates from the different product types are listed in Table 2. Out of 136 chicken and 56 turkey products, 20 (14.7%) and four (7.1%) samples respectively were *Campylobacter* positive. One of the two mixed chicken and turkey samples was positive for *Campylobacter*. *Campylobacter* was detected in four (13.3%) of the 30 natural and 14 (10.3%) of the 136 marinated poultry products. Seven (26.9%) of the 26 spiced products were positive, all being chicken with skin and bone. No *Campylobacter* was detected in two lightly salted products. The occurrence of *Campylobacter* in chicken slices and barbecue sticks was 9.4%, in chicken breast fillets 4.7% and chicken products with skin and bone 30.4%. *Campylobacter* was not detected in any of the 22 poultry products with meat of foreign origin.

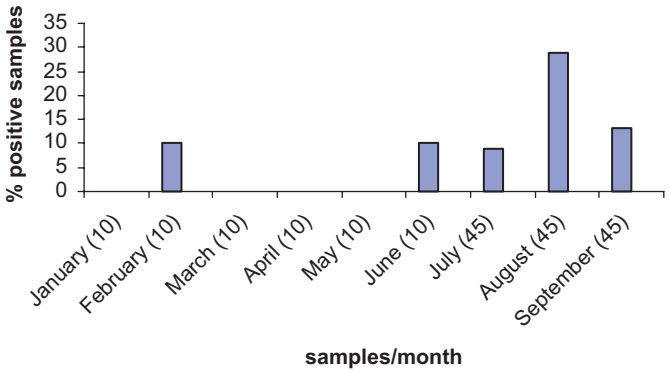


Fig. 1. Monthly distribution of *Campylobacter* positive samples in Finnish retail poultry products from January to September 2006. The numbers in parenthesis represent the number of samples taken per month.

In August, there was a peak with a 28.9% prevalence of *Campylobacter* in 45 investigated samples (Fig. 1). Between January and May, *Campylobacter* was detected in only one of 50 samples.

3.2. Comparison of the culture and PCR method

Eighteen (9.3%) of 194 samples were positive using the conventional culturing method and 24 (12.4%) were positive using the PCR method for *Campylobacter* (Table 3). The results of the culture and PCR were concordant in 186 samples, representing 96.4% of the samples. One sample (1/18), gave a negative result for PCR when the result of the culture method was positive. Seven samples gave a positive result when the culture result was negative (7/176). Approximately 400 bp of the PCR product from five of these samples were sequenced and all sequences were 99% or 100% equal to *C. jejuni*. The diagnostic specificity for the comparison of the PCR to culture by selective enrichment was 0.96 with a level of agreement of 0.96.

Table 3
Comparison of PCR results and culture for the detection of the *Campylobacter* in marinated and non-marinated poultry products after enrichment

PCR	Culture		
	Positive	Negative	Total
Positive	17	7	24
Negative	1	169	170
Total	18	176	194

Table 4
Comparison of detection limit between culture methods and PCR methods

Size of the inoculum in spiked samples (cfu/ml)	Direct culture	Direct PCR	Enrichment culture	Enrichment PCR
7×10^5	+	+	+	+
7×10^4	+	+	+	+
7×10^3	+	+	+	+
7×10^2	+	+	+	+
70	+	–	+	+
7	–	–	+	+
0.7	–	–	+	+

3.3. Specificity of the PCR assays

The specificity of the C412F-16S rRNA-campR2 primer set was tested against a panel of *Campylobacter* and non-*Campylobacter* DNA templates (Table 1). The PCR assay detected *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, *C. helveticus*, and *C. hyointestinalis*, but none of the other *Campylobacter* species tested. No signal was observed for any of the *Arcobater*, *Helicobacter*, or other non-*Campylobacter* species tested. The specificity of the MD16S1–MD16S2 PCR assay was tested by Denis et al. (1999). The assay detected all tested strains of *C. jejuni*, *C. coli* and *C. hyoilei*, but gave no reaction for non-C*ampylobacter* strains tested in the study (Denis et al., 1999).

3.4. Comparison of the detection limit between the culture and PCR method

Table 4 shows the results of direct and enrichment culture of spiked samples as well as PCR performed on DNA isolated directly from the same spiked samples or from the enrichments culture of the samples. The detection limit of both enrichment culture and enrichment PCR was less than 1 cfu/ml of sample rinse, while the detection limit of direct culture was 70 cfu/ml. For the direct PCR detection the limit was 700 cfu/ml of sample rinse.

4. Discussion

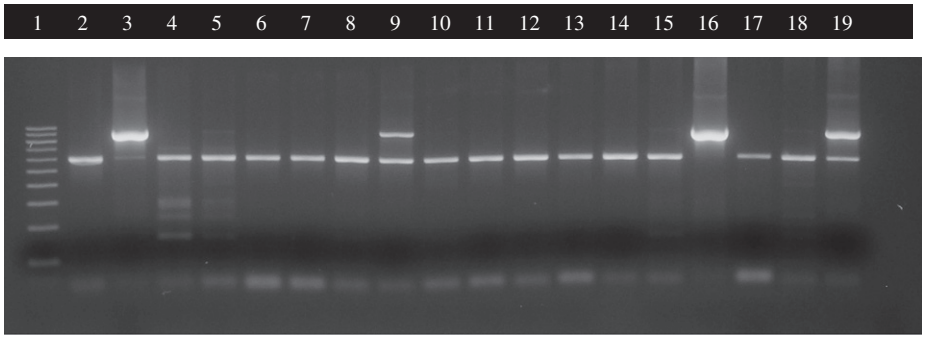
The low prevalence of *Campylobacter* in retail poultry products observed in the present study is consistent with earlier studies carried out in Finland (Hänninen et al., 2000; Anonymous, 2006a). However, these findings are relatively low compared to other countries. In Germany, Luber et al. (2005) reported the occurrence of 67.6% and 11.3% *Campylobacter* on the surface and in the deep muscle tissue of broiler legs respectively. Alter et al. (2004) detected 6.2% and 30.3% *Campylobacter* in 419 turkey and 198 chicken retail products respectively. Nielsen et al. (2006) investigated Danish retail poultry products, including domestic and imported meat, and found *Campylobacter* in 38.7% of 460 chicken and in 27.5% of 204 turkey products. Dominguez et al. (2002) reported 49.5% *Campylobacter* occurrence in 198 chicken meat samples from the Spanish retail market. The low occurrence of *Campylobacter* in Finnish retail poultry products obviously reflects the low prevalence of the organisms in the broiler slaughter batches in Finland. Prevalences between 2.9% and 7.4% have been reported from examination of all broiler slaughter batches during the summer months, when the prevalence is highest in Finland (Perko-Mäkela et al., 2002; Anonymous 2006b, c).

In August, there was a peak with a 28.9% prevalence of *Campylobacter* (Fig. 1). A seasonal variation in chicken flocks has also been observed in the other Nordic countries (Wedderkopp et al., 2000; Bang et al., 2003; Hansson et al., 2004; Hofshagen and Kruse, 2005) and the Netherlands (Bouwknegt et al., 2004). In contrast, Logue et al. (2003) discovered *Campylobacter* more frequently in the cooler months (winter and early spring) in the US. They suggested that the difference in the seasonal occurrence of *Campylobacter* in poultry might be associated with the geographical locations where sampling occurred.

In the present study, *Campylobacter* was also detected in marinated poultry products with a prevalence of 21.1% and 9.5% for turkey and chicken products respectively. Atanassova et al. (2007) reported six out of 16 marinated turkey products from German retail shops as *Campylobacter* positive. Perko-Mäkelä et al. (2000) studied the survival of *C. jejuni* in plain marinade and in both marinated and non-marinated chicken drumsticks and meat slices. In the marinade, a decrease of the inoculated *C. jejuni* level was observed; however, there was no difference between the marinated and non-marinated meat. The authors concluded that marinating may not have an effect on the survival of *Campylobacter*. This may be due to the buffering capability of meat quickly neutralizing the pH of the acidic marinade (Perko-Mäkelä et al., 2000). However, in the present study no *Campylobacter* was detected in 22 marinated sliced chicken products. All these samples were from meat of foreign origin. Foreign meat is frozen when imported to Finland which could be the reason that these samples were negative for *Campylobacter*.

The diagnostic specificity in the comparison between the PCR and the cultural detection by selective enrichment was 0.96 with a level of agreement of 0.96. This is a good agreement between the two methods. One sample of 18 gave a negative result in PCR when the result of the culture method was positive. This false-negative result may be explained by the fact that the size of the subsample used for the culture method is larger than in the PCR method; 25 ml of the rinsing fluid enriched in 225 ml Bolton broth compared to 1 ml for DNA extraction in the PCR method. However, for this reason comparing direct PCR with enrichment culture may be difficult.

Seven samples gave a positive result with PCR after enrichment, whereas the culture result was negative. However, sequencing of the PCR product from five of these samples gave sequences being 99% or 100% equal to *C. jejuni*. The reason for these negative culture results might be the abundant growth of the background flora on mCCDA plates. Six of the seven samples were products with skin likely to contain more contaminating flora than samples without skin (Josefsen et al., 2003). Background flora was detected in 5% of all samples and sometimes it was so abundant that it made the detection of *Campylobacter* impossible. Mateo et al. (2005) reported the same problem with overgrowth in 52.9% of 68 samples and identified *Escherichia coli* in some cases. However, they used Preston broth for selective enrichment. Bolton broth proved to be the best compromise between the inhibition of competitors and growth of *Campylobacter* (Baylis et al., 2000), but this may depend on the material investigated (Josefsen et al., 2003; Tangvatcharin et al., 2005).



Lane 1: 100 bp-DNA molecular weight marker; lane 2: PCR-negative control (internal control); lane 3: PCR-positive control (857 bp); lanes 4 – 8: *Campylobacter*-negative samples; lane 9: *Campylobacter*-positive sample; lanes 10 – 15: *Campylobacter*-negative samples; lane 16: *Campylobacter*-positive sample; lanes 17 – 18: *Campylobacter*-negative samples; lane 19: *Campylobacter*-positive sample.

Fig. 2. Gel electrophoresis patterns of PCR products of *Campylobacter* from enrichment samples of naturally contaminated marinated and non-marinated poultry products at the Finnish retail level using C412F and 16S rRNA campR2 primers.

Susceptibility to inhibitory substances, which can be found in high levels in foods, is a great disadvantage of PCR (Abu Al-Soud and Radstrom, 1998; Abu Al-Soud and Radstrom, 2000; Rossen et al., 1992). Lilja and Hänninen (2001), studying marinated chicken products and using the Buoyant Density Centrifugation (BDC) method for sample preparation, reported problems probably caused by emulsifiers used in the oil-spice. Rossen et al. (1992) used a pre-treatment step with hot sodium hydroxide/sodium dodecyl sulfate to reduce the effect of food inhibitory substances. In the present study, a pre-centrifugation step was performed in order to exclude most of the lipids and fat from the marinade and the chicken skin. As DNA isolation was performed manually with a DNA isolation kit for automated DNA isolation, further optimization compared to manufacturer's instructions was necessary to make the manual DNA isolation as sensitive as the automated isolation. Vigorous vortexing of the samples in lysis buffer was found to be the most important step. To optimize DNA isolation from marinated poultry products, one possibility could be to add fat digesting enzymes to the bacterial pellet just prior to DNA isolation. A nested PCR method could lower the detection limit, however, when the number of *Campylobacter* is very low, it is a question of statistics if any bacteria will appear in a 1 ml sample.

To control the PCR reaction in the different samples studied in this assay, an internal control PCR was run simultaneously with the target DNA (Fig. 2). In both PCR reactions, performed on DNA isolated directly from the samples and on DNA isolated from the enrichment media, the internal control gave a band of same intensity showing no evidence of inhibition of the PCR reaction. However, the detection limit of the direct PCR was about 700 cfu/ml (Table 4). This is high compared to other direct PCR assays for *Campylobacter*. Lund et al. (2003) reported a detection limit of approximately 40 cfu/ml in fecal material and Yang et al. (2003) of 100 cfu/ml in the same material using a real-time PCR assay. As inhibition of the PCR reaction does not seem to be the problem, it may also be possible that *Campylobacter* are preferably located in the fatty part of the sample and as this part is removed before DNA isolation many bacteria might be lost. On the other hand, the fat and or protein still present in a sample after pre-treatment could interfere with DNA isolation. As the detection limit of the present direct PCR was too high compared to the normally quite low amount of *Campylobacter* in food and retail poultry samples, it was necessary to perform a combination of enrichment and PCR assay.

In the present study, we used a new combination of primers (C412F and 16S rRNA campR2). A tendency was seen that this primer set captured more of the samples that were culture negative and negative with the MD16S1 and MD16S2 primers (results not shown). However, the differences were not statistically significant.

5. Conclusions

The PCR method used shortens time compared to microbiological analyses and can be therefore used for detection of *Campylobacter* spp. in poultry products. However, enrichment of the samples is necessary due to the low occurrence of *Campylobacter* spp. in Finnish poultry products at the retail level.

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