Comparison between conventional and qPCR methods for enumerating Campylobacter jejuni in a poultry processing plant

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Abstract

Campylobacter jejuni is worldwide recognised as a human foodborne pathogen. It is widely present in poultry meat and slaughterhouses, but little is known about its fate during the processing of poultry meat preparations. In stress conditions, this pathogen can enter into a viable but non-culturable state, where quantitative PCR (qPCR) becomes more convenient for its detection. In this study, two different pairs of primers, targeting the *rpoB* and the *hipO* genes, were compared for its detection and quantification by PCR. Two calibration curves were prepared: one for the meat samples and the other for the environmental samples. *rpoB* primers showed higher sensitivity with a quantification limit of 1 log cfu/g or ml. Microbial Assessment Scheme (MAS) was used to select the Critical Sampling Locations (CSLs) along the poultry processing line. Forty-six out of 48 samples were positive by qPCR after enrichment (t = 48h) while only 6 samples were positive by ISO 10272-1:2006. Forty-three samples showed positive signal without enrichment (t = 0h), however only 16 samples could be quantified. These results showed the high prevalence of *C. jejuni* in the poultry industry and the need for new, rapid and sensitive techniques, such as qPCR, for the detection and quantification of *C. jejuni* in meat and environmental samples.

Introduction

The global incidence of campylobacteriosis has increased over recent years. The last report published by the European Centre for Disease Prevention and Control (ECDC), in 2007, pointed to an increased incidence of campylobacteriosis in the EU, in comparison with 2006, of around 15%; a rise from around 40 to 47 cases per 100,000 inhabitants. It is the most commonly reported cause of gastrointestinal disease in the EU (ECDC, 2009). In the United States, 13 cases per 100,000 inhabitants were reported by FoodNet (CDC, 2010), although it is estimated that two-to-three million *Campylobacter*-related illnesses occur per year (Friedman et al., 2000; Miller and Mandrell, 2005). Furthermore, Nachamkin (2003) has estimated an annual global incidence rate of between 400–500 million illnesses. *Campylobacter* infection is serious but usually self-limiting, although it can also lead to long-term sequels such as reactive arthritis and Guillain-Barré syndrome. *Campylobacter* is ubiquitous in nature and, as a consequence, it is frequently found in farm animals, in the environment and on many raw foods. Bacterial numbers can be very high on certain key foods including raw poultry meat. Although all commercial meat poultry species can carry campylobacters, the risk of campylobacteriosis is greater in chicken meat (Pires et al., 2010), because of high levels of chicken consumption, among other reasons (Humphrey et al., 2007).

The *Campylobacter* genus currently includes 16 species and six subspecies. *C. jejuni* is by far the major cause of recognized *Campylobacter* outbreaks and sporadic illness, together with *C. coli* (thermophilic campylobacters). Generally, it is recognized that *C. jejuni* is more commonly associated with poultry (Murphy et al., 2006) than *C. coli,* which is more related to pork (Doorduyn et al., 2010, Mayr et al., 2010). Nevertheless, other *Campylobacter* species are beginning to emerge in specific regions of the world (Humphrey et al., 2007).

Classical detection methods are slow and sometimes unreliable due to the complex growth requirements of *Campylobacter*. For this reason, there has been a growing interest in the development of molecular tools to allow quick and unambiguous detection and identification of thermophilic *Campylobacter* species in food safety controls (Bonjoch et al., 2010; Mayr et al., 2010). Conventional methods currently in use are time-consuming and laborious, requiring prolonged incubation periods and selective enrichment to reduce the growth of background flora. It has been observed that enrichment broths used in traditional microbiological testing often fail to recover *Campylobacter* spp. from food, thereby the real prevalence of these pathogenic microorganisms is hardly understood (Habib et al., 2008). Additionally, *Campylobacter* species can enter a VBNC state. Molecular methods must therefore be developed to increase detection sensitivity as a useful alternative to traditional enrichment testing (Nogva et al., 2000). PCR-based methods can be designed to detect genera (Linton et al., 1996; Marshall et al., 1999) or groups of species, such as thermophilic *Campylobacter* (Fermér and Engvall, 1999; Klena et al., 2004; Thunberg et al., 2000), or they can be designed to speciate *Campylobacter* present in a sample (Bonjoch et al., 2010; Burnett et al., 2002; Mayr et al., 2010). With the accumulation of genomic data, new methods have been described that amplify genes unique to a given species. These include, for example, the *hipO* gene of *C. jejuni* (Keramas et al., 2003; Steinhauserova et al., 2001; Wang et al., 2002).

Although, a significant number of studies have developed and used different quantitative PCR (qPCR) methods for detecting *Campylobacter* spp., fewer use this methodology for quantification of *Campylobacter* spp. in food samples (Botteldoorn et al., 2008; Hong et al., 2007; Josefsen et al, 2010; Yang et al., 2003), and determination of surface contamination by campylobacters on an industrial scale. In this study, the

sensitivity of two different pairs of primers in the detection and quantification of *C. jejuni* in poultry and environmental samples has been tested. The most sensitive method was chosen and applied throughout the poultry processing plant, comparing its results with those of the culture-based method. More sensitive methods can help in a better design of preventive measures, and/or intervention processes to increase food safety with specific reference to *Campylobacter jejuni* presence in foods.

Material and Methods

Campylobacter jejuni strains

In this study, different strains of *C. jejuni* isolated from poultry samples have been used as controls: *C. jejuni* CaTA007 (kindly provided by Gaiker Centro Tecnológico, Bilbao, Spain), CaTA008 (kindly provided by the Departamento de Inmunología, Microbiología y Parasitología, Facultad de Farmacia, Universidad del País Vasco, Vitoria, Spain) and CECT 7572 (Spanish Type Culture Collection) isolated from human faeces. Additionally, two strains of *Listeria monocytogenes*, CECT 4032 and LTA002, isolated from lamb (Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, Burgos, Spain) and *Escherichia coli* CECT 729 were used as negative controls. The bacterial isolates were recovered from -70ºC storage and grown on agar plates or broth. *C. jejuni* strains were grown on Columbia Blood Agar (Oxoid, Basingstoke, Hampshire, England) supplemented with 5% horse blood (Oxoid). Plates were incubated at 41.5 ºC for 48 h under microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) generated by CampyGen® (Oxoid). Non-campylobacters were incubated in brain heart infusion (BHI, Oxoid) at 37ºC for 24 h.

Sampling methodology

In order to apply both the conventional and the qPCR technique throughout the poultry chain, some principles of the Microbial Assessment Scheme (MAS) (Jacxsens et al., 2009; Sampers et al., 2010) were used. The Critical Sampling Locations (CSL), sampling frequency and sampling method are summarized in Table 1. In this study, sampling began with the entrance of chicken carcasses after slaughtering and continued up until the production of fresh and raw poultry products.

Sample preparation

For the destructive sampling method (meat samples), 25 g were taken aseptically and homogenised with 225 ml of sterile Bolton broth (Oxoid) supplemented with Bolton broth selective supplement (Oxoid) for *C. jejuni* detection and Buffer Peptone Water (BPW, AES Chemunex, Bruz, France) for *C. jejuni* quantification (culture-based method). Although Bolton broth has to be supplemented with blood as recommended in ISO 10272-1:2006, some studies (Al-Soud and Rådström, 2001; Josefsen et al., 2004; Mercier et al., 1990; Rantsiou et al., 2010) suggested that blood components can produce a possible inhibitory effect on PCR reactions. In order to check whether the absence of blood supplement in Bolton broth influences the growth of campylobacters during the enrichment step, a simple test was performed to compare its suppression before the application of the ISO method or qPCR analysis. Bolton broth without blood was used in this study, the results of which are discussed below.

Sterile abrasive sponges (Envirosponge^{TM}, Biotrace international) were used for sampling the carcasses, working surfaces and personnel gloves, putting the sponges after sampling into a sterile plastic bag containing 100 ml of sterile Bolton broth

supplemented as described above. Sponges were homogenised for 2 min in a sterile plastic bag in a lab blender (Stomacher 400, Seward, London, UK).

For the conventional method, serial decimal dilutions were prepared in sterile Buffered Peptone Water (BPW) (AES Chemunex), then 100 µl were spread onto modified charcoal cefoperazone deoxycholate agar (mCCDA) prepared with *Campylobacter* blood-free selective agar base (Oxoid) supplemented with CCDA selective supplement (Oxoid) and onto a *Campylobacter* agar base (Karmali, Oxoid) with *Campylobacter* Selective Supplement (Karmali, Oxoid). Selective plates were incubated at 41.5 ºC for 48 h in a microaerophilic atmosphere. Samples homogenized in Bolton broth were also enriched at the same conditions for detection as described in ISO 10272-1:2006 (ISO, 2006).

For quantitative PCR and prior to the enrichment step, about 40 ml of the homogenate in Bolton broth were transferred to a 50 ml sterile tube and the solids were allowed to settle for about 5 min; for sponges, samples were allowed to settle out in the same bag. Further 1/10 dilution in BPW was carried out in the case of meat samples and 1 ml was centrifuged (Centrifuge 5415R, Eppendorf, Hamburg, Germany) at 13,400 rpm for 10 min at 4ºC. The resulting pellets were stored at -70ºC awaiting DNA extraction. Samples in Bolton broth were also incubated at 41.5ºC for 48h for detection by qPCR, then 1 ml of the enriched broth, diluted (1/10 in BPW for meat samples only) and pelleted, was used for DNA extraction and amplification.

DNA extraction

Nucleic acid extraction for *Campylobacter jejuni* was performed using the Master Pure^{TM} Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA) following the manufacturer's instructions, as suggested by Rantsiou et al. (2010).

Detection and quantification of Campylobacter jejuni by PCR

The detection and quantification of *C. jejuni* was performed using two different pairs of primers (Table 2). The procedure described by Rantsiou et al. (2010) was followed for the *rpoB* gene; while certain modifications to the procedure described by Keramas et al. (2003) were carried out for the *hipO* gene. Samples were first subjected to detection by qPCR at 48h after sample enrichment, and those with positive signal were quantified using the corresponding DNA obtained before sample enrichment and the calibration curves constructed as described below. Amplifications were performed in a final volume of 25 μl. One μl of DNA, extracted using method described above, was amplified with the specific primers (Table 2). The Euroclone FluoCycleTM SYBR® Green Mix kit (Genycell Biotech, Pero, Italy) was used with a $MgCl_2$ concentration of 8 mM. Primer (Sigma-Aldrich®, Madrid, Spain) concentrations can be found in Table 2. All the amplifications were performed in an iCycler iQ™ Thermal Cycler machine (Bio-rad Laboratories, California, USA). The amplification profile consisted of 50 cycles: 30s at 95 ºC; 30s at 62 ºC; and finally 30s at 72 ºC, as described by Rantsiou et al. (2010).

Calibration curves

Two calibration curves were constructed for the purposes of quantification for each qPCR procedure described above: one in Bolton broth for environmental samples and the other in minced poultry meat for meat samples. Serial dilutions of an overnight culture in Bolton broth of *C. jejuni* CaTA008, containing approximately 10^6 cfu/ml were prepared in BPW, then 1 ml of each dilution was subjected to DNA extraction. Ten g of minced poultry meat were inoculated with 1 ml of each dilution for the meat curve. The final concentration of the cells in the meat varied from 10^5 to 10^1 cfu/g. Ninety ml of Bolton broth supplemented as described above was added to the inoculated

meat and homogenized in a lab blender (Stomacher 400, Seward). One ml of each homogenate was diluted in 9 ml of BPW and 1 ml of the dilution was collected for DNA extraction and amplified in triplicate. Each serial dilution and homogenate was subjected to enumeration on mCCDA plates. Calibration curves were constructed plotting the signals produced (threshold cycle, C_t) by the DNA against the $\log_{10}$ cfu/g or ml. Correlations coefficients (R^2) and amplification efficiency (AE) were calculated as previously described (Higuchi et al., 1993).

Results and Discussion

Blood and other ingredients were added to conventional formulations of *Campylobacter* media to neutralize the toxic effects of compounds produced in the presence of oxygen and light, as stated in the ISO detection method (Corry et al., 1995) On the contrary, many studies have shown that some blood components, primarily heme, hemoglobin, and lactoferrin are PCR inhibitory (Al-Soud and Rådström, 2001; Josefsen et al., 2004; Mercier et al., 1990; Rantsiou et al., 2010). Our results showed no significant difference between the counts ($p>0.05$) obtained by either ISO (6.39 log cfu/ml) and or by qPCR (6.62 log cfu/ml) methods, when blood was omitted in the enrichment broth. Similar conclusions have been advanced by other authors (Bolton et al., 2002; Paulsen et al., 2005). On the basis of these results, it was decided to prepare samples using Bolton enrichment broth without blood for both methods.

Both pairs of primers had previously been optimized (Keramas et al., 2003 and Rantsiou et al., 2010). Moreover, in this study all three control strains of *Campylobacter jejuni* tested were positive by qPCR, while both strains of *L. monocytogenes* and the single strain of *E. coli* tested negative, using both the *hipO* and the *rpoB* genes.

Two calibration curves (meat and environmental samples) were constructed for each of the two targeted genes (Figure 1) to verify the effectiveness and the sensitivity in the detection and quantification of *C. jejuni* for both pairs of primers. As shown, quantification limits of 1 log cfu/ml and 2 log cfu/g were obtained for the environmental and the meat samples, respectively, when the *hipO* gene was the target (Figure 1a and 1b); while a quantification limit of 1 log cfu/ml o g was obtained for both types of samples, when the *rpoB* gene was the target. In all probability, the *hipO* amplification gave a lower quantification limit due to lower amplification efficiency, probably because of a large PCR product (Kubista et al., 2006). Other authors have also quantified *C. jejuni* by qPCR, by spiking food samples with different *C. jejuni* concentrations, to obtain similar quantification limits of 1 log cfu/g for meat products (Bonjoch et al., 2010; Rantsiou et al., 2010). After the calibration curves had been constructed, the qPCR method was used to quantify *C. jejuni* in some of the samples collected from different CSLs along the processing line.

Thirteen samples were randomly selected from all the CSLs to compare the quantification results obtained with both pairs of primers (Table 3). Counts obtained with both pairs of primers were of the same order of magnitude with a maximum difference between both methods of around 0.5 log cfu/g or ml. Only two samples could not be quantified with *hipO* primers because the detection limit for meat samples was less sensitive than the one obtained with *rpoB* primers.

Once the most suitable pair of primers for the quantification of *C. jejuni* in poultry and environmental samples was selected, the sensitivity of the qPCR method was compared with the ISO method for the 48 samples taken in the poultry processing plant following the MAS sampling plan (Table 4).

Forty-six out of 48 (95.83 %) enriched samples (t = 48h) tested positive by qPCR (Table 4), while 43 (89.53 %) were positive when non-enriched samples were analysed (t = 0h). However, these results are inconsistent with those obtained by conventional method where only 6 out of 48 samples were positive (12.50 %). Other studies have reported similar results when using either molecular methods such as qPCR (Rantsiou et al., 2010) or traditional microbiological approaches (Habib et al., 2008). These results can be explained by accepting that *C. jejuni* is a fastidious microorganism, sensitive to temperature variations, incubation time and also incubation atmosphere (Humphrey et al., 2007). Likewise, *C. jejuni* is present on food and in water at much lower levels than in faecal samples, and those present on food may have been injured by exposure to heating, chilling, freezing or other conditions related to processing and storage (Humphrey and Cruikshank, 1985; Rosenquist et al., 2006). Therefore, an enrichment step is required both to detect small numbers of bacteria and to resuscitate damaged cells. However, pre-enrichment may increase outgrowth of the normal foodborne microflora, resulting in suppression of the growth of *Campylobacter* (Uyttendaele and Debevere, 1996). Thus, according to Jasson et al. (2009) an overgrowth of an *Escherichia coli* strain resistant to the antibiotics added to the Bolton broth and the mCCDA plates could suppress the growth of *C. jejuni* and this could be the reason for negative results by ISO methods in comparison with the positive results by PCR. Moreover, a wide number of studies have also concluded that *Campylobacter* can change to a viable but non-culturable (VBNC) form when exposed to stress conditions, concealing itself from detection by conventional methods (Beumer et al., 1992; Humphrey et al., 2007). However, it cannot be excluded that VBNC cells may recover and exit from this state becoming potentially pathogenic for humans. From this

perspective, the PCR method may successfully be used for food-poisoning-related risk assessments (Mayr et al., 2010).

The data from the present study showed that qPCR was more sensitive than the ISO 10272-1:2006 in the detection of *C. jejuni*. These results agree with other studies where conventional methods arrived at an underestimation as against molecular methods, although the differences were not as great as those found in this study (Botteldoorn et al., 2008; Hong et al., 2007; Yang et al., 2003). Debretsion et al. (2006) found that 65 samples out of 84 (77%) obtained from chicken rinses were positive by qPCR assay, whereas only 27 (32%) samples were positive by direct plating on selective media. Likewise, Rantsiou et al. (2010) found that 87% of analyzed food samples were *Campylobacter jejuni* positive by PCR while none tested positive using conventional methods. Moreover, the qPCR method allows *C. jejuni* detection and quantification in chicken meat and environmental samples within a few hours instead of the 6 days required for determination of the presence of *Campylobacter* spp. by ISO 10272-1:2006.

Sixteen out of 43 positive non-enriched samples could be quantified by qPCR. Carcasses were the most contaminated samples in this poultry processing plant (Table 4). *C. jejuni* was also present in thighs, minced meat and packaged hamburger. Similar results were obtained by Sampers et al. (2008) using conventional methods. Applying qPCR, Botteldoorn et al. (2008) detected 8.25 log cfu per carcass of *Campylobacter* spp. at slaughterhouse level, after the cooling step. In chicken samples purchased in markets, Yang et al. (2003) obtained 5.0×10^8 cfu/g in 27 chicken breasts and 4.6×10^6 cfu/ml in 16 chicken thighs as mean counts, and Hong et al. (2007), an average of 2.17 log cfu/ml of *C. jejuni* in chicken rinses.

C. jejuni was also detected on most of the processing surfaces of the poultry line and on operator's gloves (Table 4). Although it is well documented that cross-contamination with *C. jejuni* in the slaughter step could happen (Berndtson et al., 1996, Peyrat et al., 2008; Rosenquist et al., 2006), few studies have been performed in plants processing chicken meat preparations (Cools et al., 2005). The authors, detected *C. jejuni* by conventional methods on conveyor belts, knives, gloves and cutting boards during the production shift. In this study, among the environmental samples, only those taken in the carcass portioning step (CSL 2- thighs, CSL 5-portioning table and CSL 8-operator's gloves) could all be quantified by qPCR. The contamination of these three CSLs could indicate cross-contamination between poultry meat, cutting tables and gloves while operators were processing the product. In the present study, the presence of *C. jejuni* was also determined after cleaning and disinfection procedures -time 1 of each visit- (Table 4), as has been observed in other studies (Cools et al., 2005; Peyrat et al., 2008).

Conclusions

Though easy to perform and not technically demanding, the plate counting method can only recover culturable cells and may not be able to detect highly stressed cells, which can easily underestimate the threat of *C. jejuni* that is present after low temperature storage or intervention strategies that have been applied to food. Thus, the qPCR method is more convenient for the detection and quantification of *C. jejuni* from meat poultry and environmental samples obtained directly from a poultry company processing line. The sensitivity of the two qPCR procedures is greater in comparison with conventional methods. Data obtained with both qPCR procedures from the same samples showed similar values. Moreover, *rpoB* primers were more sensitive than *hipO* ones, when the meat samples were analysed.

The use of qPCR approaches could represent a more realistic situation of *C. jejuni* prevalence in the poultry processing line, which can be useful to establish new preventive measures in order to increase food safety in this kind of products.

qPCR methods save time and are more sensitive for detection and quantification of *C. jejuni* than conventional methods and could therefore be used, having addressed technical issues and training for lab personnel, as routine control methods on an industrial scale.

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Table 1: Sampling scheme according to Microbial Assessment Scheme (MAS) procedure along the poultry processing line.

CSL[a]	1	2	3	4	5	6	7	8
Processing step	Carcass at arrival (reception of raw material)	Carcass portioning	After poultry meat grinding	Final product after packaging and chilling	Cutting table at portioning	Table at processing	Table surface at packaging	Operator's gloves at portioning
Frequency	3 visits, 1 sample n=3				3 visits, 3 samples at start–middle and end of the shift n=9			
Sampling method	25 g of neck skin	Surface swabbing of 25 cm^2 of poultry thighs	25 g of minced hamburger meat	25 g of packaged poultry hamburger	Surface swabbing of 25 cm^2 of the table			Surface swabbing of 25 cm^2 of the gloves

[a]CSL: Critical Sampling Location

Table 2: Sequence of different primers used to detect *Campylobacter jejuni*

Gen	Primer	Secuence (5'→ 3')	Concent. in the mix	Reference
rpoB	Cj_rpoB1	GAGTAAGCTTGCTAAGATTAAAG	400 nM	Rantsiou et al., 2010
	Cj_rpoB2	AAGAAGTTTTAGAGTTTCTCC	60 nM	
hipO	HIP-FW	GTACTGCAAAATTAGTGGCG	400 nM	Keramas et al., 2003
	HIP-REV	GCAAAGGCAAAGCATCCATA	300 nM	

Table 3: Comparative qPCR methods between *rpoB* and *hipO* genes (n=6)

			rpoB		***hipO***	
CSL[a]	**Visit**	**Time[b]**	**Log cfu/cm^2**	**Log cfu/g**	**Log cfu/cm^2**	**Log cfu/g**
Neck skin	1			1.52 (0.02)[c]		NC[d]
	2			3.74 (0.19)		3.75 (0.10)
Thighs	2		3.75 (0.05)		3.32 (0.06)	
Minced burger meat	1			1.43 (0.38)		NC
Minced packaged meat	2			2.01 (0.48)		2.49 (0.09)
	3			2.00 (0.53)		2.40 (0.16)
Portioning table	2	3	1.74 (0.04)		1.80 (0.10)	
Processing table	1	2	NC		NC	
	2	3	NC		NC	
Packaging table	1	3	NC		NC	
	3	3	NC		NC	
Operator´s gloves	2	1	NC		NC	
		3	2.32 (0.12)		2.05 (0.08)	

[a]CSL: Critical Sampling Location
[b]Time: 1- Before the beginning of the shift; 2- in the middle of the shift; 3- At the end of the shift.
[c]Mean value (Standard deviation)
[d] NC: Detected but non quantifiable, because data is below the quantification limit.

Table 4: Detection and quantification of *Campylobacter jejuni* in poultry processing plant by standard culture and real-time qPCR

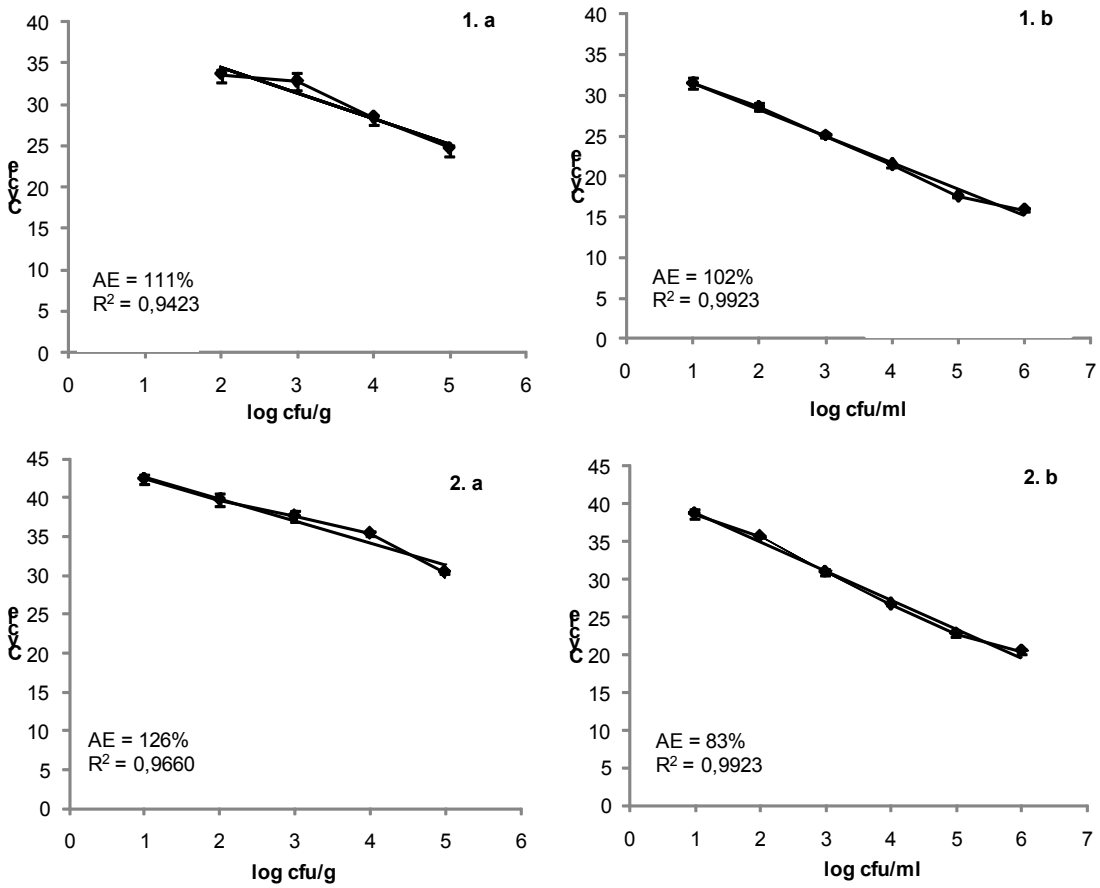
				ISO[a]	real-time PCR[b]	
	CSL[c]	Visit	Time[d]		Surface Samples (Log cfu/cm^2)	Meat Samples (Log cfu/g)
1	Neck skin	1		-		2.46
		2		-		3.55
		3		-		3.75
2	Thighs	1		-	NC	
		2		-	2.40	
		3		-	1.53	
3	Minced burger meat	1		-		NC
		2		-		1.94
		3		-		2.36
4	Packaged poultry hamburger	1		-		1.62
		2		-		NC
		3		-		2.36
5	Portioning table	1	1	-	NC	
			2	-	1.25	
			3	-	1.41	
5	Portioning table	2	1	-	NC	
			2	-	1.04	
			3	-	1.09	
5	Portioning table	3	1	-	NC	
			2	-	NC	
			3	-	N/A	
6	Processing table	1	1	-	NC	
			2	-	NC	
			3	-	NC	
6	Processing table	2	1	-	NC	
			2	-	NC	
			3	+	NC	
6	Processing table	3	1	-	NC	
			2	+	NC	
			3	-	NC	
7	Packaging table	1	1	-	NC	
			2	-	NC	
			3	-	NC	
7	Packaging table	2	1	-	NC	
			2	-	NC	
			3	-	NC	
7	Packaging table	3	1	-	NC	
			2	+	NC	
			3	+	NC	
8	Operator's gloves	1	1	-	1.03	
			2	-	NC	
			3	-	1.42	
8	Operator's gloves	2	1	+	NC	
			2	-	NC	
			3	+	1.42	
8	Operator's gloves	3	1	-	NC	
			2	-	NC	

	3	-	N/A

[a] - : Not detected by ISO; + : Detected by ISO
[b] N/A: Not detected by real-time PCR; NC: detected by real-time PCR but not quantifiable
[c]CSL: Critical Sampling Location
[d]Time: 1- Before the beginning of the shift; 2- in the middle of the shift; 3- At the end of the shift.

Figure 1: Standard curves for *C. jejuni* quantification. 1.a. *hipO* standard curve for meat poultry samples. 1.b. *hipO* standard curve for surface samples. 2.a. *rpoB* standard curve for meat poultry samples. 2.b. *rpoB* standard curve for surface samples (AE: amplification efficiency; R^2: correlation coefficients).

Figure 1


1. a
AE = 111%
R2 = 0,9423
log cfu/g
1. b
AE = 102%
R2 = 0,9923
log cfu/ml
2. a
AE = 126%
R2 = 0,9660
log cfu/g
2. b
AE = 83%
R2 = 0,9923
log cfu/ml