

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Modified enrichment strategies coupled with molecular and conventional methods to detect and quantify *Campylobacter jejuni* in chicken meat from the market

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/141758> since 2016-07-13T09:35:34Z

Published version:

DOI:10.1111/jfs.12081

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is the accepted version of the following article:

Rohonczy, K., Rantsiou, K. and Cocolin, L. (2013), Modified Enrichment Strategies Coupled with Molecular and Conventional Methods to Detect and Quantify *Campylobacter jejuni* in Chicken Meat from the Market. *Journal of Food Safety*, 33: 497–502. doi: 10.1111/jfs.12081

which has been published in final form at

<http://dx.doi.org/10.1111/jfs.12081>

Modified enrichment strategies coupled with molecular and conventional methods to detect and quantify *Campylobacter jejuni* in chicken meat from the market

Kata Rohonczy^a, Kalliopi Rantsiou^b, Luca Cocolin^{b*}

^a *WESSLING Hungary Ltd.*

^b *Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Italy*

*Corresponding author: Luca Cocolin, via Leonardo da Vinci, 44, 10095, Grugliasco, Turin, Italy. Phone: +39-011-6708553, Fax: +39-011-6708549, email lucasimone.cocolin@unito.it

Running title: *C. jejuni* in poultry by means of combined enrichment and qPCR

Abstract

Campylobacter spp. are currently the bacterial foodborne pathogen that causes the highest number of gastrointestinal diseases in developed countries, according to the World Health Organization. The aim of this study was to compare the performance of four *Campylobacter* enrichment broths: Bolton broth, Bolton broth with blood, Preston broth and Preston broth with blood using both culture-dependent methods and an optimized quantitative PCR (qPCR) protocol. The enrichment in Bolton Broth with blood allowed most positive samples for *Campylobacter jejuni* to be detected when 40 chicken meat samples from the market were tested. Correlation between cultural methods and molecular methods was poor. Only in a few cases could *C. jejuni* and *Campylobacter* spp. be identified by PCR. Out of 480 colonies isolated throughout the enrichment process, only 7 colonies could be identified as *Campylobacter* spp., three of which were *C. jejuni*. Enrichment of chicken meat samples with Bolton broth added with blood, coupled with qPCR resulted to be the most suitable method to detect samples contaminated with *C. jejuni*.

Practical Applications

Detection and identification of *Campylobacter* spp. in food is still a challenge and there is lack of consensus on the methodology that should be used in order to recover these fastidious microorganisms. The application of molecular methods, such as the amplification of a target gene sequence by PCR, in order to rapidly and unequivocally detect and identify foodborne pathogens in foodstuffs, offers a valid alternative to traditional microbiological testing. In this study we investigated the performances of combined enrichment strategies with a specific *C. jejuni* quantitative PCR (qPCR) protocol. The molecular approach resulted to be superior with respect to traditional plating and was able to detect a higher number of *C. jejuni* positive

samples. Among the enrichment broths tested, Bolton broth added with blood resulted to be the most suitable for the detection of *Campylobacter* spp. in poultry meat.

Keywords

Campylobacter jejuni, qPCR, enrichment broth, poultry meat, culture independent methods

Introduction

Campylobacter spp. are encountered as most common foodborne pathogen causing enteritis in many developed countries. *Campylobacter* infections still top the list of zoonotic diseases in the European Union. In 2007, infections from *Campylobacter* were among the most frequently reported zoonotic diseases in humans throughout the European Union, with 200,507 cases compared to 175,561 in the previous year, thus showing an increase of 14.2 %. In foodstuffs, *Campylobacter* was found above all in raw poultry meat, with an average of 26 % of samples showing contamination (Anonymous, 2008). In 2008, campylobacteriosis continued to be the most commonly reported gastrointestinal bacterial pathogen infection in humans in the European Union, with 190,566 confirmed cases, even though the number of notified cases decreased by 5.0 % compared to 2007. In foodstuffs, the highest proportion of positive *Campylobacter* samples was once again reported for fresh poultry meat, with an average of 30.1 % of positive samples. *Campylobacter* has also frequently been detected in live poultry, pigs and cattle (Anonymous, 2010).

The animal reservoir is the gastrointestinal tract of dogs, cats and other pets that can carry the organism. The transmission of *C. jejuni* to humans occurs through the ingestion of contaminated food or water, including unpasteurized milk and undercooked poultry, or through direct oral contact with fecal material from infected animals or people. The type of

illness associated with *C. jejuni* infection in humans is intestinal infection (Humphrey *et al.* 2007).

In many cases, the condition of the samples being tested and the fastidious nature of many *Campylobacter* species make the detection of campylobacters in laboratory settings very complicated (Chaban *et al.* 2009). A number of factors make *Campylobacter* a problematic agent to control: (i) it is a zoonotic agent that is asymptomatic in broilers (as well as in other birds), (ii) the sampling and testing methods that are available for its detection and quantification give variable results, thus surveillance data from different sources may not be comparable, (iii) compared to other foodborne pathogens, little is known regarding the physiology of *Campylobacter* and its ability to survive common food processing conditions, and iv) *Campylobacter* can enter into a viable not culturable state (Tholozan *et al.* 1999) making its recovery by conventional methods impossible. Furthermore, the previous belief that thermophilic campylobacters are sensitive to conditions they encounter outside their host does not seem to be supported by epidemiological data. Recently a comprehensive review on current methodologies to isolate and identify *Campylobacter* spp. from foods has been published, summarizing the most effective protocols to isolate campylobacters (mainly *C. jejuni* and *C. coli*) from food, primarily poultry products (Gharst *et al.* 2013).

Many enrichment broths have been proposed to detect *Campylobacter*. Preston, Bolton, Exter, CEB, Park and Sanders broths are currently used in food microbiology and their performance has been compared in several studies (Vidal *et al.* 2013; Williams *et al.* 2012, 2009; Habib *et al.* 2011; Richardson *et al.* 2009; Ugarte-Ruiz *et al.* 2012;) and a new Food Pathogen Enrichment (FPE) broth, which supports the growth of campylobacters, without lysed blood and CO₂ and to the same degree as Bolton and Preston broths, was developed (Hayashi *et al.* 2013). However, there is still no scientific consensus on which enrichment broth to use, although ISO 10272-2006 (ISO, 2006) indicates the use of Bolton broth, instead of Preston

broth, for selective enrichment.

The application of molecular methods to rapidly and unequivocally detect and identify foodborne pathogens in foodstuffs offers a valid alternative to traditional microbiological testing. The challenge nowadays is how to apply these methods, which are primarily based on the amplification of a target gene sequence by PCR, without previous enrichment or culturing, in order to quantify foodborne pathogens directly in foods. Until recently, the methods adopted to detect and quantify *Campylobacter* spp. in foods were applied after an enrichment step, due to their low numbers; however direct quantification of *C. jejuni* in chicken rinses has also been performed (Debretson *et al.* 2007; Josefsen *et al.* 2004; Nogva *et al.* 2000; Oliveira *et al.* 2005; Sails *et al.* 2003; Yang *et al.* 2003). In this study we tested four *Campylobacter* enrichment broths, namely Bolton broth, Bolton broth with blood, Preston broth and Preston broth with blood, coupled with a previously described quantitative PCR (qPCR) protocol (Rantsiou *et al.* 2010) in order to define an analytical strategy to properly detect *C. jejuni* in food.

Materials and Methods

Strains and media

Campylobacter jejuni ATCC 33291 (Oxoid, Milan, Italy) was used to construct calibration curves in poultry meat. It was propagated in Brain Heart Infusion broth (BHIB) supplemented with 5 % defibrinated horse blood (Oxoid) and plated on a *Campylobacter* Blood Free Selective Agar Base supplemented with CCDA Selective Supplement (mCCDA, Oxoid) in microaerophilic conditions (Campygen, Oxoid) at 37 °C for 24 h.

Four enrichment broths were used for the analysis of the samples: Bolton Broth supplemented with Bolton Broth Selective Supplement (Oxoid), with and without 5 % defibrinated horse

blood (Oxoid), Preston supplemented with Campylobacter Selective Supplement Preston (Oxoid), with and without 5 % defibrinated horse blood (Oxoid).

DNA extraction

Master-Pure™ Complete DNA and RNA Purification kits (Euroclone, Milan, Italy) were used for DNA extraction from the cultures and food samples, according to the manufacturer's instructions, as suggested by Rantsiou *et al.* (2008).

Poultry meat sampling and analysis

Forty samples of individually packed poultry were purchased from supermarkets in the Piedmont region, in Northwest Italy. Twenty five g of meat were aseptically removed from each package, mixed with 225 ml Ringer's solution (Oxoid) and homogenized using a stomacher (PBI, Milan, Italy). Ten ml of the homogenized solution was then added to 90 ml of each enrichment broth. The homogenate sample, as well as the 1 to 10 dilution in Ringer's solution, were plated in mCCDA medium and a Brilliance Campycount medium (Oxoid) (for both 0.1 ml). The plates were incubated at 42 °C for 48 h in microaerophilic conditions using the Campygen system (Oxoid). The four enrichment broths, after addition of the homogenate, were incubated for 6 hours at 37 °C and at 42°C for 48 hours. One ml of the 10⁻¹ dilution was transferred to a 1.5 ml Eppendorf tube and subsequently used for DNA extraction (t=0). DNA was also extracted from a ten-fold dilution of each enrichment broth after 6 (t₆), 24 (t₂₄) and 48 (t₄₈) hours, respectively. A full loop of the enrichment broths was also streaked in mCCDA at each time point and incubated at 42° C for 48 h under microaerophilic conditions.

Identification of the colonies

After incubation, the colonies on the plates were observed carefully to search for the typical *Campylobacter* spp. aspect. If detected, at least 5 colonies were isolated, and when fewer suspected colonies were present, all of them were isolated. Isolation was carried out by streaking on mCCDA and this was followed by incubation at 42°C for 48 h. *Campylobacter* spp., *C. jejuni* and *C. coli* isolates were identified using a molecular identification protocol proposed by Denis *et al.* (1999).

Quantitative PCR amplification

Quantitative PCR was performed using the SYBR Green I chemistry set previously described by Rantsiou *et al.* (2010). Cj_rpoB1 (5'-GAGTAAGCTTGGTAAGATTAAAG-3') and Cjs_rpoB2 (5'-AAGAAGTTTTAGAGTTTCTCC-3') primers were used to amplify part of the *rpoB* gene of *C. jejuni*. A positive and a negative control were loaded in each run. The positive control was represented by DNA extracted from a pure culture of *C. jejuni* ATCC 33291, standardized at 100 ng/μl. One μl of sterile, PCR-grade DNA water (Sigma, Milan, Italy) was added for the negative control. A melting curve analysis was carried out at the end of each run.

Construction of calibration curves in poultry meat

C. jejuni ATCC 33291 was streaked on brain heart infusion agar (BHIA, Oxoid) supplemented with 5 % defibrinated horse blood and, after incubation at 42°C for 48 h under microaerophilic conditions, the cells were scraped off the agar surface with 1 ml of Ringer's solution (Oxoid) using a plastic, sterile spreader. The cell suspension was serially diluted in Ringer's solution and counted on BHIA plates incubated at 37 °C for 24-48 h. Calibration curves were constructed by inoculating 1 ml of each dilution of *C. jejuni*, from 1 to 10⁸ colony forming units (cfu)/ml, in 25 g of poultry meat. Ringer's solution (225 ml) was added

and samples were homogenized using a stomacher for 2 min at maximum speed. Subsequently, 1 ml of each sample was diluted ten-fold and 1 ml of the dilution was used for DNA extraction, as described above. One μ l of the DNA was used in the qPCR amplifications and calibration curves were constructed by plotting the threshold cycle (Ct) against \log_{10} cfu/g.

Determination of the qPCR detection limit in the enrichment broths used in this study

Each dilution of *C. jejuni*, from 1 to 10^8 cfu/ml, was inoculated in 9 ml of each enrichment broth and 1 ml was recovered and diluted with 9 ml of Ringer's solution. One ml of each diluted enrichment broth was used for DNA extraction. One μ l of the DNA was used in qPCR amplifications to determine the limit of quantification of *C. jejuni* in the four enrichment broths used in this study.

Results and discussion

The goal of this study was to compare the performance of four *Campylobacter* enrichment broths, namely Bolton broth, Bolton broth with blood, Preston broth and Preston broth with blood, using both culture dependent methods and an optimized qPCR protocol. For this purpose, forty samples of individually packed poultry were purchased from supermarkets in the Piedmont region, in Northwest Italy and subjected to analysis. A calibration curve was constructed, using strain *C. jejuni* ATCC 33291, in order to be able to quantify the *C. jejuni* populations before the enrichment step. Moreover, the limit of detection of the qPCR protocol was determined for each broth, due to the possibility of the qPCR being inhibited by the components of the selective enrichment broths. Calibration curve in poultry meat confirmed the quantification limits previously described by Rantsiou *et al.* (2010) and Melero *et al.* (2011), in which populations of as few as 10 cfu/g or ml could be quantified (Fig. 1). When

direct qPCR analysis was conducted on the DNA extracted at t_0 , 7 of the 40 samples (17.5%) gave an amplification signal that highlighted the presence of *C. jejuni*. After quantification using the calibration curve, the counts were determined to be between 10 and 100 cfu/g in all of the 7 samples (data not shown).

The use of an enrichment step for the detection of campylobacters has attracted many researchers and a number of studies that compare their performance are available in the literature (Habib *et al.* 2011; Richardson *et al.* 2009; Ugarte-Ruiz *et al.* 2012). Most of them have investigated the two most popular broths, namely Bolton and Preston. The effect of incubation conditions and the advantage of the addition of blood in selective broths for the recovery of *Campylobacter* spp. have also been evaluated (Williams *et al.* 2009). It has been demonstrated that the base components and selective supplements of the enrichment broths can inhibit the *Campylobacter* cells over the incubation time (Baylis *et al.* 2001), and it is therefore important to understand whether the selective broth is able to recover these cells or not. To the authors' knowledge, this is the first time that a culture-dependent approach has been coupled with a culture-independent strategy, i.e. a qPCR protocol, specific for *C. jejuni*, in order to adequately detect and quantify this pathogen in poultry meat. First, the detection limit of the qPCR protocol was determined in the enrichment broths used in the study in order to take into account possible inhibitions on the amplification by the components of the broths, especially when blood was added. The detection limits are reported in Table 1, where the respective Ct value for each *C. jejuni* dilution in the four enrichment broths is shown. As can be observed, the Ct values for Bolton broths, both with or without blood, were somewhat delayed compared to the Preston broths, with the exception of the 10^8 cells. It is interesting to notice that the addition of blood did not affect the amplification process, and this confirms the results reported in a previous study that had the aim of understanding the effect of blood

addition to Bolton broth on qPCR efficiency (Melero *et al.* 2011). Generally, *C. jejuni* counts of 100 cfu/g were detected in all four enrichment broths.

The results obtained by qPCR using DNA extracted from the four enrichment broths at t_6 , t_{24} and t_{48} are summarized in Table 2, where the number of positive poultry samples is shown. As can be observed, already after 6 hours of enrichment, the number of positive samples increased from 7 to an average of 10, regardless of which broth was used, with Preston offering the highest number of positive results. At 24 hours, in all the enrichments, apart from Bolton with blood, the number of samples giving a qPCR signal decreased, and then rose again at 48 h. This result has already been described by other authors (Habib *et al.* 2008; 2011) for the detection of *Campylobacter* spp. using Bolton broth. It is possible that, in these conditions, the competing microbiota outnumbers *C. jejuni* and prevents the amplification of its DNA, as previously reported by Rantsiou *et al.* (2010). In agreement with Habib *et al.* (2011), after 48 h enrichment, the number of positive *C. jejuni* samples was higher for all the broths considered in this study. A constant increase was detected in Bolton with blood and a number of 19 samples (47.5%) containing *C. jejuni* was reached. The enrichments containing blood performed better than the respective ones without its addition, a result that correlates well with a previous study by Williams *et al.* (2009) in which the presence of blood in Bolton broth improved the isolation of *Campylobacter*, when used to analyze chicken carcass rinse samples.

The number of samples showing suspected *Campylobacter* spp. colonies for each enrichment broth at the different sampling points considered in this study is reported in Table 3. Twenty nine out of 40 analyzed chicken meat samples did not show colonies with the typical *Campylobacter* spp. aspect at t_0 , while counts spanning from 10 to 10^3 cfu/g were obtained for the other 11 samples (data not shown). The colony counts between mCCDA and the Brilliance Campycount media correlated well, apart from 4 samples, for which suspected

colonies were only observed on mCCDA agar (Tab. 3). In all cases, the number of samples containing colonies with a typical aspect increased during enrichment. Colonies assumed to belong to *Campylobacter* spp. were isolated at each sampling step and identified by means of mPCR. A total of 480 isolates were collected and identified. Only 7 colonies gave a positive PCR signal: five of them were isolated without enrichment (3 from Brilliance Campycount medium and 2 from mCCDA) while 2 were isolated after 24 hours of enrichment in Bolton broth plus blood. Three colonies were identified as *C. jejuni*, while the rest were identified as *Campylobacter* spp. On the basis of these results, 4 samples were confirmed to contain *Campylobacter* spp. at t_0 , while only in 2 samples *Campylobacter* spp. were recovered during enrichment (Tab. 3).

The results obtained by means of qPCR did not compare well with the results obtained by means of traditional methods. Almost half of the samples were determined to contain *C. jejuni* through molecular methods, but this result was not confirmed by traditional plating. A high number of samples on the plates showed possible *Campylobacter* spp. colonies, however they were not confirmed to belong to the genus after mPCR. This aspect is probably correlated directly to the specificity of the selective media used in this study and the ease of recognition of the suspected *Campylobacter* colonies. While the Brilliance Campycount medium is a chromogenic agar in which campylobacters show dark red individual colonies, *Campylobacter* spp. grows on mCCDA in a grey color and in colonies of various shapes, thus making the differentiation between typical and atypical colonies difficult (Habib *et al.* 2011). Moreover, it has been stated that mCCDA allows *Escherichia coli* that produce extended-spectrum-beta-lactamase to grow (Jasson *et al.* 2009); these crowded in the plates and make the isolation of campylobacters difficult. In this study, Brilliance Campycount agar was only used for direct enumeration without enrichment, while mCCDA was also employed with enrichment. For this reason, most of the 480 colonies came from the latter selective agar.

The qPCR strategy used after enrichment accurately detected *C. jejuni* in a high percentage of poultry samples from the market, while the conventional analysis failed to detect it. This study once more highlights the differences in the performance of media used to isolate *Campylobacter* spp. from food samples. Further investigations are necessary to completely elucidate the best culturing media to use in order to be sure of detecting and quantifying *Campylobacter* spp. in poultry, although it was demonstrated here that Bolton broth added with blood was able to identify the highest number of samples containing *C. jejuni* after 48 h of enrichment.

Acknowledgements

We wish to thank Andrea Musso from the University of Turin, Prof. Anna Maraz and Dr. Agnes Belak from the Covinus University of Budapest and Wessling Hungary Ltd. Kata Rohonczy was supported during her stay at the University of Turin by a scholarship from the Federation of European Microbiological Societies (FEMS).

References

ANONYMOUS. 2008. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and foodborne outbreaks in the European Union in 2007. EFSA J. 223.

ANONYMOUS. 2010. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and foodborne outbreaks in the European Union in 2008. EFSA J. 1496.

BAYLIS, C.L., MACPHEE, S., MARTIN, K.W., HUMPHREY, T.J., and BETT, R.P. 2001. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. J. Appl. Microbiol. 89, 884-891.

CHABAN, B., MUSIL, K.M., HIMSWORTH, C.G., and HILL, J.E. 2009. Development of cpn60-based real-time quantitative PCR assays for the detection of 14 *Campylobacter* species and application to screening of canine fecal samples. Appl. Environ. Microbiol. 75, 3055-3061.

DEBRETSION, A., HABTEMARIAM, T., WILSON, S., NGANWA, D., and YEHUALAESHET, T. 2007. Real-time PCR assay for rapid detection and quantification of *Campylobacter jejuni* on chicken rinses from poultry processing plant. Mol. Cell. Probes 21, 177-181.

DENIS, M., SOUMET, C., RIVOAL, K., ERMEL, G., BLIVET, D., SALVAT, G., and COLIN P. 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett. Appl. Microbiol. 29, 406-410.

GHARST, G., OYARZABAL, O.A., and HUSSAIN, S.K. 2013. Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. J. Microbiol. Methods 95, 84-92.

JASSON, V., SAMPERS, I., BOTTELDOORN, N., LÓPEZ-GÁLVEZ, F., BAERT, L., DENAYER, S., RAJKOVIC, A., HABIB, I., DE ZUTTER, L., DEBEVERE, J., and UYTENDAELE, M. 2009. Characterization of *Escherichia coli* from raw poultry in

Belgium and impact on the detection of *Campylobacter jejuni* using Bolton broth. *Int. J. Food Microbiol.* *135*, 248-253.

JOSEFSEN, M.H., JACOBSEN, N.R., and HOORFAR, J. 2004. Enrichment followed by quantitative PCR both for rapid detection and as a tool for quantitative risk assessment of food-borne thermotolerant campylobacters. *Appl. Environ. Microbiol.* *70*, 3588-3592.

HABIB, I., UYTTENDAELE, M., and DE ZUTTER, L. 2011. Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat. *Food Microbiol.* *28*, 1117-1123.

HABIB, I., SAMPERS, I., UYTTENDAELE, M., BERKVEN, D., and DE ZUTTER, L. 2008. Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. *Appl. Environ. Microbiol.* *74*, 5483-5489.

HAYASHI, M., KUBOTA-HAYASHI, S., NATORI, T., MIZUNO, T., MIYATA, M., YOSHIDA, S., ZHANG, J., KAWAMOTO, K., OHKUSU, K., MAKINO, S., and EZAKI, T. 2013. Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food Microbiol.* *163*, 41-46.

HUMPHREY, T., O'BRIEN, S., MADSEN, M. 2007. Campylobacters as zoonotic pathogens: a food production perspective. *Int. J. Food Microbiol.* *117*, 237-257.

INTERNATIONAL ORGANISATION FOR STANDARDIZATION (ISO). 2006. ISO

10272-1 Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Enrichment Method. ISO, Geneva, Switzerland.

MELERO, B., COCOLIN, L., RANTSIOU, K., JAIME, I., and ROVIRA, J. 2011. Comparison between conventional and qPCR methods for enumerating *Campylobacter jejuni* in a poultry processing plant. *Food Microbiol.* 28, 1353-1358.

NOGVA, H.K., BERGH, A., HOLCK, A., and RUDI, K. 2000. Application of the 5'-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 66, 4029-4036.

OLIVEIRA, T.C.R.M., BARBUT, S., and GRIFFITHS, M.W. 2005. Detection of *Campylobacter jejuni* in naturally contaminated chicken skin by melting peak analysis of amplicons in real-time PCR. *Int. J. Food Microbiol.* 104, 105-111.

RANTSIOU, K., LAMBERTI, C., and COCOLIN, L. 2010. Survey of *Campylobacter jejuni* in retail chicken meat products by application of a quantitative PCR protocol. *Int. J. Food Microbiol.* 141, S75-S79.

RANTSIOU, K., ALESSANDRIA, V., URSO, R., DOLCI, P., and COCOLIN, L. 2008. Detection, quantification and vitality of *Listeria monocytogenes* in food as determined by quantitative PCR. *Int. J. Food Microbiol.* 121, 99-105.

RICHARDSON, L.J., COX, N.A., BAILEY, J.S., BERRANG, M.E., COX, J.M., BUHR, R.J., FEDORKA-CRAY, P.J., and HARRISON, M.A. 2009. Evaluation of TECRA broth,

Bolton broth, and direct plating for recovery of *Campylobacter* spp. from broiler carcass rinsates from commercial processing plants. *J. Food Prot.* 72, 972-977.

SAILS, A.D., FOX, A.J., BOLTON, F.J., WAREING, D.R.A., and GREENWAY, D.L.A. 2003. A real-time PCR assay for the detection of *Campylobacter jejuni* in foods after enrichment culture. *Appl. Environ. Microbiol.* 69, 1383-1390.

THOLOZAN, J.L., CAPPELIER, J.M., TISSIER, J.P., DELATTRE, G., and FEDERIGHI, M. 1999. Physiological characterization of viable-but-nonculturable *Campylobacter jejuni* cells. *Appl. Environ. Microbiol.* 65, 1110–1116.

UGARTE-RUIZ, M., GOMEZ-BARRERO, S., PORRERO, M.C., ALVAREZ, J., GARCIA, M., COMERON, M.C., WASSENAAR, T.M., and DOMINGUEZ, L. 2012. Evaluation of four protocols for the detection and isolation of thermophilic *Campylobacter* from different matrices. *J. Appl. Microbiol.* 113, 200–208.

VIDAL, A.B., RODGERS, J., ARNOLD, M., and CLIFTON-HADLEY, F. 2013. Comparison of different sampling strategies and laboratory methods for the detection of *C. jejuni* and *C. coli* from broiler flocks at primary production. *Zoonoses Public Hlth.* 60, 412-425.

WILLIAMS, L.K., SAIT, L.C., COGAN, T.A., JØRGENSEN, F., GROGONO-THOMAS, R., and HUMPHREY, T.J. 2012. Enrichment culture can bias the isolation of *Campylobacter* subtypes. *Epidemiol. Infect.* 140, 1227-1235.

WILLIAMS, L.K., JØRGENSEN, F., GROGONO-THOMAS, R., and HUMPHREY, T.J. 2009. Enrichment culture for the isolation of *Campylobacter* spp: effects of incubation conditions and the inclusion of blood in selective broths. *Int. J. Food Microbiol.* 130, 131-134.

YANG, C., JIANG, Y., HUANG, K., ZHU, C., YIN, Y. 2003. Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water. *FEMS Immunol. Med. Microbiol.* 38, 265-271.

Figure 1. Calibration curve of *Campylobacter jejuni*, serially diluted and inoculated in poultry meat. DNA samples were loaded in triplicate in a qPCR reaction and means and standard deviations of the C(t) values were calculated to construct the calibration curve. The correlation coefficient (R^2) is also shown.

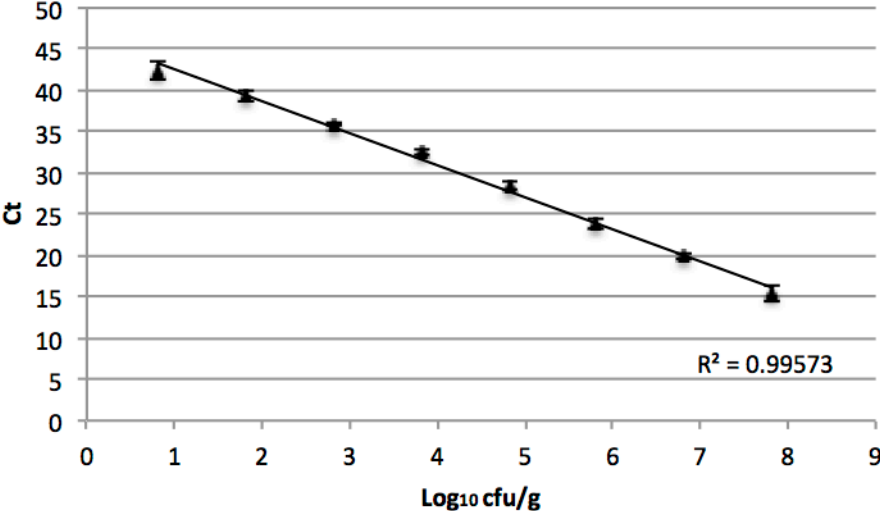


Table 1. Cycle threshold (Ct) for serially diluted *C. jejuni* cells in the four enrichment broths used in this study. The results are presented as the mean of triplicate results \pm standard deviation (SD)

Cell counts	Bolton Ct \pm SD	Bolton +blood Ct \pm SD	Preston Ct \pm SD	Preston+blood Ct \pm SD
10 ⁸	14.73 \pm 1.02	14.35 \pm 1.40	15.45 \pm 0.72	15.45 \pm 1.11
10 ⁷	20.13 \pm 0.42	20.06 \pm 0.62	17.65 \pm 1.98	17.47 \pm 0.62
10 ⁶	25.36 \pm 1.25	23.94 \pm 1.01	23.02 \pm 3.01	20.79 \pm 0.07
10 ⁵	28.63 \pm 1.00	29.26 \pm 2.03	26.74 \pm 1.43	26.41 \pm 1.67
10 ⁴	34.14 \pm 0.23	33.45 \pm 3.21	31.48 \pm 0.42	30.60 \pm 2.52
10 ³	38.74 \pm 2.02	37.50 \pm 1.42	35.54 \pm 2.02	34.49 \pm 0.43
10 ²	42.32 \pm 1.87	41.89 \pm 2.57	37.06 \pm 3.43	39.02 \pm 1.54

Table 2. Number of positive samples detected for the presence of *C. jejuni* by qPCR after 6 (t_6), 24 (t_{24}) and 48 (t_{48}) enrichment hours.

Enrichment broths	Sampling times		
	t_6	t_{24}	t_{48}
Bolton	11	7	16
Bolton with blood	10	15	19
Preston	13	8	12
Preston with blood	12	6	16

Table 3. Number of samples which show colonies with the typical *Campylobacter* spp. aspect before and after 6 (t_6), 24 (t_{24}) and 48 (t_{48}) enrichment hours for the four broths considered in this study. The number of samples that contained *Campylobacter* spp. after identification of the isolated colonies by mPCR are identified in brackets.

Sampling times			
Selective media	t_0		
mCCDA	11 (1)		
Brilliance Campycount medium	7 (3)		
Enrichment broths	t_6	t_{24}	t_{48}
Bolton	7 (0)	22 (0)	28 (0)
Bolton with blood	3 (0)	22 (2)	25 (0)
Preston	8 (0)	9 (0)	14 (0)
Preston with blood	4 (0)	9 (0)	16 (0)