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Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water

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Abstract

Campylobacter jejuni is a leading human food-borne pathogen. The rapid and sensitive detection of *C. jejuni* is necessary for the maintenance of a safe food/water supply. In this article, we present a real-time polymerase chain reaction (PCR) assay for quantitative detection of *C. jejuni* in naturally contaminated poultry, milk and environmental samples without an enrichment step. The whole assay can be completed in 60 min with a detection limit of approximately 1 CFU. The standard curve correlation coefficient for the threshold cycle versus the copy number of initial *C. jejuni* cells was 0.988. To test the PCR system, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of *C. jejuni*. 30.6% (92/300) of chicken meat samples, 27.3% (82/300) of milk samples, and 13.6% (41/300) of water samples tested positive for *C. jejuni*. This result indicated that the real-time PCR assay provides a specific, sensitive and rapid method for quantitative detection of *C. jejuni*. Moreover, it is concluded that retail chicken meat, raw milk and environmental water are commonly contaminated with *C. jejuni* and could serve as a potential risk for consumers in eastern China, especially if proper hygienic and cooking conditions are not maintained.

Keywords: Real-time polymerase chain reaction; LightCycler; TaqMan probe; *Campylobacter jejuni*; Quantitative detection

1. Introduction

Campylobacter jejuni is recognized worldwide as a leading cause of diarrhea and food-borne gastroenteritis [1,2]. This organism is carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. In most cases, the host is a carrier that does not exhibit symptoms, but it may have acquired immunity through an earlier *C. jejuni* infection [3]. *C. jejuni* cells may enter the environment, including drinking water, through the feces of infected animals, birds, or humans. Consumption of contaminated food and water with untreated animal or human waste accounts for 70% of *C. jejuni*-related illnesses each year. Unpasteurized milk, meat and poultry have the potential to serve as a source of contamination [4–7]. *C. jejuni* was predominantly associated with gastrointestinal infections. Occasional complications of the infection can be manifested as meningitis, pneumonia, miscarriage and a severe form of Guillain-Barré syndrome [3,8]. This is especially prevalent in developing countries [3].

The infective dose of *C. jejuni* cells is very small and it has been estimated that as few as 500 cells could cause human illness [9,10]. This means that even a very small number of *C. jejuni* cells in water or food present a potential health hazard. Thus, sensitive methods are needed to detect *C. jejuni* contamination in food and drinking water sources.

Unfortunately, there are several problems concerning detection of *C. jejuni* cells in food and water using culture methods. The two most important difficulties are the small numbers and the slow growth rate of the organism. The traditional methods currently used are time-consuming and laborious, requiring prolonged incubation (1–2 days) and selective enrichment to reduce the growth of background flora, and biochemical identification. Moreover, *C. jejuni* cells may also enter a viable but non-culturable (VNC) state due to starvation and physical stress [11].

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A number of polymerase chain reaction (PCR) assays have been developed for the detection of *C. jejuni* in poultry meat, milk and water samples [12–16]. Oyofo and Rollins [13] attempted to detect *C. jejuni* by PCR directly in filter-concentrated samples. This assay could detect as few as 10–100 viable *C. jejuni* cells per 100 ml of filtered sample. However, this level of sensitivity was obtained using artificially contaminated water, and the assay was not applied to the detection of *Campylobacters* in naturally contaminated samples. Waage et al. [15] detected *C. jejuni* by PCR with a level of sensitivity between 3 and 30 cells per 100 ml of water. Purdy et al. [14] used a semi-nested PCR assay to detect *Campylobacter* in 20 samples of surface water as well as 20 sewage final effluents. The result of the PCR assay was correlated with the result of a traditional culture method. In another study, a PCR enzyme-linked immunosorbent assay was applied to the detection of *C. jejuni* and *C. coli* in environmental water samples [16]. However, the methods described above needed enrichment culture. In fact, the effectiveness of PCR for quantitative detection of *C. jejuni* in naturally contaminated samples has never been established. In the present study, we have established a real-time assay targeting the 358-bp amplicon of the VS1 gene for quantitative detection of *C. jejuni* using a TaqMan probe. For this purpose we used LightCycler (Roche Molecular Biochemical, Vision 3.5), a temperature-controlled microvolume fluorimeter [17]. Naturally contaminated poultry, milk and environmental water samples in eastern China were then analyzed by the real-time PCR assay without prior enrichment.

2. Materials and methods

2.1. Bacterial isolates and media

Eleven isolates of *C. jejuni*, including the *C. jejuni* type strain *C. jejuni* ATCC 33560 (American Type Culture Collection), were used to test the specificity of the primers and probe. The isolates were collected mostly from poultry in China between 1996 and 1998. The bacterial strains used as negative controls were as follows: *C. coli* (ATCC 33559), *C. lari* (ATCC 358221), *C. fetus* (ATCC 27374), *Escherichia coli* (ATCC 25922), *Helicobacter canis* (NCTC 12739^T, National Collection of Type Culture), *Arcobacter butzleri* (NCTC 12481^T), *Helicobacter hepaticus* (ATCC 51448^T). The medium used was *Campylobacter* selective agar base (Merck, CM02248, Germany). Medium was supplemented with *Campylobacter* selective supplement (Merck, CM02249, Germany), defibrinated sheep blood, and *Campylobacter* enrichment broth (Bolton formula, Oxoid, CM0983). Oxoid Bolton *Campylobacter* enrichment broth was prepared using the defibrinated sheep blood, Oxoid Bolton enrichment broth base, and selective supplement according to the manufacturer's instructions. *A. butzleri* and *E. coli* were grown aerobically on Oxoid Bolton enrichment broth without *Campylobacter* selective supplement at 37°C. *Helicobacter* spp. were grown microaerobically on Oxoid Bolton enrichment broth without *Campylobacter* selective supplement at 37°C.

2.2. Poultry, water and milk samples

Three hundred frozen chicken meat samples were purchased from different local supermarkets in eastern China. Each chicken piece, in its original package, was placed in an individual sterile polyethylene bag and transferred to the laboratory in a cool box within 1 h of purchase. The cool box was maintained at 3–5°C. Upon arrival at the lab, each piece was immediately placed in a separate sterile plastic bag using fresh, sterile disposable gloves, and stored at 4°C before being analyzed within 24 h. Three hundred raw milk samples were collected from different dairy farms and 300 environmental water samples, including surface water and ground water, were collected (2 l per sample) in eastern China for analysis. Upon collection, 5 ml of 1 M sodium thiosulfate was added per liter of water sample. The samples were collected in sterile 3-l bottles, transported to the laboratory at ambient temperature, and stored at 4°C before being analyzed within 24 h.

2.3. Microscopic analysis of cell morphology and biochemical tests for isolation and identification of C. jejuni

The isolation procedure for *C. jejuni* from meat, milk and water was carried out based on the procedure described by Hunt and Abeyta [18,19]. Briefly, 200 ml of 0.1% sterile peptone water was added to the polyethylene bags containing individual chicken meat. The sealed bags were hand massaged for 3 min. The rinse solution was subsequently filtered through sterile cheesecloth followed by centrifugation at 16 000 × *g* for 15 min at 4°C. The supernatant fraction was discarded and the pellet suspended in 12 ml of 0.1% peptone water. For enrichment, 3 ml of the suspended pellet was inoculated into 100 ml of enrichment broth followed by incubation microaerobically (85% nitrogen, 10% oxygen, and 5% carbon dioxide) at 30°C for 3 h with agitation (150–200 rpm on a Model 50 water bath [Precision Sc., Winchester, VA, USA]). The incubation was then continued at 37°C for 2 h with agitation followed by a 24-h enrichment period. For the enrichment step, the cultures were transferred to a 42°C microaerobic incubator and incubated statically.

Raw milk samples were tested at the collection site. A sterile pipette was used to place a test portion onto pH test paper (pH range of 6–8). If the pH was below 7.6, sterile 1 N NaOH was added to adjust it to 7.5 ± 0.2. Aliquots of 50 g were centrifuged at 20 000 × *g* for 40 min. The supernatant was discarded and the pellet (not fat layer) sus-

pended in 10 ml enrichment broth. It was then transferred to 90 ml enrichment broth. This was followed by a 24-h enrichment step in which the cultures were transferred to a 42°C microaerobic incubator and incubated statically.

Water samples (2 l) were filtered through 45-μm positively charged Zetapor filters (47 mm diameter). As a result of opposite charge attraction, the negatively charged Gram-negative organisms were more effectively retained in the filter. The filter was then soaked in 100 ml enrichment broth such that the broth covered the filter. Another sterile filter was placed in the unit and the filtration process continued. For each sample one filter was used, and in the event of clogs it was removed with sterile gloves and forceps. As *Campylobacter* is very sensitive to dry conditions and high salt concentration, care was taken to prevent filters from drying out during the process. The filter was then transferred to broth, immediately followed by a 24-h enrichment in a 42°C microaerobic incubator (static incubation).

Aliquots of 50 μl enriched sample were streaked onto *Campylobacter* selective agar and incubated microaerobically at 42°C for 24–48 h. The resultant colonies were examined visually, and at least one presumptive *Campylobacter* sp. colony per plate was selected for further testing. Colonies that appeared as curved, gull wing-shaped, Gram-negative rods were tentatively identified as *Campylobacter* spp. and streak purified onto Merck agar medium. Catalase and oxidase tests were performed, and isolates with appropriate reaction were identified to the species level using API Campy strips (Biomerieux, Marcy l'Etoile, France).

2.4. DNA extraction for the real-time PCR

Real-time PCR analysis was performed without an enrichment step. A short treatment procedure for the contaminated poultry, milk and environmental water samples was carried out as described above. Template DNAs were extracted from pre-enrichment cultures using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA) without prior enrichment. Alternatively, control strain DNAs were extracted from pure cultures. Finally, DNA concentration was determined with a spectrophotometer and the DNA samples stored at 2–8°C.

2.5. Design of primers and probe

Oligonucleotide primers VS15 and VS16 [20] from the *C. jejuni* VS1 sequence (GenBank accession number X71603) were used in the real-time PCR assay. The primer sequences were as follows: VS15, 5′-GAATGAAATTTTAGAATGGGG-3′ and VS16, 5′-GATATGTATGATTTTATCCTGC-3′. The TaqMan probe was designed by the Primer Express Software (Version 1.0, PE Biosystems) based on nucleotide sequence between two primers. The VS gene and probe sequences were aligned with other sequences reported in GenBank using CLUSTAL W (Version 1.8). The probe sequence was as follows: 5′-TTTAACTTGGCTAAAGGCTAAGGCT-3′. The probe was 5′ end-labelled with 6-carboxyfluorescein (FAM) and 3′ end-labelled with 6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine (TAMRA). The theoretical T_m values of 0.5 mM of primer VS15 and primer VS16 when reconstituted in 50 mM monovalent cations were calculated to be 62°C and 66.8°C, respectively, with the optimal PCR annealing temperature calculated to be 56°C. The theoretical T_m of the probe under the same condition was 73°C. The T_m of the fluorogenic probe was approximately 5–7°C higher than that of the amplification primers, enabling the probe to hybridize with the target region of the amplicon during the annealing/extension stage of each PCR cycle. The primers and probe were synthesized by TaKaRa Biotechnology (Dalian) and by Shanghai Shengyou, respectively.

2.6. Real-time PCR

Amplification reactions (20 μl) contained 2 μl DNA sample, 1×TaqMan Buffer (Mg^{2+}-free), 5 mM $MgCl_2$, 200 μM dATP, dCTP, and dGTP, 400 μM dUTP, 0.1 μM *C. jejuni*-specific probe, 0.4 μM of each *C. jejuni*-specific primer, 0.5 U uracil-*N*-glycosylase, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). Capillary type reaction tubes were used (Roche). Before amplification, the PCR mixture was heated to 95°C for 10 min to denature the template DNA. The amplification profile was as follows: 45 cycles of 95°C for 1 s, 56°C for 20 s, and 72°C for 30 s. The temperature transition rate was 20°C s^{-1}. The program set-up for cooling the instrument at the end of the run was 40°C for 30 min. Acquisition mode was single, set at the end of the elongation stage. Reactions and data analysis were performed in the LightCycler System (Roche Molecular Biochemical, Vision 3.5). PCR products were detected directly by monitoring the increase in fluorescence from the dye-labelled *C. jejuni*-specific DNA probe, which resulted in an increase in FAM fluorescence emission (F1) with a concomitant decrease in TAMRA emission (F2), and was measured as a ratio of F1/F2. The size of the PCR product was verified on ethidium bromide-stained 2% agarose gels in Tris acetate–EDTA buffer. DL 2,000 Marker (TaKaRa) was used as a size marker.

3. Results

3.1. Sensitivity and detection limits of real-time PCR using TaqMan

Serial dilutions of purified DNA were prepared and a CFU standard curve was constructed. DNA isolated from approximately 1×10^4 *C. jejuni* cells was serially 10-fold diluted in 1×TE buffer and subjected to PCR. As shown

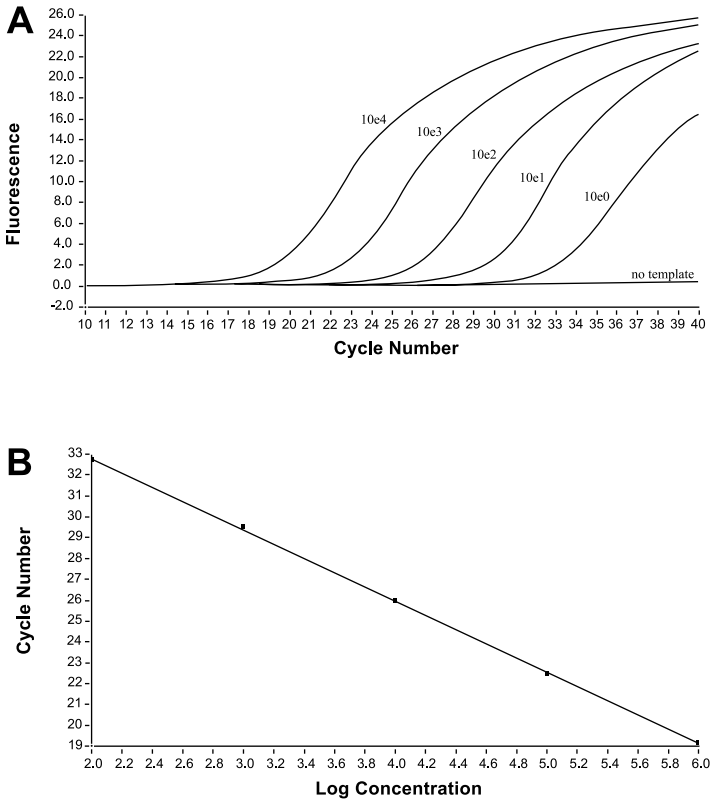

Fig. 1. A: Application curve for serial 10-fold dilutions of *C. jejuni* DNA in LightCycler. Reactions (10e4 to 10e0) contained 1.0×10^4, 1.0×10^3, 1.0×10^2, 10, and 1.0 CFU, respectively, or no template. B: Standard curve for serial 10-fold dilutions of *C. jejuni* DNA. Quantification was performed by determining the threshold cycle (C_t). C_t was plotted against the calculated copies of bacterial DNA, i.e., a 10-fold dilution of the bacterial DNA (1.0×10^4 copies in 2 μl). The straight line, which was calculated by linear regression [$C_t = -3.417$ log (number of concentration)+34.63], shows an R^2 of 0.988.

in Fig. 1A, there were 1.0×10^4, 1.0×10^3, 1.0×10^2, 10, 1.0 CFU, and no template, respectively, in the application curve for these serial dilutions of *C. jejuni* DNA. As shown in Fig. 1B, the standard curve based on the dilutions of DNA showed a linear relationship between log CFU and threshold cycles (C_t). The slope of the curve was −3.417, and the R^2 after the linear regression was 0.988. When a new serial 10-fold dilution series from the same DNA purification was used in a separate PCR experiment, the slopes in the two runs were almost identical (−3.389 vs. −3.407). When DNA from a separate isolation was used, the variation in the slopes of the standard curves was no larger than that between different serial dilutions from the same DNA isolation and the R^2 remained constant. The DNA standard curves showed a higher degree of variability among the triplicates when the amount of template decreased. DNA standard curves showed that the detection limit of the PCR assay was approximately 1 CFU per PCR from pure culture (Fig. 1B). The number of *C. jejuni* cells can be described by the following equations: $C_t = -3.417$ log CFU+34.63 and $\mathrm{CFU} = 10^{[(C_t-34.63)/-3.417]}$. Based on serial dilutions of purified DNA from *C. jejuni* in chicken meat, milk and water, the sensitivity for detecting *C. jejuni* in these sources was found to be 6–15 CFU per PCR.

3.2. Specificity of primers and probe

Specific PCR primers and probe were designed for *C. jejuni*. The probe region was chosen to optimize specificity and amplification efficiency. The 358-bp DNA fragment and the probe were subjected to homology searches, which revealed the primers and probe to be specific to this organism. The specificity of the primers and probe was subjected to an empirical screening in which a total of 11 *C. jejuni* isolates, including the type strain, were found

Table 1
Results of detection for *C. jejuni* in poultry, water and milk by real-time PCR and culture method

Samples (No. of samples)	No. of positive samples		Positive ratio (%)	
	PCR	Culture method	PCR	Culture method
Chicken breast (90)	27	21	30	23.3
Drumstick (60)	16	13	26.7	21.7
Gizzard (60)	22	16	36.7	26.7
Chicken heart (30)	6	6	20	20
Chicken wing (60)	21	21	35	35
Surface water (150)	29	18	19.3	12
Ground water (150)	12	6	8	4
Milk (300)	82	78	27.3	26

to respond positively. The specificity of the primers and probes was also tested against three strains of other *Campylobacter* species as well as a set of four species belonging to other genetically related or common food-borne organisms/pathogens (see Section 2), all of which were found to be negative. In addition, a qualitative PCR with the amplification primers was performed for the selected strains. The results confirmed that the amplification primers and probe were specific to *C. jejuni*. PCR amplicons were analyzed on 2% agarose gels. The results showed a fragment of the expected length of 358 bp for *C. jejuni*. Moreover, no non-specific PCR products were detected.

3.3. Screening market samples by real-time PCR and API Campy identification system

As an application of the real-time PCR system and API Campy identification system, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of *C. jejuni*. As shown in Table 1, for real-time PCR detection, 30.6% (92/300) of chicken meat samples, 27.3% (82/300) of milk samples, and 13.6% (41/300) of water samples were found to be positive for *C. jejuni*. Using the API Campy system, 25.6% (77/300) of chicken meat samples, 26% (78/300) of milk samples, and 8% (24/300) of water samples were found to be positive for *C. jejuni*. The results for *C. jejuni* detection in poultry, water and milk by real-time PCR and culture methods showed significant statistical differences ($r = 0.64$, $P < 0.05$). All culture-positive samples were real-time PCR-positive as well; however, 36 samples positive by PCR were culture-negative. Quantitative results for *C. jejuni* in chicken meat, milk and water by real-time PCR are presented in Table 2.

4. Discussion

4.1. Specific detection and quantification of C. jejuni

C. jejuni is recognized as a leading human food-borne pathogen. There is a requirement for rapid, quantitative and accurate detection of the organism. In the present study, a real-time PCR system was devised and applied to specifically detect and quantify *C. jejuni*. Colonization and infection appear to be dependent on motility and full-length flagella. In this regard, the flagellum gene *flaA* appears to be essential [21]. However, the variation between the different strains is too extensive; the *flaA* gene is unsuitable for design of the primers and probe necessary for a real-time PCR assay [11]. A VS gene region (GenBank accession number X71603) comprising an 1189-bp DNA fragment was isolated from a *C. jejuni* CIP 70.2 (Collection de l'Institut Pasteur, France) cosmid library and found to be specific to this organism [20]. Oligonucleotide primers VS15 and VS16 from the *C. jejuni* VS1 sequence were used in the real-time PCR assay in the present study. The TaqMan probe for the real-time PCR was designed by Primer Express (version 1.0, PE Biosystems). The specificity of the probe was tested by homology searches of nucleotide databases (GenBank). When this assay was applied, it was positive for all isolates of *C. jejuni* tested (11 isolates, including type strain ATCC 33560) and negative for all other *Campylobacter* spp. (three isolates) and several other bacteria (four species tested). This demonstrates the high specificity of this primer–probe set. Furthermore, the amplification primers were specific for *C. jejuni*, avoiding potential artifacts in a mixed population.

The R^2 after the linear regression indicated a good correlation between the amount of template (log CFU) and the amount of product (represented by C_t) in the standard curves ($R^2 = 0.988$). The linearity of the standard curves and the fact that the PCR operates with constant efficiency confirmed that the assay was well suited to quantitative measurements of *C. jejuni*. The detection limit of the present PCR assay was estimated to be approximately 1 CFU per PCR from bacterial culture. However, the sensitivity for detecting *C. jejuni* in chicken meat, milk and water was 6–15 CFU per PCR. These indicated the possible presence of inhibitory substances in these samples. Our limit of detection is similar to those reported by others using a fluorogenic 5′-nuclease PCR assay for endpoint detection. Bassler et al. obtained a detection level for *Listeria monocytogenes* of approximately 50 CFU per PCR [22], while Chen et al. showed a detection limit of as low as 2 CFU per PCR from a pure culture of *Salmonella enterica* serovar Typhimurium [23].

Table 2
Quantitative detection of *C. jejuni* in poultry, water, milk by real-time PCR

Sample type	No. of positive samples	Amount (CFU ml^{-1})[a]
Chicken breast	27	$5.0\times10^8 \pm 3.2\times10^6$
Drumstick	16	$4.6\times10^7 \pm 1.8\times10^4$
Gizzard	22	$3.4\times10^6 \pm 2.9\times10^4$
Chicken heart	6	$3.6\times10^6 \pm 5.1\times10^4$
Chicken wing	21	$2.3\times10^6 \pm 2.7\times10^5$
Surface water	29	$1.9\times10^7 \pm 3.1\times10^4$
Ground water	12	$2.3\times10^5 \pm 3.9\times10^3$
Milk	82	$6.4\times10^7 \pm 5.3\times10^5$

[a] Mean ± S.D.

4.2. Comparison of real-time PCR and culture method for detection of C. jejuni in market samples

As an application of the real-time PCR and culture methods, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of *C. jejuni*. The results from the two methods of study supported the fact that retail chicken meat, raw milk and environmental water are commonly contaminated with *C. jejuni* in eastern China. In China, there is little chance for people to be infected with *C. jejuni* directly

through consumption of poultry and milk because they do not normally consume these in the raw state. But these raw foods can contaminate cooked food. Many people in China may be infected with *C. jejuni* through drinking contaminated ground water. If proper hygienic and cooking conditions are not maintained, these foods present a potential risk to consumers.

Our reported positive ratio of detection was similar to those of other reports in China [24,25]. But the positive ratio of real-time PCR detection was higher than for the culture method. All culture-positive samples were real-time PCR-positive; however, 36 samples that were PCR-positive were culture-negative. DNA extracted from samples was used as template in the PCR assay. These samples may have contained dead or sublethally damaged cells, which were present at a high enough concentration for the *C. jejuni* DNA to be detected in the real-time PCR assay, although they could not be recovered by enrichment culture methods. *C. jejuni* has been shown to enter a VNC state in water due to starvation and physical stress, with the cells not being recoverable using conventional enrichment culture methods. This may explain the failure of culture techniques to isolate the organisms from contaminated water samples implicated in outbreaks of infection [26,27]. The numbers of viable *C. jejuni* could decline more rapidly at higher temperature or if the sample was frozen. In the case of water, survival can also be affected by sunlight. Some of the discrepant results (PCR-positive but culture-negative) reported in this study may have arisen from the detection of VNC and dead/sublethally damaged cells of *C. jejuni* present in the samples in combination with the improved sensitivity of real-time PCR.

As shown in Table 2, an apparently high number of *C. jejuni* was detected by the real-time PCR method in the three substrates examined. These numbers refer to the concentrated suspensions obtained after centrifugation or filtration. As shown in Table 3, comparison of the numbers of *C. jejuni* detected by PCR in the culture-negative compared to the cultural-positive samples indicates that the very high numbers of *C. jejuni* detected by real-time PCR were probably either non-viable or VNC cells. These dead cells could not pose a risk to human health, but do indicate that this food or water was previously contaminated with *C. jejuni*. Therefore, quantitative detection systems are crucial to estimate the possibility of *C. jejuni* contamination in food and water samples, and such risk assessments are important for future legislative work.

Table 3
Comparisons between culture-negative and culture-positive samples among real-time PCR-positive samples for detection of *C. jejuni*

Sample type	Amount detected by real-time PCR (CFU ml^{-1})[a]	
	Culture-negative sample	Culture-positive sample
Chicken frozen meat	$3.4\times10^5 \pm 1.8\times10^4$	$4.3\times10^7 \pm 2.9\times10^6$
Milk	$1.3\times10^4 \pm 7.3\times10^2$	$7.3\times10^7 \pm 3.8\times10^5$
Water	$4.3\times10^3 \pm 3.7\times10^2$	$6.4\times10^6 \pm 2.7\times10^4$

[a] Mean ± S.D.

4.3. *Advantages and potential application of real-time PCR detection of* C. jejuni

The real-time quantitative PCR assay demonstrates several advantages over other conventional PCR approaches. The fluorogenic assay is a convenient, self-contained process. The only necessary steps are the reaction set-up and the tube sealing. Unlike other quantitative PCR methods, real-time PCR does not require post-PCR sample handling, thus avoiding potential PCR product carryover contamination. This enables the assays to be conducted much faster and in a high-throughput format. The real-time PCR method has a very large dynamic range of starting target molecule determination. Moreover, this real-time method is extremely accurate and is less labor-intensive than methods such as the immunocapture PCR assay [28].

Adaptation of real-time PCR for quantification of *C. jejuni* in naturally contaminated foods is feasible [29]. Choosing proper protocols for the isolation of bacterial DNA/cells is an important factor. A possible approach may be the use of magnetic beads for specific isolation of the bacteria, followed by DNA isolation while the bacteria are still attached to the beads [11]. Real-time PCR may be particularly useful both for routine screening for *C. jejuni* contamination and for large-scale screening during outbreaks.

In summary, the data presented here showed that the designed TaqMan probe was suitable for use in the LightCycler-based real-time PCR assay for identification of *C. jejuni*. A set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of *C. jejuni*. The results indicated that retail chicken meat, raw milk and environmental water are commonly contaminated with *C. jejuni* and could serve as a potential risk for consumers in eastern China. This is especially true if hygienic and cooking considerations are not adequately applied. The real-time PCR assay provides a specific, sensitive and rapid method for quantitative detection of *C. jejuni* in naturally contaminated poultry, milk and environmental water without a need for enrichment.

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