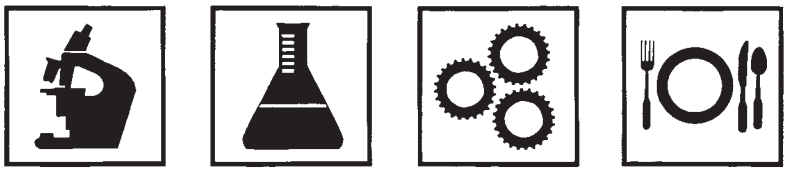
Journal of Food Protection



# Comparison of Real-Time PCR Assays for Detection, Quantification, and Differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Broiler Neck Skin Samples†

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*Journal of Food Protection, Vol. 73, No. 6, 2010, Pages 1057–1063*


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MS 09-461: Received 30 October 2009/Accepted 19 February 2010

## ABSTRACT

We tested the use of multiplex real-time PCR for detection and quantification of *Campylobacter jejuni* and *Campylobacter coli* on broiler carcass neck skin samples collected during 2008 from slaughterhouses in Switzerland. Results from an established TaqMan assay based on two different targets (*hipO* and *ceuE* for *C. jejuni* and *C. coli,* respectively) were corroborated with data from a newly developed assay based on a single-nucleotide polymorphism in the *fusA* gene, which allows differentiation between *C. jejuni* and *C. coli*. Both multiplex real-time PCRs were applied simultaneously for direct detection, differentiation, and quantification of *Campylobacter* from 351 neck skin samples and compared with culture methods. There was good correlation in detection and enumeration between real-time PCR results and quantitative culture, with real-time PCR being more sensitive. Overall, 251 (71.5%) of the samples were PCR positive for *Campylobacter,* with 211 (60.1%) in the *hipO–ceuE* assays, 244 (69.5%) in the *fusA* assay, and 204 (58.1%) of them being positive in both PCR assays. Thus, the *fusA* assay was similarly sensitive to the enrichment culture (72.4% positive); however, it is faster and allows for quantification. In addition, real-time PCR allowed for species differentiation; roughly 60% of positive samples contained *C. jejuni,* less than 10% *C. coli,* and more than 30% contained both species. Real-time PCR proved to be a suitable method for direct detection, quantification, and differentiation of *Campylobacter* from carcasses, and could permit time-efficient surveillance of these zoonotic agents.

*Campylobacter* spp. are the most common bacterial cause of human foodborne infection in Europe and in many other countries *(9)*. In 80 to 85% of human campylobacteriosis, *C. jejuni* is the causative species and *C. coli* accounts for 10 to 15% of cases, while other species like *C. upsaliensis, C. fetus,* and *C. lari* are rarely associated with human infections *(19)*.

Because *Campylobacter* is ubiquitous, and the infectious dose of a few hundred organisms is relatively low, it is an important public health concern *(2, 3)*. A main infection source for human campylobacteriosis is food of animal origin; consumption of undercooked, and handling of, raw poultry are regarded as the most important risk factors *(19, 22)*. Given that *C. jejuni* and *C. coli* are commensals in the avian gut, contamination of carcasses directly or by equipment, working surfaces, or water can occur because of automated slaughtering and processing *(13)*.

Conventional culture method is commonly used for the detection of *C. jejuni* and *C. coli (11–13)*. This method is time-consuming, and several days might be needed until first results become available. Misleading results on the level of phenotypic discrimination between different species are also possible. Differentiation between *C. jejuni* and *C. coli* is based on a positive hippurate hydrolysis for *C. jejuni*; however, not all *C. jejuni* strains are positive in this reaction *(6)*. For these reasons, other detection and identification methods (mainly on a genetic basis) have been established. Previously described real-time PCR approaches were aimed at detection of *C. jejuni (5, 21, 25, 27), C. jejuni* and *C. coli (1, 10, 15, 24),* or different species of *Campylobacter (17)*. They were evaluated by using samples of different origin, for example, chicken rinses *(5, 10),* chicken feces *(17),* food *(25, 27),* environmental sources *(24),* or human feces *(15, 16)*. Variation between *Campylobacter*-specific PCRs utilizing different targets is known *(4),* and therefore selection of a single PCR might well influence the outcome of results when determining prevalence of *Campylobacter*. Thus, we established an alternative, single-gene–based real-time PCR to be applied for direct detection and simultaneous identification of *C. jejuni* and *C. coli* on chicken neck skin samples and compared it with the one of Hong et al. *(10),* the latter seemingly the most suitable for that purpose. Both were evaluated concerning their performance by using type and field strains, and they were applied further in a study on the presence of *Campylobacter* on broiler neck skin. In parallel, generated data on the quantitative and qualitative presence of *Campylobacter,* as judged by colony counting and enrichment culture, were taken to assess the real-time PCR results. The newly developed single-gene, real-time PCR for *C. jejuni* and *C. coli* was compared with an established one, and both were further evaluated for direct detection, quantification, and differentiation of these two species from chicken neck skin samples.

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† This article is published in memory of Andrea Schnider who died on 8 March 2010.

## MATERIALS AND METHODS

**Bacterial strains.** Performance of the real-time PCR was compared with the *Helicobacter pullorum* type strain CCUG 33837$^T$ and 14 *Campylobacter* type strains. They included *C. jejuni* subsp. *jejuni* (CCUG 11284$^T$), *C. jejuni* subsp. *doylei* (LMG 8843$^T$), *C. coli* (CCUG 11283$^T$), *C. fetus* subsp. *fetus* (ATCC 27374$^T$), *C. fetus* subsp. *venerealis* (NCTC 10354$^T$), *C. lari* (CCUG 23947$^T$), *C. hyointestinalis* (CCUG 14169$^T$), *C. lanienae* (NCTC 13004$^T$), *C. mucosalis* (ATCC 43264$^T$), *C. upsaliensis* (CCUG 14913$^T$), *C. showae* (CCUG 30254$^T$), *C. concisus* (ATCC 33237$^T$), *C. helveticus* (ATCC 51209$^T$), and *C. sputorum* (LMG 7795$^T$). Strains were cultured on tryptone soy agar with 5% sheep blood (TSA; Oxoid, Pratteln, Switzerland) at 37°C, under microaerobic conditions (5% $O_2$, 5% $CO_2$, 80% $N_2$, and 10% $H_2$) for 48 h. DNA was extracted with the peqGOLD Bacterial DNA Kit (PEQLAB Biotechnologie GmbH, Axonlab, Baden, Switzerland), as recommended by the manufacturer, performing just one elution step with 200 μl of distilled water. The final DNA concentration ranged from 0.1 to 1.6 μg/ml.

Furthermore, DNA from 100 previously phenotypically and genetically well-characterized *Campylobacter* field strains (50 of each *C. jejuni* and *C. coli*) was used to compare specificity of the real-time PCR approaches *(14)*. These strains originated from various hosts including poultry, pig, human, cattle, cat, and dog, and the strains were selected to represent phylogenetically the types most different as determined by multilocus sequence typing and *flaA* sequencing *(14)*.

**Broiler neck skin sampling and sample preparation.** Samples were collected in the framework of a European Union (EU) baseline study on the prevalence of *Campylobacter* in broiler flocks *(7)*. Neck skins from carcasses of different flocks were sampled every week during 2008 at five slaughterhouses in Switzerland, which together slaughter more than 80% of domestic broiler production. Three hundred fifty-one samples were available for this study. Neck skin was taken from a freshly slaughtered broiler, at least one sample per flock. The samples were sent cooled in a sterile plastic container to the laboratory and were processed no later than 72 to 80 h after sampling. In the laboratory, 27 g of neck skin was transferred into a sterile plastic bag, and 243 ml of buffered peptone water (BPW; Oxoid) was added, resulting in a 1:10 (wt/vol) dilution. The content was then stomached for 1 min in a Stomacher 400 circulator (Seward, Ltd., West Sussex, UK). The remaining broiler neck skins and a 1.2-ml aliquot of the stomached BPW sample were stored at −20°C.

For qualitative detection of thermotolerant *Campylobacter* by a conventional culture method, according to International Organization for Standardization (ISO) technical specification 10272-1 *(11),* 10 ml of the stomached BPW sample was added to 90 ml of Bolton broth with 5% laked horse blood and modified Bolton selective supplement (Oxoid), incubated in microaerobic conditions at 37°C for 4 to 6 h, and then at 41.5°C for 40 to 48 h. A loopful of each enriched sample was streaked onto modified charcoal-cefoperazone-desoxycholate agar (mCCDA; Oxoid) as well as a Karmali plate (Oxoid) and incubated at 41.5°C for 40 to 48 h, under microaerobic conditions. Presumptive-thermotolerant *Campylobacter* colonies were subcultured on TSA and incubated microaerobically at 41.5°C for 24 to 48 h. Isolates were confirmed by microscopy, by a positive oxidase reaction, and the absence of growth on TSA after 40 to 44 h of aerobic incubation at 41.5°C as well as microaerobic growth at 25°C. To distinguish the *Campylobacter* spp., the following tests were performed: detection of catalase, sensitivity to nalidixic acid, resistance to cephalothin, hydrolysis of indoxyl acetate, and hippurate hydrolysis *(11, 20)*.

For quantitative determination by conventional culture method, according to ISO technical specification 10272-2 *(12),* 1 ml of the BPW sample was equally distributed and plated on three standard-sized mCCDA plates. This was done in duplicate. Further, 0.1 ml of each dilution step from a 10-fold dilution series down to $10^{-3}$ was plated on an mCCDA plate. Plates were incubated at 41.5°C for 40 to 48 h, under microaerobic conditions. Colonies were counted on plates where fewer than 150 suspected *Campylobacter* colonies had grown. Five colonies were subcultured for confirmation of *Campylobacter,* without differentiating the species, if qualitative detection gave a positive result.

**DNA isolation and lysate preparation from broiler neck skin.** A DNA extraction done with the QIAamp DNA Mini Kit (QIAGEN, Hombrechtikon, Switzerland) was performed on 200 μl of the stomached BPW neck skin samples, according to the manufacturer's recommendation, except that for elution, 50 μl of distilled water was used. Isolated DNA was stored at −20°C.

For direct detection of *Campylobacter,* remaining batches of broiler neck skin material were defrosted at room temperature and rinsed with 1 to 2 ml of 0.9% NaCl by applying a sterile syringe plug to squeeze and mix the sample. One milliliter of liquid (meat juice) was pipetted off, transferred to a 1.5-ml Eppendorf tube, and centrifuged for 15 s at 400 × *g* to sediment large particles. Two hundred microliters of meat juice supernatant was used for DNA isolation, utilizing the QIAamp DNA Mini Kit, with an elution volume of 50 μl of distilled water (resulting in a fourfold concentration). Isolated DNA was stored at −20°C. From the meat juice supernatant, a lysate was prepared as well. Lysis buffer contained 100 mM Tris HCl (pH 8.5), 0.05% Tween 20, and 240 μg/ml proteinase K in pyrogen-free water. Fifty microliters of juice was mixed with 50 μl of lysis buffer (resulting in a twofold dilution) and incubated in a thermomixer for 1 h at 60°C, and then for 15 min at 95°C. Lysates were stored at −20°C.

**Real-time PCR.** The previously described multiplex real-time PCR of Hong et al. *(10)* for the detection of *C. jejuni* and *C. coli,* which was based on the *hipO* and *ceuE* genes, respectively, was compared with the newly developed assay based on the housekeeping gene *fusA* (encoding elongation factor G). Primers and probes (Table 1) for the new approach were designed with Primer Express 3.0 (Applied Biosystems, Foster City, CA). The probes for *fusA* differ by one nucleotide; thus, *C. jejuni* and *C. coli* were discriminated by a single-nucleotide polymorphism (SNP). Probes were labeled with the fluorescent reporter dyes FAM and NED for *C. jejuni* and *C. coli,* respectively, and quenched by a minor groove binder–nonfluorescent quencher (MGB). Primers were synthesized by Microsynth (Balgach, Switzerland) and probes by Applied Biosystems and were stored aliquoted at −20°C. The same fluorescent labels were chosen for the published PCR *(10),* i.e., FAM for the *C. jejuni*–specific *hipO* probe and NED for the *C. coli*–specific *ceuE* probe (Table 1).

Each assay was carried out as duplex real-time PCR, in triplicate, with a 25-μl volume containing 1× TaqMan Universal PCR Master Mix, No AmpErase UNG (both from Applied Biosystems), 300 nM of each primer, and 200 nM each MGB probe. For the SNP-based *fusA* assay, TaqMan Genotyping Master Mix (Applied Biosystems) was used to guarantee optimal performance. Samples were run and analyzed on an ABI 7500 Real-Time PCR System (Applied Biosystems) with the 7500 software system, version 1.2.3 (Applied Biosystems). Standard thermal cycling conditions were used, which began with a step at 50°C for 2 min and one at 95°C for 10 min, and continued with 40 cycles of 95°C for 15 s and 60°C for 1 min. A threshold of 0.02

TABLE 1. *Primers and probes for real-time PCR*

| Target genes | Primers and probes | Sequence (5′→3′) |
|---|---|---|
| *hipO* (*Campylobacter jejuni*) | HipO-F | CTGCTTCTTTACTTGTTGCTT |
| | HipO-R | GCTCCTATGCTTACAACTGCTGAAT |
| | HipO-P | FAM-CATTGCGAGATACTATGCTTTG-MGBNFQ |
| *ceuE* (*Campylobacter coli*) | CeuE-F | GATAAAGTTGCAGGAGTTCCAGCTA |
| | CeuE-R | AACTCCACCTATACTAGGCTTGTCT |
| | CeuE-P | NED-CTGTAAGTATTTTGGCAAGTTT-MGBNFQ |
| *fusA* (*C. jejuni, C. coli*) | Ccj_fusA-L1 | GCCTTGAGGAAATTAAAACTGGTATT |
| | Ccj_fusA-L2 | GCCTTGAAGAGATTAAAACAGGGATT |
| | Ccj_fusA-R1 | TTTAAATGCAGTTCCACAAAGCA |
| | Ccj_fusA-R2 | TTTAAACGCTGTACCGCAAAGCA |
| | Cj_fusA-probe[a] | FAM-AAGTCTTTCTATCGTTCC-MGBNFQ |
| | Cc_fusA-probe[a] | NED-AAGTCTTTCTATTGTTCC-MGBNFQ |

[a] SNP is underlined.

was applied in the analysis for all reactions. A positive result was defined if at least two of the triplicate samples showed a cycle threshold ($C_t$) value less than 40. A negative result was defined if at least two of the triplicate samples had a $C_t$ value of 40. To assess possible PCR inhibition, all samples were tested in parallel with an internal positive control (TaqMan Exogenous Internal Positive Control, Applied Biosystems).

## RESULTS

**Comparison of real-time PCR assay performance.** To compare specific performance of the two real-time PCR approaches for *C. jejuni* and *C. coli,* 15 different type strains as well as 50 *C. jejuni* and 50 *C. coli* field strains were tested. Both multiplex real-time PCRs correctly identified type strains of *C. jejuni* and *C. coli*. With the same amount of undiluted *C. jejuni* type strain CCUG 11284$^T$ DNA (4 ng per reaction), the mean $C_t$ values from triplicate reactions were 18.8 for *hipO* and 16.2 for Cj_*fusA*. With undiluted *C. coli* type strain CCUG 11283$^T$ DNA (1.25 ng per reaction), the mean $C_t$ values from triplicate reactions were 17.7 for *ceuE* and 18.7 for Cc_*fusA*. The Cj_*fusA* reaction spuriously amplified undiluted *C. lanienae* type strain DNA, with a $C_t$ value of 38.7 by using 0.4 ng of DNA in the reaction (corresponding to about $4 \times 10^5$ copies), and with the Cc_*fusA* assay, undiluted *C. hyointestinalis* subsp. *hyointestinalis* type strain DNA amplified with a $C_t$ value of 39.5 by using 3.4 ng of DNA in the reaction (corresponding to about $2 \times 10^6$ copies). All other species tested remained negative after 40 cycles with undiluted genomic DNA.

All 100 *C. jejuni* and *C. coli* field strains were correctly identified by the *hipO–ceuE* assay, with $C_t$ values between 2.4 and 27.7, reflecting different DNA concentrations of the samples. Real-time PCR based on *fusA* detected accurately 99 of the field strains, with $C_t$ values ranging from 4.9 to 31.4. One of the *C. coli* isolates was identified as *C. jejuni* by the *fusA* assay.

To compare the dynamic range of the two PCR assays, serial 10-fold dilutions in water were performed with genomic DNA of type strains *C. jejuni* CCUG 11284$^T$ and *C. coli* CCUG 11283$^T$. Both assays showed a linear increase of $C_t$ values with the 10-fold dilutions, with linearity down to the detection limit at $10^{-7}$ dilution (1.6 pg/ml), corresponding to about two copies in the reaction. *r* values of both assays were close to 1.0. The *hipO–ceuE* PCR yielded *r* values of 0.994 for *hipO* and 0.995 for *ceuE*. The *r* values for Cj_*fusA* and Cc_*fusA* were 1.0 and 0.997, respectively. Slope values were as follows: 3.068 for *hipO,* 3.267 for *ceuE,* 3.428 for Cj_*fusA,* and 3.467 for Cc_*fusA*.

**Detection of *Campylobacter* from stomached BPW samples.** Broiler skins stomached in BPW were first tested as basic material, which was also used for conventional quantitative culture investigation, allowing direct comparison of CFU and $C_t$ values. However, results were not satisfactory, given that amplification curves were ambiguous and the internal positive control was often negative (9.4%), indicating that factors in the samples had hampered PCR.

Comparison of 10-fold dilution series of *C. jejuni* and *C. coli* in pure water, pure BPW, and negative neck skin BPW samples showed only a minor difference in $C_t$ values between spiked water and spiked, pure BPW. $C_t$ values of spiked negative neck skin BPW samples were around 2 and 5.5 times higher than were those of spiked water or pure BPW. Hence, PCR inhibitors must be present in the stomached BPW–broiler skin samples but not in BPW itself.

In order to improve sample quality and to reduce inhibitors, a DNA extraction was done from the BPW samples. For that purpose, samples were grouped according to CFU per gram, determined by conventional culture (0 to $10^1$, $10^1$ to $10^2$, $10^2$ to $10^3$, $10^3$ to $10^4$, $10^4$ to $10^5$, and $10^5$ to $10^6$ CFU/g). Overall, 92 samples representative of all groups were tested with the *fusA* and *hipO–ceuE* PCR assays. Sensitivity compared with quantitative culture was very low. From the 73 samples that were culture positive, 16 were negative in the *fusA* assay, and 21 were negative with the *hipO–ceuE* real-time PCR. Given the overall problems with the BPW samples, we decided to continue with directly testing neck skins.

**Detection and prevalence of *Campylobacter* on neck skin samples.** From all 351 neck skin samples, DNA isolation by using a commercial kit as well as a simple lysate preparation from meat juice was performed and tested in the *hipO–ceuE* real-time PCR. In 173 cases, samples purified with the kit or simple lysates were found to be

TABLE 2. *Comparison of the* hipO–ceuE *with the* fusA *assays*

| Assay | Cj_*fusA* | Cc_*fusA* | Cj_*fusA*–Cc_*fusA* | Negative | Total |
|---|---|---|---|---|---|
| *hipO* | 93 | 0 | 28 | 6 | 127 |
| *ceuE* | 1 | 7 | 12 | 1 | 21 |
| *hipO–ceuE* | 12 | 3 | 48 | 0 | 63 |
| Negative | 37 | 1 | 2 | 100 | 140 |
| Total | 143 | 11 | 90 | 107 | 351 |

positive for *Campylobacter* (*C. jejuni, C. coli,* or both). One hundred thirty-one samples showed concordantly negative results with both methods. In 9 cases, *Campylobacter* was detected only with lysate-purified samples, whereas 38 samples showed a positive result only when purified with the kit. When $C_t$ values of the positive results from kit and lysate were compared, those of the lysate were lower on average by 2 values, reflecting the lower concentration of target DNA in the lysate compared with purified DNA.

The *hipO–ceuE* real-time PCR results obtained with the kit-purified samples were then compared with quantitative culture results. One hundred seventy-four samples were found to be positive for *Campylobacter* with the PCR as well as in culture, and 125 were negative with both methods with a detection limit of the quantitative culture method at 10 CFU/g, due to the initial 1:10 (wt/vol) dilution. In 37 cases, a real-time PCR–positive, but culture-negative (i.e., <10 CFU/g), result was determined, whereas 15 samples were positive in culture but negative in real-time PCR (Table 2). Thus, prevalence of *Campylobacter* resulting from quantitative culture analysis was 53.8%, whereas prevalence assessed by the *hipO–ceuE* real-time PCR was 60.1%.

The kit-purified broiler neck skin DNA samples were also tested with the newly developed *fusA* gene–based real-time PCR. When the *fusA* real-time PCR was compared with quantitative culture results, 182 samples were determined positive for *Campylobacter* in PCR as well as in culture, and 100 were negative with both methods. Real-time PCR–negative, culture-positive samples were found in 7 samples; in contrast, 62 real-time PCR–positive, culture-negative results were obtained. Prevalence of *Campylobacter* based on the *fusA* real-time PCR was therefore determined to be 69.5%.

Results of the two real-time PCR approaches using kit-purified DNA were then compared, including the possibility of species discrimination given (Table 2). With the *hipO–ceuE* assay, 127 samples contained *C. jejuni,* 21 contained *C. coli,* and 63 revealed co-presence of both species. With the *fusA* assay, *C. jejuni* was found in 143 samples, and in 11 samples, *C. coli* was detected. Co-presence of *C. jejuni* and *C. coli* was determined in 90 samples. From 351 tested samples, 204 were found positive for *Campylobacter* and 100 negative with both real-time PCR approaches. Seven samples were positive only with the *hipO–ceuE* real-time PCR, and in 40 samples, only the *fusA* assay was able to detect *Campylobacter*. Of the 204 positive samples, congruent species results were found in 93 cases for *C. jejuni,* 7 for *C. coli,* and 48 for *C. jejuni* as well as *C. coli*. $C_t$ values were compared between the *hipO–ceuE* and *fusA* PCRs (Fig. 1). The trendline showed an *r* value of 0.871. $C_t$ values from *fusA* were in general slightly higher than they were with the other approach.

When noting the results of the qualitative enrichment culture, the prevalence was determined as 72%, of which 65.4% was *C. jejuni,* 25.6% *C. coli,* and 9.0% both species. Taking advantage of the discrimination power of both real-time PCR assays, we found that roughly 60% of the positive samples contained *C. jejuni* (60% with *hipO–ceuE,* 58% with Cj_*fusA*), less than 10% contained *C. coli* (10% with *hipO–ceuE,* 5% with Cc_*fusA*), and more than 30% of positive samples contained both species (30% with *hipO–ceuE,* 37% with *fusA*). All samples determined positive in the quantitative culture were also positive in the qualitative enrichment culture. Moreover, of the 162 negative samples (<10 CFU/g) in the quantitative culture, 65 were positive in the enrichment culture, 36 were positive in the *hipO–ceuE* assay, and 60 were positive in the *fusA* real-time PCR.

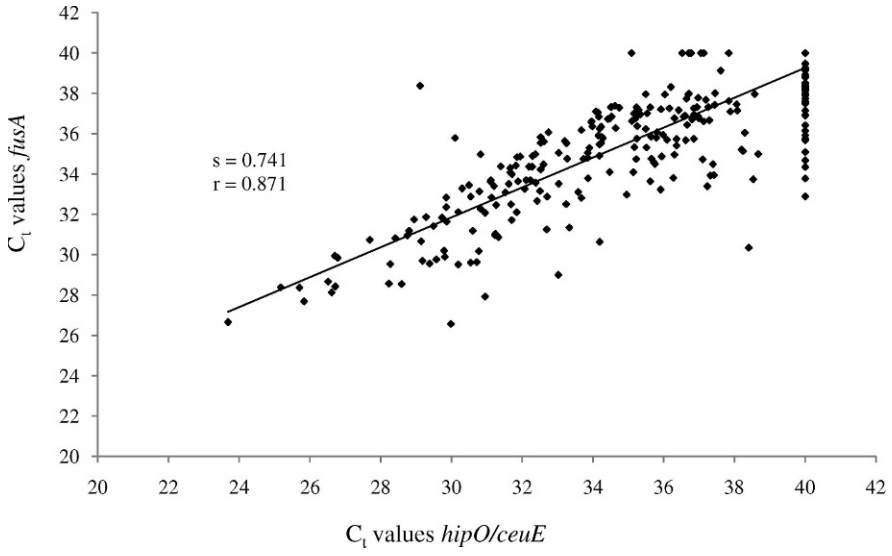


FIGURE 1. *Comparison of* $C_t$ *values of the* hipO–ceuE *with the* fusA *assays from 351 broiler neck skin samples. The* r *value and the slope (*s*) are indicated.*

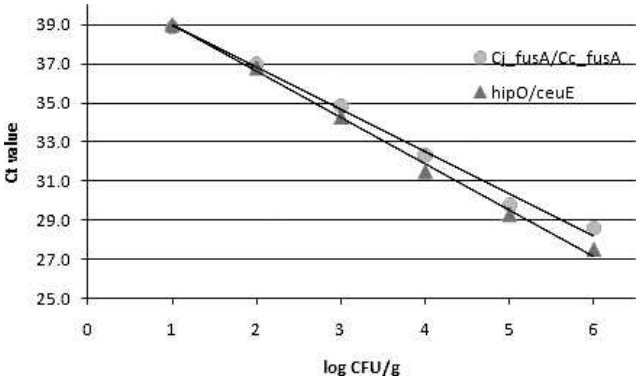


FIGURE 2. *Log of CFU per gram plotted against mean* $C_t$ *values of the* hipO–ceuE *(▲) and the* fusA *(●) assays. Log numbers indicate ranges (0 to* $10^1$*,* $10^1$ *to* $10^2$*,* $10^2$ *to* $10^3$*,* $10^3$ *to* $10^4$*,* $10^4$ *to* $10^5$*,* $10^5$ *to* $10^6$*).*

**Quantification of *Campylobacter* on neck skin samples.** An approximation on the load of *Campylobacter* determined by culture can be achieved by PCR and is shown in Figure 2. Ranges containing 0 to $10^1$, $10^1$ to $10^2$, $10^2$ to $10^3$, $10^3$ to $10^4$, $10^4$ to $10^5$, and $10^5$ to $10^6$ CFU/g were taken, and the corresponding means of $C_t$ values were plotted against the corresponding log range determined by culture. An *r* value greater than 0.99 was observed between $C_t$ values and CFU per gram for both real-time PCR assays, and the $C_t$ values indicate the approximate range of *Campylobacter* on the carcasses.

## DISCUSSION

A new real-time PCR assay was designed and evaluated, which allowed the simultaneous detection, quantification, and differentiation of *C. jejuni* and *C. coli,* based on a single gene by using SNP. It was compared with a recently established real-time PCR based on two individual genes for *C. jejuni* and *C. coli,* respectively. Because of the close genetic relationship of *C. jejuni* and *C. coli,* differentiation by a single PCR can be problematic. Burnett et al. *(4),* using six different conventional PCR assays, showed that results were not always concordant. Therefore, the redundancy obtained by applying more than one real-time PCR assay on the broiler neck skin sample set investigated helped to corroborate the findings, and inclusion of an amplification control permitted identification of false negatives. Moreover, results from PCR were compared with conventional culture assays in order to better assess the usefulness of the real-time PCR assays.

The SNP-based, *fusA* real-time PCR assays performed similarly to that of Hong et al. *(10),* based on *hipO* and *ceuE*. When tested with type strains and previously characterized field isolates, the specificity of the *hipO–ceuE* assay was slightly better than it was with the *fusA* approach. Whereas the *hipO–ceuE* assay correctly identified the *C. jejuni* and *C. coli* strains and did not give signals with other species, the *fusA* assay resulted in a weak and very late ($C_t > 38$) amplification of *C. lanienae* type strain with the *C. jejuni*–specific probe as well as with the *C. hyointestinalis* subsp. *hyointestinalis* type strain by using the *C. coli*–specific probe. However, these signals were observed with a high concentration of purified genomic DNA (about $10^6$ genome copy numbers in the reaction). This indicates either an unspecific and inefficient amplification or possibly an artifact. Moreover, these species are not present in poultry, and therefore this does not present a problem for false-positive results *(6, 23)*. The detection limit of the assays for both species was as few as 2 genome equivalents per reaction. The assays showed optimal performance for both species and linearity over 8 log of DNA dilutions.

The real-time PCR assays were used in parallel to screen broiler neck skin samples collected during the EU baseline study in 2008 on the prevalence of *Campylobacter* in broiler carcasses, in which Switzerland took part in the framework of the bilateral approach *(7)*. Detection of *Campylobacter* from the stomached BPW samples used for the culture counting proved not to be reproducible, due to the presence of inhibitors that either hampered PCR performance or even completely inhibited the amplification. DNA isolation from the BPW samples did give reproducible results, and no inhibition was observed. However, sensitivity was very low compared with quantitative culture. Since stomached neck skin BPW as the most direct sample for comparison with culture counting was not applicable, we investigated the usefulness of directly testing neck skin sample juice. Two template preparation methods were tested for this kind of sample. A simple and cheap lysate proved useful for detecting *Campylobacter* in broiler neck skin meat juice, and this test simplifies and reduces laboratory work. When compared with a standard DNA purification using a commercial column-based kit, similar results were obtained, with more samples being positive only with the kit than only with the lysate. This indicates that with the lysate there still seem to be more inhibitors present than with the kit-purified samples; however, with the latter, one might lose DNA in some cases. Nevertheless, by DNA isolation, a concentration of target DNA can be achieved, whereas with a lysate preparation, a dilution has to be accounted for. Therefore, the $C_t$ values with the lysate were slightly higher than they were with the kit-purified samples.

The use of neck skin meat juice had the advantage of directly testing the original material without 10-fold dilution; however, it came with the disadvantages that samples for PCR did not originate from exactly the same location of the neck skin samples and the weight of the remaining neck skin used for preparing the sample varied. Nevertheless, a direct comparison of quantitative PCR results with CFU per gram was possible; hence, testing meat juice can indicate *Campylobacter* load on the neck skin. This is interesting in the context of determining the *Campylobacter* load on carcasses, an aspect becoming more important, given that not only the presence but also the number of *Campylobacter* might be used to assess food quality *(8)*. Establishing standard protocols and defining threshold values for *Campylobacter* loads is needed to come to an international consensus.

The prevalence for *Campylobacter* found with the *fusA* assay was about 10% higher as compared with the *hipO–ceuE* test. In the quantitative culture assay with a detection

limit of 10 CFU/g, a prevalence of 54% was determined, whereas with an enrichment step, the culturally determined prevalence was 72%. Therefore, the *fusA* real-time PCR might be better, and certainly faster, for detection of *Campylobacter* than is the culture counting method, and results in values are comparable to the enrichment culture determination. The newly developed *fusA* assay therefore proved more sensitive in the detection of *Campylobacter* in neck skin meat juice than did the published *hipO–ceuE* assay. It has to be kept in mind, however, that the PCR will also detect DNA from dead bacteria, which could mislead in assessing the infectious dose based on viable cells as determined by culture. On the other hand, culture can be equally biased, seeing as cells might die during transport and processing samples for culture. Moreover, viable but nonculturable cells in which metabolic activity is minimized and growth interrupted in a way that culture is not successful, and false-negative results are obtained, could be a problem *(18)*.

Species identification based on culture results is rarely done for routine prevalence studies or quantitative culture determination of *Campylobacter*. Moreover, this identification is biased, as single or only a few colonies are further identified, and therefore samples containing both species can be missed *(11)*. Thus, the discrimination power of real-time PCR can lead to slightly different but more trustful results due to simultaneous detection of both species without selection bias. *C. coli* was detected in 40% of positive Swiss broiler carcasses and can be found alone or in co-presence with *C. jejuni*. This underscores that even though *C. jejuni* is considered the major pathogen causing gastroenteritis in humans, *C. coli* should not be neglected. In a recent study performed in Scotland, more than 50% of human cases caused by *C. coli* could be attributed to consumption of contaminated chicken meat *(26)*. Therefore, the contribution of *C. coli* prevalent in chicken-to-human clinical cases is probably underestimated because the two species are not always clearly identified, as is possible with the real-time PCR approach we describe herein. On the other hand, a differentiation between the closely related species *C. coli* and *C. jejuni* might only be of epidemiological interest, since this is not clinically relevant.

In conclusion, real-time PCR proved to be a fast and easy method for direct detection, differentiation, and quantification of *C. jejuni* and *C. coli* on broiler carcasses. To the authors' knowledge, the *fusA* assay presented in this study is the first described real-time PCR for *C. jejuni* and *C. coli,* based on an SNP. We can conclude that the assay of Hong et al. *(10)* and the *fusA* approach are reliable for detection and enumeration of *Campylobacter* from chicken neck skin without an enrichment step, with the *fusA* approach being more sensitive, achieving similar prevalence values as does the enrichment culture–based method. Template preparation can be critical; however, a simple lysate prepared from meat juice might be sufficient for certain purposes. For *Campylobacter* surveillance programs, the here-applied real-time PCR can be useful to control measures taken to reduce *Campylobacter* contamination on chicken carcasses.

## ACKNOWLEDGMENTS

We thank Elisabeth Lüthi, Stefanie Ackermann, and Stefanie Kaderli for excellent technical help. This research was supported by a Swiss Federal Veterinary Office grant (1.08.12).

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