Quantitative detection of *Campylobacter jejuni* on fresh chicken carcasses by real-time PCR

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Problems with traditional direct plating on mCCDA

- Overgrowth by other species
- Growth of campylobacter-like colonies
- Swarming
Extraction of total DNA from chicken rinse

Chicken rinse 400 ml

10 ml Filtration (100 µm)

5 µl of the elute (200 µl) as template

Total DNA extraction (Qiagen DNeasy tissue kit)

Real-time PCR

Filtration (100 µm)
Application of the 5′-Nuclease PCR Assay in Evaluation and Development of Methods for Quantitative Detection of Campylobacter jejuni

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Campylobacter jejuni is recognized as a leading human food-borne pathogen. Traditional diagnostic testing for C. jejuni is not reliable due to special growth requirements and the possibility that this bacterium can enter a viable but nonculturable state. Nucleic acid-based tests have emerged as a useful alternative to traditional enrichment testing. In this article, we present a 5′-nuclease PCR assay for quantitative detection of C. jejuni and describe its evaluation. A probe including positions 381121 to 381206 of the published C. jejuni strain NCTC 11168 genome sequence was identified. When this probe was applied, the assay was positive for all of the isolates of C. jejuni tested (32 isolates, including the type strain) and negative for all other Campylobacter spp. (11 species tested) and several other bacteria (41 species tested). The total assay could be completed in 3 h with a detection limit of approximately 1 CFU. Quantification was linear over at least 6 log units. Quantitative detection methods are important for both research purposes and further development of C. jejuni detection methods. In this study, we used the assay to investigate to what extent the PCR signals generated by heat-killed bacteria interfere with the detection of viable C. jejuni after exposure at elevated temperatures for up to 5 days. An approach to the reduction of the PCR signal generated by dead bacteria was also investigated by employing externally added DNAses to selectively inactivate free DNA and exposed DNA in heat-killed bacteria. The results indicated relatively good discrimination between exposed DNA from dead C. jejuni and protected DNA in living bacteria.
The efficiency of the PCR reaction

DNA from chicken rinse; slope = -3.43, E = 0.96
Detection limit

Theoretical limit = 4 bacteria per ml chicken rinse
Detection of a low number of *C. jejuni* by both direct plating and real-time PCR

Samples of spiked chicken rinse

<table>
<thead>
<tr>
<th>CFU/plate or CCE/assay</th>
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- **Plate count**
- **Q-PCR**
Detection of a low number of *C. jejuni* by both direct plate and real-time PCR

![Graph showing detection of *C. jejuni* by plate count and Q-PCR](image-url)

- **CFU/ml or CCE/ml**
- **Samples of spiked chicken rinse**

- Plate count
- Q-PCR
Correlation between direct plating and q-PCR

\[ R^2 = 0.98 \]
\[ Y = 0.51 + 1.0X \]
30 positive carcasses sampled at slaughter

\[ Y = 0.88 \times 0.1; \quad R^2 = 0.76 \]
Analysis of 45 carcasses from retail

<table>
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<th>Plate count</th>
<th>Real-time PCR</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<td>Positive</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>25</td>
</tr>
</tbody>
</table>
Carcasses sampled at retail

\[ Y = 1.6X - 1.4; \quad R^2 = 0.58 \]
Conclusions

• The sensitivity of the developed method is similar to direct plating.

• The correlation of the two methods is good when spiked or samples directly from slaughter are analysed.

• The correlation is less good with samples from retail, probably due to larger variation of dead and/or VNC *Campylobacter* between the samples.

• The purified DNA can be used for detection and quantification of other species, *e.g.* *Salmonella.*
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