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Different tolerance of loop-mediated isothermal amplification and polymerase chain reaction to inhibitors in chicken carcass rinse and feces for detecting *Campylobacter jejuni*

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Introduction

Campylobacter spp, particularly *C. jejuni*, are a leading cause of human foodborne gastroenteritis worldwide, with poultry being the major reservoir. There is a need for developing rapid methods to detect this organism in poultry for baseline studies and the reduction of this pathogen in poultry. Polymerase chain reaction (PCR) is a commonly used molecular procedure to detect pathogens including foodborne campylobacters. However, inhibition of PCR due to inhibitors in various types of samples has been problematic. Loop-mediated isothermal amplification (LAMP) procedure has been shown to be equivalent to or more sensitive than PCR and be less affected by the inhibitory substances compared with PCR. This study investigated the relative tolerance of LAMP and real-time PCR detecting *C. jejuni* to inhibitor(s) in chicken carcass rinse and feces.

Methods

Sample preparation. 1) *C. jejuni* (NCTC11168) was spiked into PBS or chicken meat rinse, lettuce and chicken fecal homogenates to determine detection limit. 2) 10-fold concentrated non-enriched chicken meat rinses with known culture results were used to compare a LAMP and a real-time PCR assays. The culture results were obtained using combined procedures of direct Campy-Cefex plating and enrichment procedures (1, 2). 3) Purified and crude DNA extracts were prepared using DNeasy Blood and Tissue Kit (Qiagen) and EX-F extraction kit **(**Eiken Chemical Co. Ltd., Japan/SA Scientific, USA) respectively.

LAMP assay. The LAMP procedure (3) detects the putative oxidoreductase gene of *C. jejuni* with 6 primer set. A commercial LAMP kit detecting *C. jejuni*/*coli* (Eiken/SA Scientific) was used as reference confirmation method. For all samples, the LAMP assay was performed using a real-time PCR thermocycler to keep consistent temperature (63^0C), and to measure the by-product (insoluble magnesium pyrophosphate) using a fluorescent dye (calcein) in real time for 60–70 min. A block heater and conventional PCR thermocycler were tested as practical heat sources. For selected samples, agarose gel electrophoresis was conducted to confirm the presence of typical ladder patterns of the end product.

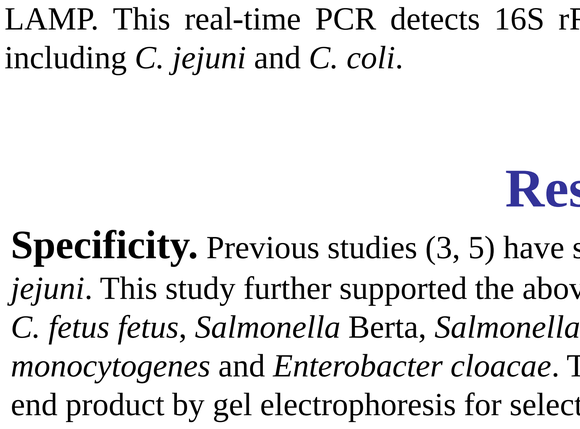
Real-time PCR. A real-time PCR (RT-PCR) (4) was used for comparison with the LAMP. This real-time PCR detects 16S rRNA gene sequence in *Campylobacter* spp. including *C. jejuni* and *C. coli*.

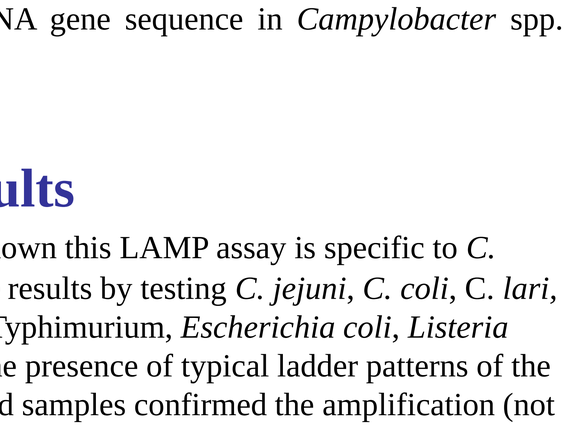
Results

Specificity. Previous studies (3, 5) have shown this LAMP assay is specific to *C. jejuni*. This study further supported the above results by testing *C. jejuni*, *C. coli*, C. *lari*, *C. fetus fetus*, *Salmonella* Berta, *Salmonella* Typhimurium, *Escherichia coli*, *Listeria monocytogenes* and *Enterobacter cloacae*. The presence of typical ladder patterns of the end product by gel electrophoresis for selected samples confirmed the amplification (not shown).

Detection limit (1). For purified DNA extract from spiked chicken meat rinse, the detection limit for LAMP was 10^2-10^3 CFU)/mL (less than 10 CFU per reaction), and 10^1-10^2 CFU/mL for the real-time PCR in 2 experiments (Fig. 1) with similar results for vegetable and fecal homogenates (not shown). The commercial kit also consistently detected 10^1-10^2 CFU/mL.

Fig 1. Detection limit for purified DNA from spiked chicken rinse. Positive at $2x10^2$ (10 CFU/reaction) – 10^8 CFU/mL for both LAMP and real-time PCR.

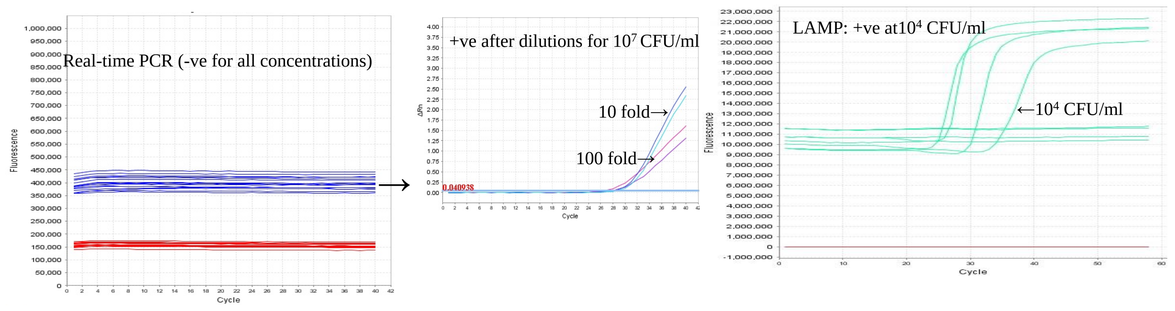




Results (cont'd)

Detection limit (2).
For crude DNA extract from spiked chicken meat rinse, the real-time PCR detected 10^3 CFU/mL, and 10^5 CFU/mL for the LAMP. For crude DNA extract from spiked chicken feces, the LAMP detected 10^4 CFU/ml, the real-time PCR was negative at 10^1 –10^7 CFU/mL unless the crude DNA extract at 10^7 CFU/mL was 10-100 fold diluted in water (Fig. 2).

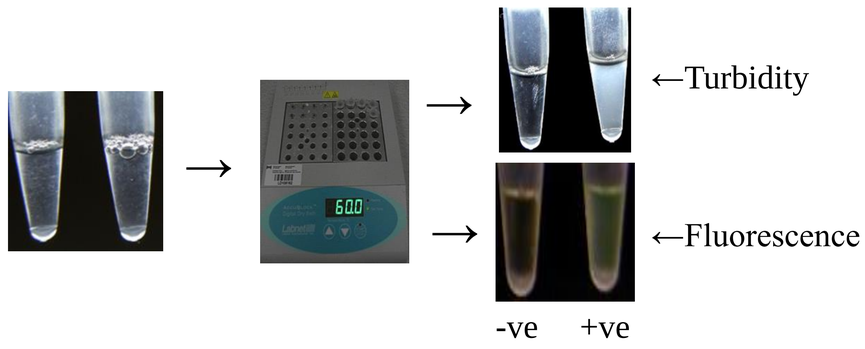
Fig. 2. Detection limit for crude DNA extract of spiked chicken feces.



Testing of natural samples. Among 42 natural chicken rinse samples tested, 28 were positive by culture methods. The real-time PCR and the LAMP showed positive for 14 and 16 of 28 culture positives respectively.

Feasibility for simple testing conditions. Selected samples were tested using a block heater and a conventional PCR thermocycler with the same results as that by using the real-time PCR thermocycler. The end products (turbidity or fluorescence) could be visualized by the naked eye or under UV (Fig. 3).

Fig. 3. Testing using simple equipment



Summary

- The LAMP and real-time PCR procedures evaluated showed similar detection limits for samples with purified DNA extracts.

- The LAMP and real-time PCR assays were affected by different inhibitors. Particularly the inhibition effect of chicken fecal substance(s) was more prominent for real-time PCR than LAMP when using crude DNA in feces.

- The LAMP using crude fecal DNA preparation could potentially be a less expensive and simple method.

- This LAMP assay could be an alternative test amenable for use under various laboratory conditions.

References

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Acknowledgements

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