FDA validated molecular method to detect C. cayetanensis in food samples

Vegetables

CASE STUDY

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Food Microbiology 76 (2018) 497–503

Overview

Keyword: Cyclospora cayetanensis, Fresh produce, Prepared dish, qPCR
Aim of the study: Evaluate the performance of the FDA method for detection of C. Cayetanensis in fresh product items
Application: qPCR
Sample name: Carrots, basil, parsley, cabbage & carrot mix
Sample type: Fresh and prepared produce
Material: FastDNA™ Spin Kit for Soil, FastPrep-24™ Instrument
Buffer: Sodium Phosphate Buffer and MT Buffer (from the FastDNA™ Spin Kit for Soil)

Protocol and Parameters

1. Add up to 850 µL of pooled pellet collected after the washing procedure of infected food samples to a Lysing Matrix tube containing the mix of beads of Lysing Matrix E (1.4mm ceramic beads, 0.1mm silica beads and one 4mm glass bead)
2. Add 122 µL MT buffer
3. Add 978 µL Sodium Phosphate Buffer. Screw on cap securely.
4. Transfer the samples to the FastPrep-24™ bead beater and homogenize at a setting of 6.5 m/s (approximately 4000 rpm) for 60 seconds. Immediately remove the sample holder containing the tubes from the instrument and place on ice for 3 minutes. Return the sample holder to the bead beater and repeat the bead beating and the incubation on ice as above.
5. Remove the tubes from the sample holder and centrifuge at 14,000 × g for 15 minutes.
6. Transfer the supernatant to a clean 2 mL tube. Add 250 µL PPS and mix by inverting by hand 10 times.
7. Centrifuge at 14,000 × g for 5 minutes then transfer supernatant to a clean 15 mL Falcon tube containing 1.0 mL of resuspended Binding Matrix.
8. Place on a rotator or invert by hand for 2 minutes and then allow silica matrix to settle for 3 minutes. Centrifuge the 15 mL tubes briefly at 1000 × g for 1 minute in a swinging bucket rotor.
9. Remove and discard a total of 1.4 mL of supernatant from each tube in two 700 µL aliquots.
10. Resuspend the matrix in the remaining supernatant and transfer approximately 700 µL to a SPIN Filter in a catch tube. Centrifuge at 14,000 × g for 1 minute. Empty the catch tube and add any remaining resuspended mixture to the SPIN Filter and spin as before. Empty the catch tube again.
11. Add 500 µL prepared SEWS-M to each filter. Gently resuspend each by pipetting up and down.
12. Centrifuge at 14,000 × g for 1 minute. Empty catch tube and replace
13. Centrifuge at 14,000 × g for 2 minutes to dry the matrix. Discard the catch tube and replace with a new catch tube.
14. Air dry the filter for 5 minutes at room temperature.
15. Add 75 µL DES to the matrix in the spin filter. Resuspend the Binding Matrix by gently stirring with a small pipet tip. Incubate for 5 minutes in a heat block at 55°C.
16. Centrifuge at 14,000 × g for 1 minute to recover the eluted DNA and then discard the SPIN Filter.
17. Store the DNA samples at 4°C for up to 2 days or at -20- or -80 °C for longer term prior to performing the Real-Time PCR detection step.

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Conclusion

The FastPrep-24™ homogenizer used in combination with the FastDNA™ Spin Kit for Soil is shown to be an effective method for the lysis of C. Cayetanensis oocysts from infected food matrices and isolation of their DNA. The extracted DNA was used successfully in real-time PCR assays that were able to detect as few as 5 oocysts in 25g of food samples.

Mean copy number of the 18S rRNA gene determined per qPCR reaction in carrots, cabbage and carrots mix, parsley, and basil after seeding samples with 200 Cyclospora cayetanensis oocysts.

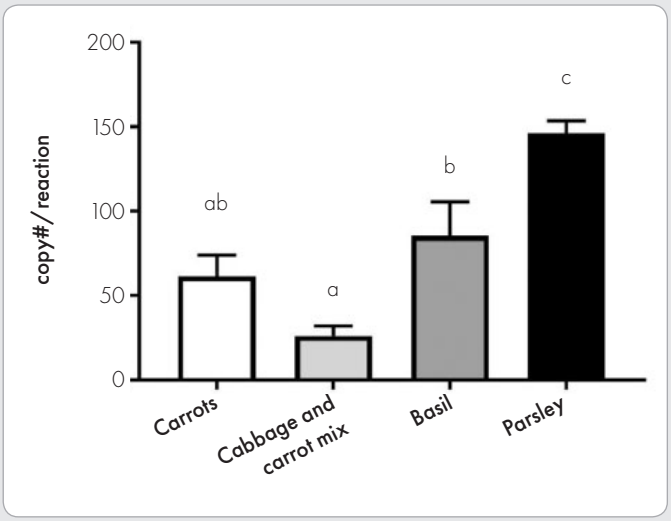

Figure 1.
Comparaison of mean copy number of the 18S rRNA gene determined per qPCR raction (2µl of DNA/reaction) in carrots, cabbage and carrots mix, parsley, and basil after seeding samples with 200 *Cyclospora cayetanensis* oocysts. Arbitrary letters a, b and c, were indicated over columns. Different letters over the columns indicate statiscally significant differences among matrices ($P < 0.05$). Significant differences were observed between cabbage and carrots mix samples compared to both basil and parsley samples, and in parsley compared to all other matrices. No significant differences were observed between carrots and cabbage and carrots mix or between carrots and basil. The standard error is represented by error bars.

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