Detection of *Cyclospora cayetanensis*, *Echinococcus multilocularis*, *Toxocara* spp. and microsporidia in fresh produce using molecular methods: – A review

B. Bartosova⁴, B. Koudela, I. Slana

⁴ Veterinary Research Institute, Hudcova 296/70, Brno 621 00, Czech Republic

Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, Brno 612 42, Czech Republic

Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, Brno 612 42, Czech Republic

**Article info**

Article history:
Received 17 December 2020
Received in revised form 9 April 2021
Accepted 23 April 2021

Keywords:
PCR
Vegetables
Fruit
Parasite
Food safety

**Abstract**

The current trend for a healthy lifestyle corresponds with a healthy diet, which is associated with regular and frequent consumption of raw fruit and vegetables. However, consumption of ready-to-eat (RTE) food without heat treatment or sufficient washing may pose a risk to consumers. Among the well-known protozoan parasites associated with RTE food and water are *Cryptosporidium* spp., *Giardia duodenalis* and *Toxoplasma gondii*. These belong among prioritized parasitic pathogens, as they are associated with numerous disease outbreaks in humans all around the world. Nevertheless, other parasitic agents such as *Cyclospora cayetanensis*, *Toxocara cati*, *Toxocara canis*, *Echinococcus multilocularis* and zoonotic microsporidia should not be neglected. Although these selected parasites belong to phylogenetically diverse groups, they have common characteristics associated with fresh produce and each of them poses a health risk to humans.

Ensuring healthy food is produced requires the standardization of laboratory methods for the detection of parasitic agents. This article reviews the molecular methods currently used in laboratories for detection of *Cyclospora cayetanensis*, *Toxocara cati*, *Toxocara canis*, *Echinococcus multilocularis* and zoonotic microsporidia in fresh produce.

© 2021 The Authors. Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Contents**

1. Introduction ........................................................ 2
2. Identification of selected parasites ...................................... 2
   2.1. *Cyclospora cayetanensis* ............................................... 2
   2.2. *Echinococcus multilocularis* ............................................ 2
   2.3. Microsporidia. ................................................................. 6

---

* Corresponding author at: Veterinary Research Institute, Hudcova 296/70, Brno 621 00, Czech Republic.

E-mail address: bartosova@vri.cz. (B. Bartosova).

https://doi.org/10.1016/j.fawpar.2021.e00124

2405-6766/© 2021 The Authors. Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

On the farm-to-fork pathway there are many sources of possible contamination for ready-to-eat (RTE) food. RTE foods such as fresh vegetables, fruit, herbs and sprouts, which are usually eaten without cooking or heating, are quite susceptible to parasite contamination. Parasitic infections are often referred to as neglected diseases, although such infections may have serious consequences for human health. In the past few decades there has been an effort to reduce the global consumption of “modern diets” which are based on unhealthy fried meals and semi-finished products. These negatively influence human health and contribute to the growth in obesity worldwide. Nowadays diets are undergoing changes due to the inclusion of more fruit and vegetables in common dishes (Ramos et al., 2013). It is therefore logical that the number of outbreaks is also increasing. Identifying foodborne or waterborne outbreaks is difficult in cases involving retail purchases of produce over a large geographical area (Dawson, 2005). In many countries, the rising consumption of fresh smoothies and shakes in addition to the availability of various pre-prepared salad mixes for direct consumption without the need for any preparation contributes to the possibility of foodborne infection. It is clear that the consumption of raw, unwashed fresh products may be risky for humans in terms of the possibility of infection by certain pathogens (Tefera et al., 2018). Nowadays, waterborne parasites, together with foodborne parasites, are in the forefront of interest (European Food Safety Authority and European Centre for Disease Prevention and Control, 2018).

Monitoring and risk assessment of known water-related zoonotic parasites such as Cryptosporidium spp., Giardia duodenalis and Toxoplasma gondii are important, but this could be expanded to include other less monitored and well-known parasites. Many articles discussing Cryptosporidium spp., Giardia duodenalis and Toxoplasma gondii detection have already been published. The aim of this review is to present the molecular methods of selected parasites, namely protozoa Cyclospora cayetanensis, helminths Toxocara cati and Toxocara canis, Echinococcus multilocularis, one of the smallest tapeworms, and zoonotic representatives of microsporidia (Plutzer and Karanis, 2016). It includes pre-treatment steps, DNA isolation and PCR assays used on fresh produce.

The parasites that we focus on in this study are excreted with feces into the environment as environmentally resistant stages (ERS): oocysts, cysts, eggs, spores. These ERS possess unique abilities in terms of environmental survival (weeks or months) and resistance to various temperatures and humidity. Furthermore, due to their small sizes (2–90 μm), low specific gravity, low infectious dose and resistance to routinely used chemical disinfections, there is substantial waterborne transmission (Thevenet et al., 2017; Ortega and Sanchez, 2010; Dumitre and Darde, 2003; Thevenet et al., 2005). Possible sources of infection include water (water for irrigation, water used for washing produce and other food processing), wastewater from human settlements, soil and soil environments including fecal waste from warm-blooded animals, manure and other fertilizers, animals and insects or handling by pickers and handlers (Berger et al., 2010; Dixon, 2016; Tefera et al., 2018; Geldreich, 1996; Dudlova et al., 2015). Nevertheless, all these sources are also common for many other pathogens, not only parasites. Fresh produce may be contaminated by ERS from a variety of parasites and use of the appropriate laboratory method is crucial for identification. Generally, examination of fecal samples is easier because of the high concentration of excreted parasites. Examination of food and water samples, however, requires more complex methods involving concentrating, pre-processing, isolation and detection steps. The concentration step is crucial but unfortunately varies depending on the type of matrices and parasitic species (Steele et al., 2003; Chandra et al., 2014). Studies show that the fluctuations in the prevalence of vegetables and fruit contamination are related to differences in plant morphology and structure, as well as in the way that these products are grown (Berrouch et al., 2020). For example, strawberries have an uneven surface with trichomes, which allow the ERS to adhere to the surface in contact with contaminated water or soil. In contrast, tomatoes have a smooth surface, which may tend to reduce the rate of ERS adhesion (Giangaspero et al., 2015). Rapid, specific and accurate detection of the agents of interest is necessary in order to monitor their occurrence in food and water, especially when an outbreak occurs. The use of molecular methods fulfills these requirements. Furthermore, the ability for PCR-based methods to simultaneously perform pathogen detection in a single reaction reduces operating costs. However, microscopic methods, although time consuming and not entirely species-specific, should not be ruled out. They are still used in low and middle-income countries for parasitic detection due to their low operating costs (Zarlenga and Trout, 2004).

For this publication The Web of Science® (WOS) Core Collection database (Thomson Reuters) and PubMed were used to retrieve publications related to the topic of molecular detection of selected parasitic agents and fresh produce. Only relevant articles published in English were considered.

2. Identification of selected parasites

2.1. Cyclospora cayetanensis

Cyclospora cayetanensis is a human coccidia parasite that causes cyclosporiasis (Mansfield and Gajadhar, 2004). The disease occurs worldwide with certain seasonality. It is classified as an emerging pathogen for humans. People with this disease suffer
from diarrhea and associated anorexia, and other symptoms of gastroenteritis. The disease is more severe in immunocompromised individuals. The transmission of *C. cayetanensis* oocysts in the environment is associated with water and consumption of RTE food, as confirmed by the many cases of cyclosporiasis in the United States (Varma et al., 2003; Ortega et al., 1993; Casillas et al., 2019).

In animals, many species of *Cyclospora* have been identified. *C. cayetanensis* is the only species found affecting humans. At the end of the 1970s, *Cyclospora* was first identified as the causative agent of human disease and in 1993 Ortega et al. (1993) named it *Cyclospora cayetanensis*. Relman et al. (1996) performed phylogenetic analysis of the 18S rRNA gene of this coccidium, which showed a close relationship with other coccidia of the genus *Eimeria*. Pleniazek and Herwaldt (1997) confirmed this analysis and clarified the similarity to mammalian *Eimeria*. Oocysts of *Eimeria* (*Eimeria papillata*) were used as a surrogate for those of *C. cayetanensis* in artificial contamination experiments to detect it in fresh produce (Lalonde and Gajadhar, 2016a). The earliest paper describing the detection of *C. cayetanensis* in fresh produce is from 1998, but only one or two publications a year on *C. cayetanensis* detection in fresh produce have been published since 2013. It is common for publications to describe the detection of *C. cayetanensis* along with that of other zoonotic parasites (Orlandi and Lampel, 2000; Dixon et al., 2013; Lalonde and Gajadhar, 2016a; Temesgen et al., 2019b; Pineda et al., 2020). The advantage for *C. cayetanensis* is the existence of a validated regulatory method (FDA Method, 2004) which is regularly updated. These updates are essential, as the original method involved the use of FTA filters, which are no longer available. Even so, only one study detailed the detection of *C. cayetanensis* in cranberries using the aforementioned filters (Orlandi and Lampel, 2000). In 2017, a validated new U.S. Food and Drug Administration method (FDA BAM) was developed for detection of *C. cayetanensis* in produce using real-time PCR. Improved sample preparation together with a real time PCR assay provides a rapid, robust, and less laborious procedure for detecting *C. cayetanensis* (Murphy et al., 2017).

The types of analyzed fresh products are summarized in Table 1, which includes the quantity and selected sample matrices. The studies that focus on herbs (basil) and berries (strawberries, raspberries) use maximum amounts of 25/30 g and 50/100 g, respectively, for the analysis. Leafy greens and other kinds of vegetables are analyzed less often. The largest sample volumes were used for analyzing the external parts of melons, cucumbers or tomatoes (1000 g; Giangaspero et al., 2015). In addition to the amount of sample, it is also important to consider pre-treatment and subsequent selection of the kit for DNA isolation. A third of the studies did not include pre-treatment. Pre-treatment usually consists of freeze-thaw cycling in liquid nitrogen or bead beating. One study describes only the freeze-thaw cycling for DNA isolation from *C. cayetanensis* oocysts (Jinneman et al., 1998). In a large number of studies Qiagen kits were used for DNA isolation. Raspberries and strawberries are matrices often used for *C. cayetanensis* detection. In such matrices, PCR analysis is challenging due to the presence of many inhibitory substances and the low concentration of parasitic agents. When inhibition of qPCR is observed in the detection of *C. cayetanensis* in cilantro, Assurian et al. (2020b) recommended using various DNA clean-up commercial kits. Therefore, care must be taken in the washing of samples (Murphy et al., 2017).

As shown in Table 2, four detection targets are used for the detection of *C. cayetanensis* in fresh produce. The oldest and most commonly used target is the 18S rRNA gene. To ensure specificity and to differentiate between *Cyclospora* and *Eimeria*, nested PCR combined with restriction fragment length polymorphism analysis (RFLP) was used (Dixon et al., 2013; Shapiro et al., 2019). The 18S rRNA target was used in both nested PCR and real time PCR. When using real time PCR, the amplicon products are shorter and therefore primers are designed differently for nested PCR. The conserved nature of the 18S rRNA gene among coccidia means that there is potential for cross reactivity with other related coccidian species. This provides a challenge in developing more specific detection methods based on other targets. Other *C. cayetanensis* specific primers includes internal transcribed spacer 2 (*ITS 2*), the *hsp70* gene and internal transcribed spacer 1 (*ITS 1*; Lalonde and Gajadhar, 2008, Shields et al., 2013, Temesgen et al., 2019b). The study on berries shows that the limit of detection for qPCR analysis focused on the *ITS* region can be 10 oocysts/30 g of berry fruits and approximately 32, 12.8, and 6.4 pg of DNA roughly estimated to be equivalent to 5, 2, and 1 oocyst based on gene copy number respectively. However, the high variability in the *ITS* 1 region for further source observation and in epidemiological studies makes the design of primers and probes of all isolates demanding (Temesgen et al., 2019b).

### 2.2. *Echinococcus multilocularis*

One of the smallest tapeworms, *Echinococcus multilocularis*, is of interest in Central and Eastern Europe and is emerging in parts of North America and Asia (Bouwknegt et al., 2018; Szostakowska et al., 2014). *E. multilocularis* is responsible for a disease known as alveolar echinococcosis (AE). AE is caused by the larval stage of *E. multilocularis* which creates characteristic lesions similar to a tumor-like growth in the affected organ, predominantly the liver. The symptoms of human AE tend to be severe (e.g. weight loss, abdominal pain, general malaise and signs of hepatic failure), even potentially lethal. A major complication in diagnosis is that the cause of the disease may not be detected and the illness may last for years without proper treatment (Eckert and Deplazes, 2004). Infection in humans is caused by direct or indirect ingestion of *E. multilocularis* eggs excreted by a definitive host (fox and other canids) through its feces into the environment (soil, water). Infection among foxes is widely distributed throughout the northern hemisphere and is associated with the occurrence of intermediate hosts (rodents). The occurrence of infection is
Table 1
Cyclospora cayetanensis: sample, pre-treatment and molecular detection methods.

| Origin/item                          | Spiking (No of oocysts) | Amount (g) | Pre-treatment | DNA isolation | Detection method                      | Recovery or LOD (oocyst) | Reference                                                                 |
|--------------------------------------|-------------------------|------------|---------------|---------------|---------------------------------------|--------------------------|--------------------------------------------------------------------------|
| Raspberry wash sediment extract      | YES (50)                | 250 g      | 6 × 2 min in N₂/98 °C NO | DNA released by F-T | nested PCR                           | 25                       | Jinneman et al. (1998)                                                   |
| Raspberries                         | YES (N/A)               | 100 g      | 6 mm F-T nested PCR | PCR           |                                       | 10–30/100 g               | Orlandi and Lampel (2000)                                                |
| Basil leaves                         | YES (10³–10⁵)           | 30 g       | 8 × 1 min in N₂/98 °C NO | QiAmp DNA microkit or DNeasy® blood and tissue kit PCR | nested PCR; qPCR          | 10 in 9/15; 1 in 2/15 sampl LOD: tens of unit²; Recovery: 5/50 g           | Lalonde and Gajadhar (2008)                                              |
| Raspberries, basil, mesclun lettuce  | YES (10–4000)           | 100 g      | NO            | QiAmp DNA stool Mini Kit nested PCR | qPCR; nested PCR; qPCR | Recovery; 3%–18% LOD: 1–100 g                                        | Steele et al. (2003)                                                    |
| Washes from raspberry, basil, pesto  | YES (50 or 500, 5000)   | 25 g basil; 50 g raspberries NO | Qiagen DNeasy Blood and Tissue Kit nested PCR | qPCR           |                                       | 5/50 g; LOD: 1:01 of gene                                                | Shields et al. (2013)                                                   |
| Leafy greens                         | NO (9/544)              | 25 g       | 5 × 2 min in N₂/90 °C NO | Qiagen DNeasy Blood and Tissue Kit nested PCR | nested PCR               | N/A                       | Dixon et al. (2013)                                                      |
| Basil                                | YES (100 or 1000)       | 25 g       | NO            | FastDNA Spinning Kit nested PCR | nested PCR               | N/A                       | Chandra et al. (2014)                                                   |
| Cucumber, lettuce, fennel, celery, tomato, melon, chicory | NO (6/49) | 100 g external leaves of vegetable³; 1000 g melon or tomato NO | Nucleospin tissue/stool kit nested PCR | qPCR           |                                       | 46–1580/g                                                              | Gangaspéro et al. (2015)                                                 |
| Leafy greens (herbs, green onions), berry fruits | YES (5000 E. papillata) | 35 ± 0.5 g | 8 × 1 min in N₂/98 °C NO | QiAmp DNA Mini Kit nested PCR | qPCR; nested PCR; qPCR | 5 of E. papillata/g                                                      | Lalonde and Gajadhar (2016b)                                            |
| Leafy greens³                        | NO (6/1171)             | 35 ± 0.5 g | 8 × 1 min in N₂/98 °C NO | QiAmp DNA microkit/DNeasy® blood and tissue kit nested PCR | qPCR; nested PCR; qPCR | Recovery: 3%–18% LOD: 1–100 g                                          | Lalonde and Gajadhar (2016a)                                              |
| Packaged salads³                     | NO (1.3%/64)            | 100 g      | 15 × 1 min N₂/65 °C NO | QiAmp Plant Mini Kit nested PCR | qPCR           | 46–1580/g                                                              | Caradonna et al. (2017)                                                  |
| Perilla leaves, winter-grown cabbages, chives, sprouts, blueberries, cherry tomatoes | NO (5/44) | 20 g perilla; 30 g sprouts; 50 g rest NO | QiAquick stool mini kit nested PCR | qPCR           |                                       | 13–348/g                                                               | Sim et al. (2017)                                                       |
| cilantro, raspberries                | YES (0, 5, 10, 200)     | 25 g cilantro; 50 g raspberries BB | FastDNA SPIN Kit for Soil nested PCR | qPCR           |                                       | N/A                                                                   | Murphy et al. (2017)                                                     |
| Cilantro, raspberries³               | YES-interlaboratory (0, 5, 10, 200) | 25 g cilantro; 50 g raspberries BB | FastDNA SPIN Kit for Soil nested PCR | qPCR           |                                       | 5/25 g                                                                | Murphy et al. (2018)                                                     |
| Carrot, cabbage, basil, parsley, Coleslaw with dressing | YES (5 or 10, 200); 141 | 25 g BB | 2 × BB 4 m/s for 60 s | DNeasy® PowerSoil Kit nested PCR | qPCR/multiplex | 10/30 g                                                | Temesgen et al. (2019a, 2019b)                                           |
| Strawberries, blueberries, raspberries | YES (10, 50)           | 30 g       | 2 × 2 BB 4 m/s for 60 s | DNeasy® PowerSoil Kit nested PCR | qPCR/multiplex | 10/30 g                                                | Li et al. (2019)                                                        |
| Vegetables, fruits²                  | NO (2/1099)             | 25 g NO    | E.Z.N.A.R®® Stool DNA Kit nested PCR | nested PCR | nested PCR; qPCR; qPCR | 5/50 g                                                                  | Li et al. (2019)                                                        |
| Spinach                              | YES (10⁵–10⁸)           | 10 g       | 1 × 4 min N₂/100 °C NO | DNeasy® Blood and Tissue Kit nested multiple, qPCR, RFLP qPCR | qPCR; nested PCR; qPCR | 1–10/g                                                                | Shapiro et al. (2019)                                                    |
| Berry fruit                          | YES (20, 200)           | 30 g BB    |                           | DNeasy® PowerSoil kit/UNEX-based DNA extraction nested PCR | qPCR; nested PCR; qPCR | 10/30 g                                                                | Temesgen et al. (2020)                                                   |
| Fresh and frozen berries³            | YES (1,20)              | 50 g BB    |                           | FastDNA SPIN Kit for Soil nested PCR | qPCR           | 5/50 g                                                                  | Assurian et al. (2020a)                                                  |
| Fresh berries, berry farm soil       | YES (0–10⁵)             | 50 g NO    |                           | ZymoBIOMICS DNA Kit nested PCR | qPCR           | 1/g                                                                    | Resendiz-Nava et al. (2020)                                              |
also influenced by climatic conditions (Miterpakova et al., 2006) Since *E. multilocularis* eggs are sensitive to desiccation and high temperatures, positive canids were more often found in areas with higher humidity (Veit et al., 1995). However, eggs can remain infectious in environmental conditions with residual humidity for up to one year (Otero-Abad and Torgerson, 2013; Veit et al., 1995). To date there is little information about the risk of *E. multilocularis* infection from fresh products. With increasing numbers of foxes (with territories near to human settlements) and the popularity of RTE food consumption, there is a possible risk of AE (Bastien et al., 2018).

Microscopic identification of ERS is difficult because the eggs of all *Echinococcus* and *Taenia* species are morphologically indistinguishable. Due to the microscopic size of the eggs concentration, often by flotation is recommended. However, the diagnostic sensitivity of double flotation-based protocols in the detection of taeniid eggs is only about 50% (Liccioli et al., 2012). After DNA isolation, Taenid eggs can be differentiated to the species level with PCR-based assays. This work also showed that the eggs of all *Echinococcus* species are morphologically indistinguishable and 

| Origin/item | Spiking (No of oocysts) | Amount (g) | Pre-treatment | DNA isolation | Detection method | Recovery or LOD (oocyst) | Reference |
|-------------|-------------------------|------------|---------------|---------------|------------------|------------------------|-----------|
| Cilantro   | YES (5, 10, 200)        | 25 g       | BB            | FastDNA SPIN Kit for Soil with a FastPrep-24™ | qPCR              | Assurian et al. (2020b) |
| Strawberries | NO (1/120)                 | 30 g       | BB            | DNeasy Power Soil kit | multiplex PCR               | Pineda et al. (2020) |

LOD = limit of detection.
BB = bead beating.
a 40 oocysts/100 g raspberries, 10 oocysts/100 g basil, 1000 oocysts/100 g mesclun lettuce.
b Lettuce, fennel, celery or the external part of cucumber.
c The number of DNA copies per μl was calculated by correlating the Ct mean value, with the number of oocysts calculated assuming that an oocyst contains 15 copies of rDNA, depending on the stage of sporulation.
d Spinach, spring mix, leaf lettuce, romaine, kale, arugula, chard, collards, dandelion greens, rapini.
e Mixed salad (curly and escarole lettuce, red radish, rocket salad and carrots).
f Lettuce, coriander, celery, baby bok choy, leaf lettuce, water spinach, crown daisy, fennel plant, endive, spinach, schizonepeta, cabbage, leaf mustard, Chinese chive, chive and the stripped epidermis of cucumber, watermelon, potato, bean, green chili.
g Blackberries, strawberries, blueberries and mixed berries.
h Five DNA cleanup commercial kits: QIAquick® Purification kit, One step™ PCR inhibitor removal, Nucleospin® Genomic DNA clean up, DNA IQ™ system and DNeasy® Power Plant® Pro kit.

### Table 1 (continued)

| Origin/item | Spiking (No of oocysts) | Amount (g) | Pre-treatment | DNA isolation | Detection method | Recovery or LOD (oocyst) | Reference |
|-------------|-------------------------|------------|---------------|---------------|------------------|------------------------|-----------|
| Cilantro   | YES (5, 10, 200)        | 25 g       | BB            | FastDNA SPIN Kit for Soil with a FastPrep-24™ | qPCR              | Assurian et al. (2020b) |
| Strawberries | NO (1/120)                 | 30 g       | BB            | DNeasy Power Soil kit | multiplex PCR               | Pineda et al. (2020) |

LOD = limit of detection.
BB = bead beating.
a 40 oocysts/100 g raspberries, 10 oocysts/100 g basil, 1000 oocysts/100 g mesclun lettuce.
b Lettuce, fennel, celery or the external part of cucumber.
c The number of DNA copies per μl was calculated by correlating the Ct mean value, with the number of oocysts calculated assuming that an oocyst contains 15 copies of rDNA, depending on the stage of sporulation.
d Spinach, spring mix, leaf lettuce, romaine, kale, arugula, chard, collards, dandelion greens, rapini.
e Mixed salad (curly and escarole lettuce, red radish, rocket salad and carrots).
f Lettuce, coriander, celery, baby bok choy, leaf lettuce, water spinach, crown daisy, fennel plant, endive, spinach, schizonepeta, cabbage, leaf mustard, Chinese chive, chive and the stripped epidermis of cucumber, watermelon, potato, bean, green chili.
g Blackberries, strawberries, blueberries and mixed berries.
h Five DNA cleanup commercial kits: QIAquick® Purification kit, One step™ PCR inhibitor removal, Nucleospin® Genomic DNA clean up, DNA IQ™ system and DNeasy® Power Plant® Pro kit.
Microsporidia are obligate intracellular pathogens which form durable spores that survive in the environment and infect animals, humans or invertebrates (Wittner, 1999). There are more than 1200 species of microsporidia, however only four are known to be pathogenic to humans (Enterocytozoon bieneusi, Encephalitozoon intestinalis, E. cuniculi, E. hellem) and can cause microsporidiosis. The spores are shed with feces or urine in the environment (soil, water etc.). Infection

### Table 2
Summary of PCR oligonucleotides used for the specific detection of Cyclospora cayetanensis in fresh produce.

| Type of PCR | Target | Sequence | Amplicon size (bp) | Reference |
|-------------|--------|----------|-------------------|-----------|
| Nested      | ITS-1  | 1st: CYCIFE: 5'-TACCCAATGAAAACAGTTT-3'; CYC2RB: 5'-CAGGAGAAGCCCAAGGTTAGC-3' | 294       | Jinneman et al. (1998) |
|             | rRNA   | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' |           | Dixon et al. (2013) |
| nested      |        | 1st: F1E: 5'-TACCCAATGAAAACAGTTT-3'; R2B: 5'-CAGGAGAAGCCCAAGGTTAGC-3' | 636       | Orlandi and Lampel (2000) |
|             |        | 2nd: F3E: 5'-CCTTCCGCTGGCCTGGT-3'; R4B: 5'-CGATTCAACCCCTCTCCTG-3' | 294       | Sim et al. (2017) |
| nested      | multiplex | 1st: F1E + R2B | 636       | Shields et al. (2013) |
|             |        | 2nd: CCITS2 + CRP999; Probe-HEX: 5'-CGAAGAACACCACCCAGCAGCTTG-3' | 298       | Murphy et al. (2017, 2018) |
|             | qPCR MCA | 1st: CYCIFE: 5'-ATTGCAAACCCTCAGAGTAC-3'; CYC2RB: 5'-CGAATACTCATCCACAGC-3' | 101       | Steele et al. (2003) |
|             | qPCR    | 2nd: CYC2FE: 5'-AATTGCAAACCCTCAGAGTAC-3'; CYC2RB: 5'-CGAATACTCATCCACAGC-3' | 500       | Chandra et al. (2014) |
|             |         | 1st: ExCyC: 5'-AATTGCAAACCCTCAGAGTAC-3'; ExCyC: 5'-CGAATACTCATCCACAGC-3' | 294       | Shapiro et al. (2019) |
|             |         | 2nd: NesCyF: 5'-AATTGCAAACCCTCAGAGTAC-3'; NesCyC: 5'-CGAATACTCATCCACAGC-3' | 751–779   | Shapiro et al. (2019) |
|             | qPCR    | 1st: m18SeF: 5'-CGGATCCGCAGATAGG-3'; m18SeR: 5'-AATTCCAGCTCCAATAGTGTAT-3' | 359       | Lalonde and Gajadhar (2016a, 2016b) |
|             |         | 2nd: m18ScycF: 5'-TCGCGGTCAGTCCAATAGG-3'; m18ScycR: 5'-AATTCCAGCTCCAATAGTGTAT-3' | 315       | Shields et al. (2013) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 200       | Orlandi et al. (2008) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 101       | Murphy et al. (2017, 2018)) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 132       | Almeria et al. (2018) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 298       | Assurian et al. (2020a, 2020b) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 359       | Almeria et al. (2003) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 315       | Shields et al. (2013) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 200       | Orlandi et al. (2008) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 101       | Murphy et al. (2017, 2018)) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 132       | Almeria et al. (2018) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 298       | Assurian et al. (2020a, 2020b) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 359       | Almeria et al. (2003) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 315       | Shields et al. (2013) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 200       | Orlandi et al. (2008) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 101       | Murphy et al. (2017, 2018)) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 132       | Almeria et al. (2018) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 298       | Assurian et al. (2020a, 2020b) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 359       | Almeria et al. (2003) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 315       | Shields et al. (2013) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 200       | Orlandi et al. (2008) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 101       | Murphy et al. (2017, 2018)) |
|             | qPCR    | 1st: CYCIFE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 132       | Almeria et al. (2018) |
|             |         | 2nd: CYC2FE: 5'-CCTTCCGCTGGCCTGGT-3'; CYC2RB: 5'-CGATTCAACCCCTCTCCTG-3' | 298       | Assurian et al. (2020a, 2020b) |

MCA = melting curve analysis.

SNP = single nucleotide polymorphism.
a After nested PCR RFLP from MluI restriction enzyme was used to differentiate Cyclospora and Eimeria organisms.
b After nested PCR RFLP from BsaBI restriction enzyme was used to differentiate Cyclospora, Eimeria, Cryptosporidium, Giardia organisms.
c Primers m18SeF and m18SeR not specific only to C. cayetanensis.
d Internal amplification control artificially designed, showing no homology to sequences in GenBank.

2.3. Microsporidia

Microsporidia are obligate intracellular pathogens which form durable spores that survive in the environment and infect animals, humans or invertebrates (Wittner, 1999). There are more than 1200 species of microsporidia, however only four are known to be pathogenic to humans (Enterocytozoon bieneusi, Encephalitozoon intestinalis, E. cuniculi, E. hellem) and can cause microsporidiosis. The spores are shed with feces or urine in the environment (soil, water etc.). Infection...
becomes symptomatic predominantly in immunocompromised individuals, mostly as gastrointestinal illness in the form of mild or severe diarrhea that can become chronic. Infection of the respiratory, reproductive, muscle, excretory, and nervous systems has also been reported (Mathis et al., 2005). Human pathogenic microsporidia have been detected in drinking water, wastewater, groundwater and irrigation water (Izquierdo et al., 2011; Thurston-Enriquez et al., 2002; Ghosh and Weiss, 2009). These findings indicate that waterborne route of transmission is possible and poses a risk of direct infection or the contamination of fresh produce (Jedrzejewski et al., 2007; Thurston-Enriquez et al., 2002).

Microsporidia occurrence on fresh vegetables is rarely monitored. There are only two studies dealing with the detection of microsporidia on fresh produce using molecular methods (Table 5). Although the detection of microsporidia in water by PCR was described in 1998 (Dowd et al., 1998), all other studies dealing with fresh produce are from the last two years. These articles do not focus on the method of detection but on the testing of real samples. Javanmard et al. (2018) and Li et al. (2019) focused on sample processing and PCR method detection. Use of species-specific pair primers or general primer sets for the amplification of all Encephalitozoon spp. for genotyping in environmental samples (water, fresh produce) is beneficial and leads, for example, to

### Table 3

**Echinococcus multilocularis: sample, pre-treatment and molecular detection methods.**

| Origin/item | Amount (g) | Pre-treatment | DNA isolation | Detection method | Spiking (egg amount)/No of positive/tested | Recovery (No. of eggs) | LOD | Reference |
|-------------|------------|---------------|---------------|-----------------|-------------------------------------------|-----------------------|-----|-----------|
| Fruit, vegetable, mushroomsa | 300–500 g fruits/mushrooms, 500 g vegetables, one head of lettuce, two bunches dill or chives | NOb | Chelex-100 + Qiamp DNA mini kit | conventional PCR | NO 0/141c | N/A | N/A | Federer et al. (2016) |
| Fruit, vegetable, mushroomsb | 300–500 g fruits/mushrooms, 500 g of vegetables, one head of lettuce, two bunches of dill or chives | 3× F–Tb | Sherlock AX Kit AntyInhibitor Kit | nested PCR | NO 7/104 | 100 | N/A | Lass et al. (2017) |
| Leafy greens, berries (romaine lettuce and strawberries) | 35 g romaine lettuce, 55 g strawberry samples | BB 8× F–T | FastDNA™ SPIN Kit for soil (include BB), QiAmp® DNA Stool Mini Kit (include F–T) | qPCR | YESd | 5/sample | N/A | Frey et al. (2019) |
| Bilberries | 250 g | NOe | Tissue and Hair Extraction Kit + OneStep™ PCR Inhibitor Removal Kit | qPCR | YESd | 0/141 | N/A | Mäkkinen et al. (2019b) |
| Berries (raspberries or blueberries) | 30 g | BB | DNeasy PowerSoil Kit +2× BB 4 m/s for 60 s | Multiplex qPCR | YES (5, 10) | 5 | 5 eggs/30 g assay could detect 1 egg of EM | Temesgen et al. (2019b) |
| Strawberries | 30 g | BB | DNeasy PowerSoil Kit +2× BB 4 m/s for 60 s | Multiplex qPCR | NO 0/120 | 2 eggs/sample | N/A | Pineda et al. (2020) |
| Lettuce | 300 g; 900–1800 g (totally 158 kg -1413 lettuce heads) | NOh,i | Chelex-100 + Qiamp DNA mini kit | YES (2, 4, 10, 20, 40, 120); 2/157 | N/A | Guggisberg et al. (2020) |

F–T = cycles of freezing at −70 °C and thawing at 30 °C.
BB = bead beating.
EM = *Echinococcus multilocularis*.

a Berry, mushroom, carrot, parsley, beet, celery, radish, lettuce, dill.

b Before DNA isolation, the sample was concentrated by flotation using ZnCl₂ solution, usage of sieves.

c Detected *Echinococcus granulosus* in 2 samples.

d Spiked by *Taenia pisiformis*; lettuce: 500, 100, 50, 10, 5; strawberries 100, 50, 10, 5.

e Before DNA isolation, the sample was concentrated by usage of sieves.

f Before DNA isolation, the sample was concentrated by centrifugation.

### Table 4

**Echinococcus multilocularis: molecular detection methods.**

| Origin/item | Amount (g) | Pre-treatment | DNA isolation | Detection method | Spiking (egg amount)/No of positive/tested | Recovery (No. of eggs) | LOD | Reference |
|-------------|------------|---------------|---------------|-----------------|-------------------------------------------|-----------------------|-----|-----------|
| Fruit, vegetable, mushroomsa | 300–500 g fruits/mushrooms, 500 g vegetables, one head of lettuce, two bunches dill or chives | NOb | Chelex-100 + Qiamp DNA mini kit | conventional PCR | NO 0/141c | N/A | N/A | Federer et al. (2016) |
| Fruit, vegetable, mushroomsb | 300–500 g fruits/mushrooms, 500 g of vegetables, one head of lettuce, two bunches of dill or chives | 3× F–Tb | Sherlock AX Kit AntyInhibitor Kit | nested PCR | NO 7/104 | 100 | N/A | Lass et al. (2017) |
| Leafy greens, berries (romaine lettuce and strawberries) | 35 g romaine lettuce, 55 g strawberry samples | BB 8× F–T | FastDNA™ SPIN Kit for soil (include BB), QiAmp® DNA Stool Mini Kit (include F–T) | qPCR | YESd | 5/sample | N/A | Frey et al. (2019) |
| Bilberries | 250 g | NOe | Tissue and Hair Extraction Kit + OneStep™ PCR Inhibitor Removal Kit | qPCR | YESd | 0/141 | N/A | Mäkkinen et al. (2019b) |
| Berries (raspberries or blueberries) | 30 g | BB | DNeasy PowerSoil Kit +2× BB 4 m/s for 60 s | Multiplex qPCR | YES (5, 10) | 5 | 5 eggs/30 g assay could detect 1 egg of EM | Temesgen et al. (2019b) |
| Strawberries | 30 g | BB | DNeasy PowerSoil Kit +2× BB 4 m/s for 60 s | Multiplex qPCR | NO 0/120 | 2 eggs/sample | N/A | Pineda et al. (2020) |
| Lettuce | 300 g; 900–1800 g (totally 158 kg -1413 lettuce heads) | NOh,i | Chelex-100 + Qiamp DNA mini kit | YES (2, 4, 10, 20, 40, 120); 2/157 | N/A | Guggisberg et al. (2020) |

F–T = cycles of freezing at −70 °C and thawing at 30 °C.
BB = bead beating.
EM = *Echinococcus multilocularis*.

a Berry, mushroom, carrot, parsley, beet, celery, radish, lettuce, dill.

b Before DNA isolation, the sample was concentrated by flotation using ZnCl₂ solution, usage of sieves.

c Detected *Echinococcus granulosus* in 2 samples.

d Spiked by *Taenia pisiformis*; lettuce: 500, 100, 50, 10, 5; strawberries 100, 50, 10, 5.

e Before DNA isolation, the sample was concentrated by usage of sieves.

f Before DNA isolation, the sample was concentrated by centrifugation.
the tracing of sources of infection or to the discovery of new genotypes. Javanmard et al. (2018) aimed to evaluate the presence of zoonotic microsporidia in treated wastewater and vegetable farms (vegetable samples) irrigated with treated wastewater over the course of a year. Among detected microsporidia from examined samples of vegetables and wastewater and samples from animals and humans originated from the same region were revealed a close phylogenetic relationship. From 1099 samples of...
vegetables and fruits, Li et al. (2019) identified eight previously known genotypes and four new genotypes of *E. bieneusi*, which were named CHV1, CHV2, CHV3, CHV4. For microsporidia detection on fruit and vegetables also non-molecular methods can be used. Fluorescence in situ hybridization (FISH) assays use fluorescent probes that bind only to the parts of the DNA sequence with a high degree of sequence complementarity. The microscopy is used for assessing the DNA bounded fluorescent probe. This approach is widely used for example in human sample analysis and can be adapted for fresh produce samples (Graczyk et al., 2007; Jedrzejewski et al., 2007). A total of 80 samples of fresh produce originating from commercial grocery stores, supermarkets, street vendors and markets in Poland were tested by multiplex FISH assay, revealing human-virulent spores (*E. intestinalis, E. bieneusi* and *E. cuniculi*; Jedrzejewski et al., 2007; Table 6).

2.4. Toxocara cati and Toxocara canis

Another parasites distributed worldwide that are an examples of neglected zoonotic parasites are *Toxocara cati* and *Toxocara canis*, ascariid roundworms for which the hosts are felids and canids, respectively, that excrete eggs in feces into the environment. Over time the eggs develop into infective L3 larvae that are able to stay infective for months or even years, depending on the environmental conditions (Azam et al., 2012). The disease caused by *T. cati* and *T. canis* is called toxocariasis. The risk of infection for humans is high because cats usually bury their excrement in loose soil: thus vegetable beds are one of their most sought-after places. The prevalence of *T. canis* and *T. cati* infection in dogs and cats, respectively, ranges around 5% in Germany, the Netherlands and Australia. Even higher prevalence, from 1% to 45% in adult dogs and 3.2%–91% in cats, was observed in countries such as Portugal, Nigeria, India and China (Rostami et al., 2019). The estimated number of cats in Europe is over 106 million, and 87.5 million for dogs (PEDI/F, 2020; Ma et al., 2018). According to an extensive meta-analysis involving 109 studies, the estimated global prevalence of *Toxocara* eggs in public places is around 21%, depending on geographical longitude, latitude and relative humidity (Fakhri et al., 2018). Human toxocariasis can be symptomatic or asymptomatic depending on the anatomic site of the migrating parasite. *T. cati* and *T. canis* can migrate from the intestine by the circulatory system to throughout the body (liver, lungs, brain, eyes, muscles, CNS) and can encyst in these tissues. Clinical toxocariasis in humans often escapes attention due to its non-specific clinical presentation of the disease: visceral larva migrans, ocular larva migrans, neurotoxocariasis and covert toxocariasis (Rostami et al., 2019; Ma et al., 2018).

Over the last 20 years, several studies based on microscopic examination, discussed the detection of *Toxocara* spp. on vegetables. The results revealed a relatively high concentration of *Toxocara* spp. eggs on raw vegetables from local markets in northern Iran, Ethiopia, Poland, Libya, Turkey and Tunisia (Rostami et al., 2016; Tefera et al., 2014; Klapc and Borecka, 2012; Abougrain et al., 2010; Kozan et al., 2005; M’Rad et al., 2020). Only one molecular-based study focused on vegetables (lettuce). In their study, Guggisberg et al. (2020) purchased samples from farmer’s markets and supermarkets in Zürich. The weight of a single analyzed sample varied from 300 g up to 1800 g of lettuce. In total, approximately 158 kg of lettuce heads was analyzed. The parasitic agents assessed were *E. multilocularis, T. gondii, T. cati* and *T. canis*. The authors improved the method for concentrating pathogens using a complex sieving system which simultaneously concentrated helminth and protozoa parasites. Such a complex approach has the potential for widespread use as a recovery and isolation method.

3. Discussion

Food and vegetables are principal components of a healthy diet and have been in high demand in recent years, although eating fresh, minimally processed food is not without risk. Since 1995 until now, major outbreaks of *C. cayetanensis* infection from fresh produce occurred in USA, Canada and Mexico, linked to the fresh produce originated from endemic countries (Hadjilouka and Tsaltas, 2020). From approximately 1100 globally reported outbreaks where etiological agent was identified, 4.5% were caused by parasites (Ramos et al., 2013). Many outbreaks of foodborne parasites on fresh produce are associated with products grown in countries with poor hygiene standards, water sanitation and sanitation in general (Lalonde and Gajadhar, 2016a). In spite of cats and wild carnivores (Poulle et al., 2017). Attention should be focused on ensuring safe fruit and vegetables and good equipment. The basic prevention measures to reduce infectious parasites are treating domestic dogs and cats as well as stray carnivores with anthelmintics, thoroughly washing raw vegetables and fruit, and using adequate heat treatment on animal tissues (Ma et al., 2018). However, a key prevention measure for every household is to secure kitchen gardens from access by dogs, cats and wild carnivores (Poulle et al., 2017). Attention should be focused on ensuring safe fruit and vegetables and good water quality, as well as the possibility of inactivation of parasites prior to human consumption.

4. Conclusion

Molecular detection of parasitic agents in food is essentially in its infancy, in contrast to their detection in common matrices such as feces. There is no recommended procedure, and information on sample processing and detection methods (ISO standards) are spread throughout the literature. In an increasingly globalized world, involving movement of people, animals, food, the study and detection of many foodborne parasites is relevant and should be considered at the global level. At present, we must assume there is unlimited spread of parasites that were previously only considered a local issue. Furthermore, it is necessary to look at
foodborne parasites from a global perspective, taking into account the complex transport routes of food and globalization (Robertson et al., 2013). A summary of sample processing and detection methods seems to be the first step in harmonizing diagnostics and enabling us to compare results worldwide and map global trends regarding foodborne parasites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

A Grant from the Ministry of Agriculture of the Czech Republic No QK1810212 supported this study.

References

Abougain, A.K., Nahaisi, M.H., Madi, N.S., Saied, M.M., Chenghesh, K.S., 2010. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control 21, 760–762.

Almeria, S., da Silva, A.J., Blessington, T., Cloyd, T.C., Cinar, H.N., Durigan, M., et al., 2018. Evaluation of the US Food and Drug Administration validated method for detection of Cyclopsora cayetanensis in high-risk fresh produce matrices and a method modification for a prepared dish. Food Microbiol. 76, 497–503.

Assurian, A., Murphy, H., Ewing, L., Cinar, H.N., da Silva, A., Almeria, S., 2020a. Evaluation of the US Food and Drug Administration validated molecular method for detection of Cyclopsora cayetanensis oocysts on fresh and frozen berries. Food Microbiol. 87, 9.

Assurian, A., Murphy, H., Shipley, A., Cinar, H.N., da Silva, A., Almeria, S., 2020b. Assessment of commercial DNA clean-up kits for elimination of qPCR inhibitors in the detection of Cyclopsora cayetanensis in cilantro. J. Food Prot. 83, 1863–1879.

Azam, D., Ukplai, O.M., Said, A., Abd-Allah, G.A., Morgan, E.R., 2012. Temperature and the development and survival of infective Toxocara canis larvae. Parasitol. Res. 110, 649–656.

Bastien, M., Vaniscotte, A., Combes, B., Uhmberg, G., Germain, E., Vey, V., et al., 2018. High density of fox and cat feces in kitchen gardens and resulting rodent exposure to Echinococcus multilocularis and Toxoplasma gondii. Folia Parasitol. 65, 9.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., et al., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. 12, 2385–2397.

Bennett, S., Escotte-Binet, S., Hauk, R., Huguenin, A., Flori, P., Fournier, L., et al., 2020. Detection methods and prevalence of transmission stages of Toxoplasma gondii, Giardia duodenalis and Cryptosporidium spp. in fresh vegetables: a review. Parasitology 5, 516–532.

Bouwknecht, M., Devleesschauwer, B., Graham, H., Robertson, J.L., van der Giessen, J.W., The Euro-FBP Workshop Participants, 2018. Prioritization of food-borne parasites in Europe. 2016. Eurosurveillance 23,9.

Caradonna, T., Marangi, M., Del Chierico, F., Ferrari, N., Reddel, S., Bracaglia, G., et al., 2017. Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. Food Microbiology 67, 67–75.

Casillas, S.M., Hall, R.L., Herwaldt, B.L., 2019. Cyclosporiasis surveillance - United States, 2001–2015. Morbidity Mortality Weekly Rep. 68 (3), 1–12.

Chandra, V., Torres, M., Ortega, Y.R., 2014. Efficacy of wash solutions in recovering Cyclopsora cayetanensis, Cryptosporidium parvum, and Toxoplasma gondii from basil. J. Food Prot. 77, 1348–1354.

Dawson, D., 2005. Foodborne protozoan parasites. Int. J. Food Microbiol. 103, 207–227.

Dinkel, A., Kern, S., Brinker, A., Oehme, R., Vaniscotte, A., Giraudoux, P., et al., 2011. A real-time multiplex-PCR system for copropathogical diagnosis of Echinococcus multilocularis and host species. Parasitol. Res. 109, 493–498.

Dixon, B., Parrington, L., Cook, A., Farber, J., 2013. Detection of Cyclopsora, Cryptosporidium and Giardia in ready-to-eat packaged leafy greens in Ontario, Canada. J. Food Prot. 76, 307–313.

Dixon, B.R., 2016. Parasitic illnesses associated with the consumption of fresh produce - an emerging issue in developed countries. Curr. Opin. Food Sci. 8, 104–109.

Dowd, S.E., Gerba, C.P., Pepper, I.L., 1998. Confirmation of the human-pathogenic microsporidia Enterocytozoon bieneusi, Encephalitozoon intestinalis, and Vittaforma corneae in water. Appl. Environ. Microbiol. 64, 3323–3325.

Dudlova, A., Juris, P., Juris, K., Cislakova, L., Papajova, I., Krcmery, V., et al., 2015. Molecular detection of Toxocara canis and Toxocara cati larvae. Parasitol. Res. 110, 760–767.

Dudlova, A., Juris, P., Cislakova, L., Papajova, I., Krcmery, V., et al., 2015. Molecular detection of Toxocara canis and Toxocara cati larvae. Parasitol. Res. 110, 760–767.

Dumétére, A., Darde, M.L., 2003. How to detect...
Thevenet, P.S., Alvarez, H.M., Basualdo, J.A., 2017. Survival, physical and physiological changes of *Taenia hydatigena* eggs under different conditions of water stress. Exp. Parasitol. 177, 47–56.
Thurston-Enriquez, J.A., Watt, P., Dowd, S.E., Enriquez, J., Pepper, I.L., Gerba, C.P., 2002. Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. J. Food Prot. 65, 378–382.
Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taenid eggs in the feces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 911–920.
Varma, M., Hester, J.D., Schaefer, F.W., Ware, M.W., Lindquist, H.D.A., 2003. Detection of *Cyclospora cayetanensis* using a quantitative real-time PCR assay. J. Microbiol. Methods 53, 27–36.
Veit, P., Bilger, B., Schad, V., Schafer, J., Frank, W., Lucius, R., 1995. Influence of environmental-factors on the infectivity of *Echinococcus multilocularis* eggs. Parasitology 110, 79–86.
Wittner, M., 1999. Historic perspective on the microsporidia: expanding horizons. The Microsporidia and Microsporidiosis. American Society of Microbiology, pp. 1–6.
Zarlenga, D.S., Trout, J.M., 2004. Concentrating, purifying and detecting waterborne parasites. Vet. Parasitol. 126, 195–217.