Detection of human intestinal protozoan parasites in vegetables and fruits: a review

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Abstract
Diarrheal diseases caused by intestinal protozoan parasites are a major food-borne public health problem across the world. Vegetables and fruits provide important nutrients and minerals, but are also common sources of some food-borne human pathogenic microorganisms. The contamination of raw vegetables and fruits with human pathogenic parasites are now a global public health threat, despite the health benefits of these foods in non-pharmacological prophylaxes against diseases. A large number of reports have documented the contamination of vegetables or fruits with human pathogenic microorganisms. In this paper, we reviewed the contamination and detection methods of human pathogenic intestinal protozoans that are frequently recovered from raw vegetables and fruits. The protozoan parasites include Cryptosporidium spp., Giardia duodenalis, Cyclospora cayetanensis, Entamoeba spp., Toxoplasma gondii, Balantioides coli, Blastocystis sp., Cystoisospora belli and Enterocytozoon bieneusi. The risk factors involved in the contamination of vegetables and fruits with parasites are also assessed.

Keywords: Intestinal protozoans, Detection methods, Vegetables, Fruits, Contamination

Background
Nearly 1.7 billion cases of diarrheal disease are reported globally every year, imposing an annual socioeconomic burden on health services of 72.8 million disability-adjusted life years [1, 2]. A number of pathogens are responsible for causing diarrheal diseases, among which intestinal protozoan parasites are important contributors that can be transmitted by ingestion of the contaminated food [3, 4]. The intestinal protozoan infections are characterized by chronic to severe diarrhea, sometimes accompanied by abdominal cramping, flatulence, nausea, vomiting, anorexia, fatigue, low-grade fever and weight loss [5–7].

Vegetables and fruits provide important nutrients to humans, including various essential vitamins and minerals [8]. The ingestion of raw vegetables and fruits appear to be a quick, easy, and healthy source of nutrition. However, these fresh vegetables and fruits can be an important source of some food-borne pathogenic microorganisms, if they are contaminated [9, 10]. The contamination of raw vegetables and fruits with human parasites has recently been recognized as a global threat, despite the health benefits of these foods in non-pharmacological prophylaxes against diseases.

A number of studies documented the contamination of vegetables and fruits with human pathogenic microorganisms [11–15]. In this paper, we reviewed the detection methods and contamination of some human pathogenic intestinal protozoans that are frequently recovered from raw vegetables and fruits. The protozoan parasites include Cryptosporidium spp., Giardia duodenalis, Cyclospora cayetanensis, Entamoeba spp., Toxoplasma gondii, Balantioides coli, Blastocystis sp., Cystoisospora belli and Enterocytozoon bieneusi.

We searched PubMed and Web of Science databases, with no language restrictions, using the following search terms: ‘Cryptosporidium’ or ‘Giardia’ or ‘Cyclospora’ or ‘Entamoeba’ or ‘Toxoplasma gondii’ or ‘Balantioides...
coli’ or ‘Blastocystis sp.’ or ‘Cystoisospora belli’ or ‘Isospora belli’ or ‘microsporidian’ and ‘vegetable’ or ‘fruit’. Articles were screened using Endnote X9. For articles whose full text was unavailable or that were published in other languages, the titles and abstracts in English were screened. Articles published up to December 31st 2019 were included in this review.

Detection methods of intestinal protozoan parasites contaminating vegetables and fruits

The recovery of parasitic eggs/oocysts/cysts from contaminated vegetables and fruits with proper methods is the first and an important way for the detection of contaminating intestinal protozoa. The methods or techniques for the detection of Cryptosporidium in food samples were well reviewed by Ahmed and Karanis in 2018 [16].

Generally, a washing procedure is the first step in any recovery process. Several elution strategies have been used to isolate the parasites from vegetables and fruits. A portion (usually 50–250 g) of each vegetable or fruit sample is washed separately in a container containing some chemical solutions. The most widely used solutions are normal saline [14, 17–20] and phosphate-buffered saline [12, 21–24]. The commonly used solutions are glycine [11, 25], sodium dodecyl sulfate [26], Alconox® [27], and Tween 80 [28]. Other unusual solutions, such as 10% formal saline [29] and 0.1% peptone water [30] are also reported to isolate the contaminating parasites. Different elution methods can lead to variable recovery rates for parasites from contaminated vegetables or fruits, however, the Alconox® solution was reported to be more effective than the other commonly used solutions [27, 31].

The isolation of the detergent solution sediments is the second key step in parasite detection. Two methods are commonly used to obtain these concentrated sediments. One is the overnight sedimentation of the washing solution [19, 30]. The supernatant is discarded and the sediment is then transferred to a new tube to remove any unwanted material [32]. The other is membrane filtration (more commonly and effectively used), in which the deposit is collected by centrifugation. Membrane filtration devices include stomacher bags [23, 30], zipper bags [22, 24], sieves [18], gauze [21], or cellulose acetate membranes [28].

Finally, the sediment or deposit is screened with light microscopy, staining, immunofluorescence microscopy, or PCR to detect any parasite. More than one smear slide is usually prepared for each specimen to allow its precise detection [12, 26]. Oocysts or cysts can be detected microscopically based on their morphological features [14, 17, 20, 29], using Lugol’s iodine [12, 14, 29] or modified Ziehl-Neelsen staining (or any other staining technique) [14, 19, 26]. The extraction of the parasitic DNA from the sediment, followed by the PCR amplification of specific genes, is also efficiently used for the protozoan detection in vegetable and fruit samples [22, 24].

Contamination of vegetables and fruits with intestinal protozoan parasites

Cryptosporidium contamination

Cryptosporidium spp. are widespread protozoan parasites that infect humans and animals, and the second commonest cause of diarrhea in children after rotavirus [9]. Cryptosporidium is characterized by its extensive genetic variation that results in the existence of 38 species and more than 60 genotypes of this parasite [33]. At least 20 distinct species cause moderate or severe infections in humans, of which C. hominis and C. parvum are the major causative agents [34].

The detection of Cryptosporidium oocysts in vegetable and fruit samples with light microscopy is simple, convenient, and direct [13, 16], but it requires a high level of expertise to interpret the slides, while an immunofluorescence assay is standard practice and more sensitive [16]. Immunomagnetic separation (IMS) is used to concentrate Cryptosporidium oocysts for the efficient detection by microscopy or PCR [12, 25, 35]. The PCR amplification and sequencing of specific genes of Cryptosporidium recovered from contaminated vegetables and fruits is the most precise method of identification of human pathogenic and zoonotic species (e.g., [13, 23–25]. However, PCR is commonly used in developed countries, but most surveillance studies in developing countries involve microscopy.

The contamination of vegetables and fruits with Cryptosporidium spp. has been documented in many countries (Table 1), and the average prevalence is calculated as 6.0% (375/6210; 95% confidence interval, CI: 5.4–6.6%). Among the Cryptosporidium species, C. parvum, C. hominis, and C. ubiquitum were detected in the contaminated vegetable and fruit samples [12, 23, 25, 36]. The Cryptosporidium species are important human pathogens and major causes of human cryptosporidiosis, representing a threat to public health through food as a vehicle.

Giardia duodenalis contamination

Giardia duodenalis (synonyms: G. intestinalis, G. lamblia) is a non-invasive protozoan parasite that adhere to and colonize the upper small intestine, causing acute watery diarrhea in humans and animals [37]. It is an important zoonotic protozoan and the main cause of human giardiasis, which therefore represents a threat to public health [38]. Eight genetically distinct assemblages
| Location     | Detection method                                | Vegetable or fruit item       | No. of samples tested | No. of positive samples (%) | Cryptosporidium species (n)                  | References |
|--------------|-------------------------------------------------|-------------------------------|-----------------------|----------------------------|---------------------------------------------|------------|
| Brazil       | PCR                                             | Vegetables                    | 21                    | 2 (9.5)                    | Cryptosporidium spp. (1); C. parvum (1)     | [45]       |
| China        | PCR                                             | Lettuce                       | 200                   | 0                          |                                             | [36]       |
|              |                                                 | Coriander                     | 152                   | 0                          |                                             |            |
|              |                                                 | Celery                        | 70                    | 0                          |                                             |            |
|              |                                                 | Baby bok choy                 | 59                    | 0                          |                                             |            |
|              |                                                 | Chinese cabbage               | 47                    | 0                          |                                             |            |
|              |                                                 | Leaf lettuce                  | 44                    | 0                          |                                             |            |
|              |                                                 | Water spinach                 | 28                    | 0                          |                                             |            |
|              |                                                 | Crown daisy                   | 27                    | 0                          |                                             |            |
|              |                                                 | Fennel plant                  | 26                    | 0                          |                                             |            |
|              |                                                 | Endive                        | 25                    | 0                          |                                             |            |
|              |                                                 | Spinach                       | 20                    | 0                          |                                             |            |
|              |                                                 | Schizonepeta                  | 20                    | 0                          |                                             |            |
|              |                                                 | Cabbage                       | 18                    | 0                          |                                             |            |
|              |                                                 | Chinese chive                 | 132                   | 1 (0.8)                    | C. parvum (1)                               |            |
|              |                                                 | Chive                         | 128                   | 0                          |                                             |            |
|              |                                                 | Cucumber                      | 41                    | 0                          |                                             |            |
|              |                                                 | Watermelon                    | 15                    | 0                          |                                             |            |
|              |                                                 | Potato                        | 3                     | 0                          |                                             |            |
|              |                                                 | Bean (kidney/French bean)     | 28                    | 0                          |                                             |            |
|              |                                                 | Green chili                   | 5                     | 0                          |                                             |            |
| Costa Rica   | Direct smear, followed by light microscopy      | Cilantro leaves               | 80                    | 4 (5.0)                    | Cryptosporidium spp. (4)                    | [79]       |
|              |                                                 | Cilantro roots                | 80                    | 7 (8.7)                    | Cryptosporidium spp. (7)                    |            |
|              |                                                 | Lettuce                       | 80                    | 2 (2.5)                    | Cryptosporidium spp. (2)                    |            |
|              |                                                 | Radish, tomato, cucumbers,    | 80                    | 1 (1.2)                    | Cryptosporidium spp. (1)                    |            |
|              |                                                 | carrots                       |                       |                             |                                             |            |
| Costa Rica   | Zielh-Nielsen stain, Weber stain                | Lettuce                       | 50                    | 7 (14.0)                   | Cryptosporidium spp. (7)                    | [71]       |
|              |                                                 | Parsley                       | 50                    | 1 (2.0)                    | Cryptosporidium spp. (1)                    |            |
|              |                                                 | Cilantro                      | 50                    | 1 (2.0)                    | Cryptosporidium spp. (1)                    |            |
|              |                                                 | Strawberries                  | 50                    | 0                          |                                             |            |
|              |                                                 | Blackberries                  | 50                    | 3 (6.0)                    | Cryptosporidium spp. (3)                    |            |
|              |                                                 | Fresh fruit juices            | 61.3                  |                             | Cryptosporidium spp.                        | [80]       |
| Egypt        | Wet mount, Weber modified trichrome, modified   | Fruits and vegetables         | 360                   | 46 (12.8)                  | Cryptosporidium spp. (46)                   | [19]       |
|              | Zielh-Neelsen stains                            | Tomato                        | 100                   | 9 (9.0)                    | Cryptosporidium spp. (9)                    | [14]       |
|              |                                                 | Cabbage                       | 96                    | 0                          |                                             |            |
|              |                                                 | Green pepper                  | 66                    | 2 (3.0)                    | Cryptosporidium spp. (2)                    |            |
|              |                                                 | Carrot                        | 62                    | 7 (11.3)                   | Cryptosporidium spp. (7)                    |            |
|              |                                                 | Salad                          | 23                    | 2 (8.7)                    | Cryptosporidium spp. (2)                    |            |
| Ghana        | Zielh-Neelsen stain                             | Cabbage                       | 90                    | 18 (20.0)                  | Cryptosporidium parvum (18)                 | [12]       |
|              |                                                 | Green pepper                  | 55                    | 12 (21.8)                  | Cryptosporidium parvum (12)                 |            |
|              |                                                 | Carrot                        | 47                    | 6 (12.8)                   | Cryptosporidium parvum (6)                  |            |
|              |                                                 | Onion                          | 70                    | 9 (12.9)                   | Cryptosporidium parvum (9)                  |            |
|              |                                                 | Tomato                         | 31                    | 4 (12.9)                   | Cryptosporidium parvum (4)                  |            |
|              |                                                 | Lettuce                       | 102                   | 18 (17.6)                  | Cryptosporidium parvum (18)                 |            |
| Location | Detection method | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | Cryptosporidium species (n) | References |
|----------|------------------|------------------------|----------------------|-----------------------------|-----------------------------|------------|
| Ghana    | Sediment smears and fluorescence stain | Cabbage | 72 | 12 (16.7) | Cryptosporidium spp. (12) | [67] |
|          |                  | Lettuce | 72 | 15 (20.8) | Cryptosporidium spp. (15) | |
|          |                  | Carrot | 72 | 4 (5.6) | Cryptosporidium spp. (4) | |
|          |                  | Spring onion | 72 | 8 (11.1) | Cryptosporidium spp. (8) | |
|          |                  | Tomatoes | 72 | 1 (1.4)  | Cryptosporidium spp. (1) | |
| Ghana    | Direct wet mount, Trichrome, modified Zielh-Nielsen stain | Tiger nuts | 40 | 12 (30.0) | Cryptosporidium parvum (12) | [81] |
| India    | DAPI-stain followed by fluorescence microscopy, and PCR | Cabbage | 47 | 3 (6.4)  | Cryptosporidium parvum (3) | [13] |
|          |                  | Chili | 42 | 2 (4.8)  | Cryptosporidium spp. (2)  | |
|          |                  | Coriander | 28 | 2 (7.1)  | Cryptosporidium spp. (2)  | |
|          |                  | Cucumber | 52 | 3 (5.8)  | Cryptosporidium parvum (3) | |
|          |                  | Radish | 14 | 1 (7.1)  | Cryptosporidium spp. (1)  | |
|          |                  | Tomatoes | 56 | 6 (10.7) | Cryptosporidium spp. (6)  | |
| Iran     | Modified Ziehl-Neelsen acid-fast stain | Mint | 82 | 7 (8.5)  | Cryptosporidium spp. (7)  | [26] |
|          |                  | Leek | 90 | 3 (3.3)  | Cryptosporidium spp. (3)  | |
|          |                  | Cress | 90 | 8 (8.9)  | Cryptosporidium spp. (8)  | |
|          |                  | Green onion | 54 | 8 (14.8) | Cryptosporidium spp. (8)  | |
|          |                  | Coriander | 90 | 6 (6.7)  | Cryptosporidium spp. (6)  | |
|          |                  | Basil | 90 | 1 (1.1)  | Cryptosporidium spp. (1)  | |
| Korea    | qPCR | Vegetables | 34 | 3 (8.8)  | Cryptosporidium spp. (3)  | [72] |
|          |                  | Ready-to-eat packaged salads | 648 | 6 (0.9) | Cryptosporidium parvum/C. ubiquitous (6) | [23] |
| Korea    | Multiplex qPCR | Carrots | 3 | 1 (33.3) | Cryptosporidium parvum (1) | [22] |
|          |                  | Cabbages | 3 | 1 (33.3) | Cryptosporidium parvum (1) | |
|          |                  | Blue berries | 3 | 1 (33.3) | Cryptosporidium parvum (1) | |
|          |                  | Perilla leaves | 72 | 5 (6.9)  | Cryptosporidium spp. (5)  | [24] |
|          |                  | Winter-grown cabbage | 70 | 4 (5.7)  | Cryptosporidium spp. (4)  | |
|          |                  | Chives | 73 | 13 (17.8) | Cryptosporidium spp. (13) | |
|          |                  | Sprouts | 72 | 1 (1.4)  | Cryptosporidium spp. (1)  | |
|          |                  | Blueberries | 44 | 3 (6.8)  | Cryptosporidium spp. (3)  | |
|          |                  | Cherry tomatoes | 73 | 5 (6.8)  | Cryptosporidium spp. (5)  | |
| Norway   | Concentrated by IMS, and screening by light microscopy | Alfalfa sprouts | 16 | 0 | | [35] |
|          |                  | Dill | 7 | 0 | | |
|          |                  | Lettuce | 125 | 5 (4.0) | | Cryptosporidium spp. (5) |
|          |                  | Mung bean sprouts | 149 | 14 (9.4) | | Cryptosporidium spp. (14) |
|          |                  | Mushrooms | 55 | 0 | | |
|          |                  | Parsley | 7 | 0 | | |
|          |                  | Precut salad mix | 38 | 0 | | |
|          |                  | Radish sprouts | 6 | 0 | | |
|          |                  | Raspberries | 10 | 0 | | |
|          |                  | Strawberries | 62 | 0 | | |
| Norway   | Concentrated by IMS, and screening by light microscopy | Alfalfa | 16 | 0 | | [82] |
|          |                  | Mung bean | 149 | 14 (9.4) | | Cryptosporidium spp. (14) |
|          |                  | Radish | 6 | 0 | | |
| Peru     | Direct microscopic observation, acid-fast staining, and immunofluorescent assays | Vegetables | 14.5 | 14.5 | Cryptosporidium parvum | [83] |
(A to H) of G. duodenalis have been defined, with the occurrence of zoonotic assemblages A and B in both humans and animals. However, the other assemblages are mostly specific to animal hosts [38]. This parasite is estimated to cause ~28.2 million cases of diarrhea annually through the ingestion of contaminated foods [7]. The outbreaks of giardiasis have also been associated with a variety of processed foods. Human infections of G. duodenalis are often associated with the consumption of contaminated raw vegetables and fruits [39–41].

Giardia duodenalis cysts can be detected with light microscopy based on their morphological features [19, 42, 43], and staining with typical Lugol’s iodine is universally used for the detection of G. duodenalis cysts [12, 14, 17, 18, 29]. However, an immunofluorescence assay is usually applied for the detection of Giardia cysts in food items with more sensitivity [7]. The IMS method is also applied to concentrate G. duodenalis cysts for further detection [11, 35]. The PCR amplification and sequencing of specific G. duodenalis genes recovered from contaminated food are also commonly used for the confirmatory detection of this parasite (e.g. [28, 39, 44]).

The contamination of vegetables and fruits with G. duodenalis cysts has been reported in many countries (Table 2), and the average prevalence is estimated as 4.8% (276/5739; 95% CI: 4.2–5.4%). In contaminated vegetable and fruit samples, G. duodenalis zoonotic assemblages A and B were commonly detected [23, 28, 39, 44, 45].

Cyclospora cayetanensis is another important protist parasite, usually transmitted via food that causes human gastrointestinal cyclosporiasis [5, 46]. Globally, C. cayetanensis is an important food-borne human protozoan [5, 46]. Many reports have documented the food-borne cyclosporiasis outbreaks that were associated with the consumption of contaminated raw vegetables or fruits.

Cyclospora cayetanensis oocysts can be detected simply and directly with light microscopy provided that there are a large number of oocysts present in the vegetables and fruits [23, 37]. Modified Ziehl-Neelsen staining, and autofluorescence or immunofluorescence assays are also commonly used for their detection [12, 14, 19, 47]; however, there are no immunofluorescence assays commercially available for Cyclospora. Furthermore, PCR amplification and sequencing of C. cayetanensis genes have currently been used for the specific detection of this organism in contaminated food samples [23, 24, 48].

The contamination of vegetables and fruits with C. cayetanensis oocysts have been documented in many countries (Table 3). The average prevalence of C. cayetanensis contamination is counted as 3.9% (180/4628; 95% CI: 3.3–4.5%).

Entamoeba contamination

Among the Entamoeba spp., E. histolytica is responsible for most cases of human amebiasis and remains one of the top three causes of parasitic mortality worldwide [49]. Although some of the E. histolytica infections are asymptomatic, many infections may lead to severe amoebic colitis and disseminated disease [50]. Entamoeba spp. infections are significantly associated with the consumption of contaminated vegetables and fruits [17, 41, 51, 52].

Entamoeba spp. cysts can be detected with light microscopy based on their morphological features [29, 42, 43]. Staining with Lugol’s iodine is widely used to detect the Entamoeba spp. cysts (e.g. [12, 14, 17, 19, 52]). The PCR technique is also commonly used to detect Entamoeba spp. in food items based on amplification and sequencing of specific genes [23, 53].

Many reports have documented the contamination of raw vegetables and fruits with Entamoeba spp. cysts worldwide (Table 4). The average prevalence of Entamoeba contamination is calculated as 3.5% (199/5647; 95% CI: 3.0–4.0%). Entamoeba histolytica, E. dispar and E. coli were the most commonly detected species among

| Location | Detection method | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | Cryptosporidium species (n) | References |
|----------|----------------|------------------------|----------------------|----------------------------|----------------------------|------------|
| Poland | Separated by IMS and identified by immunofluorescence and DIC microscopy, and PCR identified | Fresh vegetables | 128 | 6 (4.7) | Cryptosporidium parvum or C. hominis (6) | [25] |
| Spain | Concentrated by IMS and stained for immunofluorescence assay | Chinese cabbage | 6 | 2 (33.3) | Cryptosporidium spp. (2) | [11] |
| | | Lollo rosso lettuce | 4 | 3 (75.0) | Cryptosporidium spp. (3) | |
| | | Romaine lettuce | 9 | 7 (77.8) | Cryptosporidium spp. (7) | |
| Total | | | 6210 | 375 (6.0) | | |
Table 2  Contamination of vegetables and fruits with *Giardia duodenalis*

| Location                        | Detection method                                      | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | Giardia duodenalis assemblages identified (n) | References |
|---------------------------------|-------------------------------------------------------|-------------------------|------------------------|-----------------------------|-----------------------------------------------|------------|
| Bangladesh                      | Iodine and normal saline wet mount                    | Vegetables              | 200                    | 2 (1.0)                     | [52](#)                                      | 52         |
| Brazil                          | PCR                                                   | Lettuce and chicory     | 11                     | 2 (18.2)                    | Assemblage BIV (2)                           | 39         |
| Brazil                          | Immunofluorescence, PCR                               | Arugula                 | 4                      | 2 (50.0)                    | Assemblage AII (2)                           | 28         |
| Brazil                          | Semi-nested PCR                                       | Chives                  | 12                     | 1 (8.3)                     | Assemblage AII (1)                           |            |
| Brazil                          | Semi-nested PCR                                       | Crisp lettuce           | 32                     | 4 (12.5)                    | Assemblage AII (4)                           |            |
| Brazil                          | Semi-nested PCR                                       | Greens collard          | 24                     | 1 (4.2)                     | Assemblage AII (1)                           |            |
| Brazil                          | Semi-nested PCR                                       | Parsley                 | 12                     | 2 (16.7)                    | Assemblage AII (2)                           |            |
| Brazil                          | Semi-nested PCR                                       | Watercress              | 12                     | 4 (33.3)                    | Assemblage AII (4)                           |            |
| Brazil                          | Semi-nested PCR                                       | Wild chicory            | 12                     | 2 (16.7)                    | Assemblage AII (2)                           |            |
| Brazil                          | PCR                                                   | Regular lettuce         | 60                     | 8 (13.3)                    | Assemblage AII (4); Assemblage B (1); Assemblage E (1); N/D (2) | 44         |
| Brazil                          | Sediment being stained in Lugol’s solution            | Crisp lettuce           | 100                    | 5 (5.0)                     | Assemblage AII (2); N/D (3)                  |            |
| Brazil                          | Sediment being stained in Lugol’s solution            | Chicory                 | 60                     | 5 (8.3)                     | Assemblage AII (3); N/D (2)                  |            |
| Brazil                          | Sediment being stained in Lugol’s solution            | Rocket                  | 20                     | 1 (5.0)                     | N/D (1)                                      |            |
| Brazil                          | Sediment being stained in Lugol’s solution            | Kale                    | 20                     | 0                           |                                                |            |
| Costa Rica                      | Direct smear, followed by light microscopy            | Vegetables              | 21                     | 10 (47.6)                   | Assemblage E (2); N/D (8)                    | 45         |
| Costa Rica                      | Direct smear, followed by light microscopy            | Lettuce                 | 100                    | 0                           |                                                | 15         |
| Costa Rica                      | Direct smear, followed by light microscopy            | Coriander               | 100                    | 1 (1.0)                     |                                                |            |
| Egypt                           | Lugol’s iodine stain                                  | Cilantro leaves         | 80                     | 4 (5.0)                     |                                                | 79         |
| Egypt                           | Lugol’s iodine stain                                  | Cilantro roots          | 80                     | 2 (2.5)                     |                                                |            |
| Egypt                           | Lugol’s iodine stain                                  | Lettuce                 | 101                    | 16 (15.8)                   |                                                | 18         |
| Egypt                           | Lugol’s iodine stain                                  | Watercress              | 116                    | 13 (11.2)                   |                                                |            |
| Egypt                           | Lugol’s iodine stain                                  | Parsley                 | 102                    | 12 (11.8)                   |                                                |            |
| Egypt                           | Lugol’s iodine stain                                  | Green onion             | 103                    | 4 (3.9)                     |                                                |            |
| Egypt                           | Lugol’s iodine stain                                  | Leek                    | 108                    | 2 (1.9)                     |                                                |            |
| Ethiopia                        | Lugol’s iodine stain                                  | Fruits and vegetables   | 360                    | 27 (7.5)                    |                                                | 19         |
| Ethiopia                        | Sediment smear under light microscope                | Fruits and vegetables   | 360                    | 36 (10.0)                   |                                                | 32         |
| Ethiopia                        | Sediment smear under light microscope                | Tomatoes                | 45                     | 1 (2.2)                     |                                                | 43         |
| Ethiopia                        | Sediment smear under light microscope                | Lettuce                 | 45                     | 4 (8.8)                     |                                                |            |
| Ethiopia                        | Sediment smear under light microscope                | Carrot                  | 45                     | 7 (15.6)                    |                                                |            |
| Ethiopia                        | Sediment smear under light microscope                | Cabbage                 | 45                     | 8 (17.8)                    |                                                |            |
| Ethiopia                        | Sediment smear under light microscope                | Green pepper            | 45                     | 6 (13.3)                    |                                                |            |
| Ethiopia                        | Sediment smear under light microscope                | Avocado                 | 45                     | 0                           |                                                |            |
| Ethiopia                        | Sediment smear and Lugol’s iodine stain             | Tomato                  | 100                    | 0                           |                                                | 14         |
| Ethiopia                        | Sediment smear and Lugol’s iodine stain             | Cabbage                 | 96                     | 16 (16.7)                   |                                                |            |
| Ethiopia                        | Sediment smear and Lugol’s iodine stain             | Green pepper            | 66                     | 4 (6.1)                     |                                                |            |
| Ethiopia                        | Sediment smear and Lugol’s iodine stain             | Carrot                  | 62                     | 4 (6.5)                     |                                                |            |
| Ethiopia                        | Sediment smear and Lugol’s iodine stain             | Salad                   | 23                     | 0                           |                                                |            |
| Ghana                           | Lugol’s iodine stain                                  | Cabbage                 | 90                     | 5 (5.6)                     |                                                | 12         |
| Ghana                           | Lugol’s iodine stain                                  | Green pepper            | 55                     | 3 (5.5)                     |                                                |            |
| Ghana                           | Lugol’s iodine stain                                  | Carrot                  | 47                     | 4 (8.5)                     |                                                |            |
| Ghana                           | Lugol’s iodine stain                                  | Onion                   | 70                     | 3 (4.3)                     |                                                |            |
| Ghana                           | Lugol’s iodine stain                                  | Tomato                  | 31                     | 2 (6.5)                     |                                                |            |
| Ghana                           | Lugol’s iodine stain                                  | Lettuce                 | 102                    | 5 (4.9)                     |                                                |            |
Table 2 (continued)

| Location          | Detection method                          | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | Giardia duodenalis assemblages identified (n) | References |
|-------------------|-------------------------------------------|-------------------------|-----------------------|-----------------------------|-----------------------------------------------|------------|
| India             | DAPI-stain followed by fluorescence microscopy, and PCR | Cabbage                 | 47                    | 1 (2.1)                     |                                                | [13]       |
|                   |                                            | Carrot                  | 25                    | 1 (4.0)                     |                                                |            |
|                   |                                            | Chili                   | 42                    | 4 (9.5)                     |                                                |            |
|                   |                                            | Coriander               | 28                    | 3 (10.7)                    | Assemblage D (1)                              |            |
|                   |                                            | Cucumber                | 52                    | 1 (1.9)                     | Assemblage D (1)                              |            |
|                   |                                            | Tomatoes                | 56                    | 2 (3.6)                     | Assemblage A (2)                              |            |
|                   |                                            | Turnip                  | 3                     | 1 (33.3)                    | Assemblage A (2)                              |            |
| Iran              | Lugol’s iodine stain                       | Vegetables              | 141                   | 11 (7.8)                    |                                               | [84]       |
| Iran              | Sediment smear under light microscopy      | Leek                    | 30                    | 3 (10.0)                    |                                               | [42]       |
|                   |                                            | Spring onion            | 22                    | 0                           |                                               |            |
|                   |                                            | Basil                   | 15                    | 1 (6.7)                     |                                               |            |
|                   |                                            | Parsley                 | 21                    | 0                           |                                               |            |
|                   |                                            | Lettuce                 | 23                    | 0                           |                                               |            |
|                   |                                            | Cress                   | 17                    | 0                           |                                               |            |
|                   |                                            | Spearmint               | 18                    | 0                           |                                               |            |
|                   |                                            | Tarragon                | 19                    | 0                           |                                               |            |
|                   |                                            | Coriander               | 24                    | 0                           |                                               |            |
|                   |                                            | Radish                  | 29                    | 0                           |                                               |            |
| Italy             | Lugol’s iodine stain and PCR               | Ready-to-eat packaged salad | 648                  | 4 (0.6)                     | Assemblage A (4)                              | [23]       |
| Jordan            | Lugol’s iodine stain                       | Lettuce                 | 30                    | 7 (23.3)                    |                                               | [20]       |
| Norway            | Concentrated by IMS, and screening by light microscopy | Alfalfa sprouts         | 16                    | 0                           |                                               | [35]       |
|                   |                                            | Dill                    | 7                     | 2 (28.6)                    |                                               |            |
|                   |                                            | Lettuce                 | 125                   | 2 (1.6)                     |                                               |            |
|                   |                                            | Mung bean sprouts       | 149                   | 3 (2.0)                     |                                               |            |
|                   |                                            | Mushrooms               | 55                    | 0                           |                                               |            |
|                   |                                            | Parsley                 | 7                     | 0                           |                                               |            |
|                   |                                            | Precut salad mix        | 38                    | 0                           |                                               |            |
|                   |                                            | Radish sprouts          | 6                     | 1 (16.7)                    |                                               |            |
|                   |                                            | Raspberries             | 10                    | 0                           |                                               |            |
|                   |                                            | Strawberries            | 62                    | 2 (3.2)                     |                                               |            |
| Norway            | Concentrated by IMS, and screening by light microscopy | Alfalfa                 | 16                    | 0                           |                                               | [82]       |
|                   |                                            | Mung bean               | 149                   | 3 (2.0)                     |                                               |            |
|                   |                                            | Radish                  | 6                     | 1 (16.7)                    |                                               |            |
| Saudi Arabia      | Lugol’s iodine stain                       | Green onion             | 50                    | 0                           |                                               | [17]       |
|                   |                                            | Watercress              | 50                    | 0                           |                                               |            |
|                   |                                            | Lettuce                 | 50                    | 0                           |                                               |            |
|                   |                                            | Cucumber                | 50                    | 0                           |                                               |            |
|                   |                                            | Cabbage                 | 50                    | 0                           |                                               |            |
|                   |                                            | Pea                     | 50                    | 0                           |                                               |            |
|                   |                                            | Tomato                  | 50                    | 0                           |                                               |            |
|                   |                                            | Carrot                  | 50                    | 4 (8.0)                     |                                               |            |
the isolates from contaminated vegetables and fruits [12, 17, 29, 42].

**Toxoplasma gondii** contamination

*Toxoplasma gondii* is a ubiquitous protozoan parasite capable of infecting virtually all warm-blooded animals [54]. According to a new nomenclature system, *T. gondii* genotypes are classified as Type I, Type II or Type III. Other atypical or exotic genotypes include Chinese 1, Type Br I, Type Br II, Type Br III, Type IV and Type 12 [55, 56]. Among the three principal routes of toxoplasmosis transmission, consumption of unwashed vegetables and fruits contaminated with cat feces is an important one that sometimes may lead to food-borne outbreaks [57]. The significant association of *T. gondii* infections with the consumption of contaminated raw vegetables is also observed in previous studies [58–60].

The detection of *Toxoplasma gondii* in contaminated vegetables and fruits is usually performed by PCR amplification [23, 61–63]. The contamination of vegetables and fruits with *T. gondii* was observed in Brazil, China, Italy and Poland (Table 5), and the average prevalence of the contamination was estimated as 3.8% (63/1676; 95% CI: 2.9–4.7%). The *T. gondii* isolates obtained from vegetables and fruits belonged to genotypes Type I and II [23, 61, 64].

**Other intestinal protozoan contaminations**

Fresh vegetables and fruits are occasionally contaminated with some other intestinal protozoans, such as *Balantioides coli*, *Cystoisospora belli*, *Blastocystis* sp. and *Enterocytozoon bieneusi*.

Several reports have documented *B. coli* contamination of vegetables, leading to global public health concerns [65]. *Balantioides coli* is usually detected on vegetables and fruits with light microscopy [14, 30, 52, 66, 67]. The contamination of vegetables with *B. coli* has been reported in Bangladesh, Brazil, Cameroon, Ethiopia, and Ghana (Table 6) and the average prevalence of the contamination is calculated as 9.3% (72/907; 95% CI: 7.6–11.0%).

*Cystoisospora belli* infection is commonly reported in tropical and subtropical areas of the world [68]. *Cystoisosporiasis* can be acquired through the ingestion of contaminated food. *Cystoisospora belli* is commonly detected with modified Ziehl-Neelsen staining, followed by microscopy [32, 43]. There are three reports on *Cystoisospora belli* contamination in vegetables and fruits in Ethiopia and Ghana (Table 6). The average prevalence of the contamination is estimated as 1.9% (19/1025; 95% CI: 1.1–2.7%).

The detection of *Blastocystis* sp. is usually based on microscopy and PCR [23]. Cell culture is also used for the detection of this parasite. The contamination of vegetables and fruits with *Blastocystis* sp. has only been documented in Brazil and Italy, with a prevalence of 4.4% (37/848; 95% CI: 3.0–5.8%) (Table 6).

*Enterocytozoon bieneusi* is an important microsporidian species infecting humans [69]. The genetic diversity of the pathogen is inferred by the analysis of

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**Table 2 (continued)**

| Location | Detection method | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | *Giardia duodenalis* assemblages identified (n) | References |
|----------|------------------|-------------------------|-----------------------|----------------------------|-----------------------------------------------|------------|
| Spain    | Concentrated by IMS and stain cysts for immunofluorescence assay | Chinese cabbage 6 | 2 (33.3) | [11] |
|          |                   | Lolro rosso lettuce 4 | 3 (75.0) |  |
|          |                   | Romaine lettuce 9 | 5 (55.6) |  |
| Sudan    | Lugol’s iodine stain | Tomatoes 36 | 1 (2.8) |  |
|          |                   | Cucumber 12 | 0 |  |
|          |                   | Armenian cucumber 16 | 0 |  |
|          |                   | Green pepper 25 | 1 (4.0) |  |
|          |                   | Cayenne pepper 7 | 0 |  |
|          |                   | Radish 24 | 1 (4.2) |  |
|          |                   | Beet 19 | 0 |  |
|          |                   | Watercress 23 | 2 (8.7) |  |
|          |                   | Lettuce 11 | 1 (9.1) |  |
|          |                   | Green onion 36 | 1 (2.8) |  |
|          |                   | Carrot 50 | 1 (2.0) |  |
| Total    |                   | 5739 | 276 (4.8) |  |

*Giardia duodenalis, G. intestinalis, G. lamblia*
### Table 3  Contamination of vegetables and fruits with *Cyclospora cayetanensis*

| Location | Detection method | Vegetable or fruit item       | No. of samples tested | No. of positive samples (%) | References |
|----------|-----------------|-----------------------------|-----------------------|-----------------------------|------------|
| **Cameroon** | Sediment smear, followed by light microscopy | Green cabbage | 30 | 0 | [66] |
| | | Red cabbage | 30 | 0 | |
| | | Lettuce | 30 | 10 (33.3) | |
| | | Cucumber | 30 | 0 | |
| | | Carrots | 30 | 0 | |
| | | Green pepper | 30 | 20 (66.7) | |
| **China** | PCR | Lettuce | 200 | 1 | [36] |
| | | Coriander | 152 | 0 | |
| | | Celery | 70 | 0 | |
| | | Baby bok choy | 59 | 0 | |
| | | Chinese cabbage | 47 | 0 | |
| | | Leaf lettuce | 44 | 1 (2.3) | |
| | | Water spinach | 28 | 0 | |
| | | Crown daisy | 27 | 0 | |
| | | Fennel plant | 26 | 0 | |
| | | Endive | 25 | 0 | |
| | | Spinach | 20 | 0 | |
| | | Schizonepeta | 20 | 0 | |
| | | Cabbage | 18 | 0 | |
| | | Leaf mustard | 11 | 0 | |
| | | Chinese chive | 132 | 0 | |
| | | Chive | 128 | 0 | |
| | | Cucumber | 41 | 0 | |
| | | Watermelon | 15 | 0 | |
| | | Potato | 3 | 0 | |
| | | Bean (kidney/French bean) | 28 | 0 | |
| | | Green chili | 5 | 0 | |
| **Costa Rica** | Zielh-Nielsen and Weber stain | Lettuce | 50 | 2 (4.0) | [71] |
| | | Parsley | 50 | 0 | |
| | | Cilantro | 50 | 0 | |
| | | Strawberries | 50 | 0 | |
| | | Blackberries | 50 | 0 | |
| **Egypt** | Weber modified trichrome and modified Zielh-Neelsen stains | Fresh fruit juices | 14.5 |  | [80] |
| **Ethiopia** | Modified Zielh-Neelsen stain | Fruits and vegetables | 360 | 18 (5.0) | [19] |
| **Ethiopia** | Modified Zielh-Neelsen stain | Fruits and vegetables | 360 | 25 (6.9) | [32] |
| **Ethiopia** | Modified Zielh-Neelsen stain | Tomato | 100 | 4 (4.0) | [14] |
| | | Cabbage | 96 | 0 | |
| | | Green pepper | 66 | 2 (3.0) | |
| | | Carrot | 62 | 0 | |
| | | Salad | 23 | 1 (4.5) | |
| **Ghana** | Direct wet mount, trichrome modified Zielh-Neelsen stain | Tiger nuts | 40 | 9 (22.5) | [81] |
| **Ghana** | Zielh-Neelsen stain | Cabbage | 90 | 5 (5.6) | [12] |
| | | Green pepper | 55 | 3 (5.5) | |
| | | Carrot | 47 | 3 (6.4) | |
| | | Onion | 70 | 3 (4.3) | |
| | | Tomato | 31 | 3 (9.7) | |
| | | Lettuce | 102 | 3 (2.9) | |
| **Italy** | qPCR | Vegetables | 49 | 6 (12.2) | [48] |
single nucleotide polymorphisms (SNPs) in the internal transcribed spacer (ITS) that resulted in nearly 500 valid genotypes of the pathogen [70]. The phylogenetic analysis of the valid genotypes recognized eleven genetic groups (Groups 1 to 11), figuring out their host specificity and zoonotic potential. Food-borne transmission of *E. bieneusi* has been documented and the contamination of vegetables and fruits with this pathogen was reported in China, Costa Rica and Poland (Table 6). The parasite was successfully detected in contaminated vegetables and fruits by staining or with fluorescence **in situ** hybridization [21, 71], and PCR amplification [36]. The average prevalence of the reported contamination was estimated as 3.6% (52/1429; 95% CI: 2.6–4.6%).

**Risk factors involved in the contamination of vegetables and fruits with parasites**

Previous studies in Ethiopia, Ghana, Brazil and Iran reported a relatively higher prevalence of intestinal parasitic infections associated with the consumption of vegetables sold at open-air markets than those associated with supermarkets [12, 14, 15]. The parasitic load in the raw vegetables of open markets was high and posed a high risk of parasitic infections. The high contamination rates recorded in the open-market samples indicate poor hygiene in these locations, which is suitable for the propagation and transmission of the parasites [72].

High risk of diarrhea among raw vegetable consumers in the Kathmandu valley of Nepal, mostly due to the use of river water by farmers for washing vegetables, suggests a need to avoid the use of river water for washing vegetables [73]. There are also many reports that highlight the contamination of surface water with parasitic infective stages in Brazil [74], Iran [75], Poland [76] and Spain [77]. The use of such contaminated surface water for washing fresh vegetables and fruits might cause parasitic contamination.

Another study in the Czech Republic reported a significantly higher contamination of *T. gondii* in vegetables collected from farm storage rooms than those from fields [64], indicating a higher chance of contamination of vegetables and fruits during processing and selling [78]. Therefore, the adaptation of good practices in every step between farm and fork, such as production, processing, storage and selling minimize the microbial contamination of vegetables and fruits.

**Conclusions**

The accidental ingestion of parasitic infective stages such as eggs, oocysts, cysts or spores with the contaminated raw vegetables or fruits causes varying intestinal diseases in humans that sometimes may lead to serious
Table 4 Contamination of vegetables and fruits with Entamoeba spp.

| Location       | Detection method                                      | Vegetable or fruit item | Number of samples tested | Number of positive samples (%) | Entamoeba species identified (n) | References |
|----------------|-------------------------------------------------------|-------------------------|--------------------------|--------------------------------|----------------------------------|------------|
| Bangladesh     | Wet mount                                             | Vegetables              | 200                      | 17 (8.5)                       | Entamoeba histolytica            | [52]       |
| Brazil         | Direct smear, followed by light microscopy            | Lettuce                 | 30                       | 3 (10.0)                       | Entamoeba coli (3)               | [85]       |
| Brazil         | Lugol's iodine stain                                 | Loose leaf lettuce<sup>a</sup> | 1                        | 1                               | Entamoeba sp.                    | [30]       |
| Brazil         |                                                      | Red lettuce<sup>a</sup> | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Curly lettuce<sup>a</sup> | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Iceberg lettuce<sup>a</sup> | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Parsley<sup>a</sup>     | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Chive<sup>a</sup>       | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Coriander<sup>a</sup>   | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Basil<sup>a</sup>       | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Arugula<sup>a</sup>     | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Chicory<sup>a</sup>     | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Kale<sup>a</sup>        | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         |                                                      | Bean sprouts<sup>a</sup> | 1                        | 1                               | Entamoeba sp.                    |            |
| Brazil         | Sediment smear, followed by light microscopy         | Vegetables              | 100                      | 32 (32.0)                      | Entamoeba spp. (32)              | [86]       |
| Brazil         | Sediment being stained in Lugol's solution           | Lettuce                 | 100                      | 9 (9.0)                        | Entamoeba histolytica (9)        | [15]       |
| Brazil         |                                                      | Coriander               | 100                      | 11 (11.0)                      | Entamoeba histolytica (11)       |            |
| Cameroon        | Lugol's iodine stain                                 | Green cabbage           | 30                       | 5 (16.7)                       | Entamoeba spp. (5)               | [66]       |
| Cameroon        |                                                      | Red cabbage             | 30                       | 3 (10.0)                       | Entamoeba spp. (3)               |            |
| Cameroon        |                                                      | Lettuce                 | 30                       | 9 (30.0)                       | Entamoeba spp. (9)               |            |
| Cameroon        |                                                      | Cucumber                | 30                       | 5 (16.7)                       | Entamoeba spp. (5)               |            |
| Cameroon        |                                                      | Carrots                 | 30                       | 3 (10.0)                       | Entamoeba spp. (3)               |            |
| Cameroon        |                                                      | Green pepper            | 30                       | 5 (16.7)                       | Entamoeba spp. (5)               |            |
| Costa Rica      | Direct smear, followed by light microscopy           | Cilantro leaves         | 80                       | 5 (6.2)                        | Entamoeba histolytica (5)        | [79]       |
| Costa Rica      |                                                      | Cilantro roots          | 80                       | 2 (2.5)                        | Entamoeba histolytica (2)        |            |
| Costa Rica      |                                                      | Lettuce                 | 80                       | 3 (3.8)                        | Entamoeba histolytica (3)        |            |
| Costa Rica      |                                                      | Radish                  | 80                       | 2 (2.5)                        | Entamoeba histolytica (2)        |            |
| Egypt           | Lugol's iodine stain                                 | Lettuce                 | 101                      | 14 (13.9)                      | Entamoeba spp. (14)              | [18]       |
| Egypt           |                                                      | Watercress              | 116                      | 9 (7.8)                        | Entamoeba spp. (9)               |            |
| Egypt           |                                                      | Parsley                 | 102                      | 8 (7.8)                        | Entamoeba spp. (8)               |            |
| Egypt           |                                                      | Green onion             | 103                      | 2 (1.9)                        | Entamoeba spp. (2)               |            |
| Egypt           |                                                      | Leek                    | 108                      | 3 (2.8)                        | Entamoeba spp. (3)               |            |
| Ethiopia        | Lugol's iodine stain                                 | Fruits and vegetables   | 360                      | 19 (5.3)                       | Entamoeba histolytica/E. dispar  | [19]       |
| Ethiopia        | Sediment smear                                       | Fruits and vegetables   | 360                      | 52 (14.4)                      | E. histolytica/dispar (52)       | [32]       |
| Ethiopia        | Lugol's iodine stain                                 | Tomato                  | 100                      | 22 (22.0)                      | E. histolytica (22)              | [14]       |
| Ethiopia        |                                                      | Cabbage                 | 96                       | 0                               | E. histolytica (7)               |            |
| Ethiopia        |                                                      | Green pepper            | 66                       | 0                               | E. histolytica (7)               |            |
| Ethiopia        |                                                      | Carrot                  | 62                       | 7 (11.3)                       | E. histolytica (7)               |            |
| Ethiopia        |                                                      | Salad                   | 23                       | 0                               | E. histolytica (7)               |            |
| Location     | Detection method                  | Vegetable or fruit item | Number of samples tested | Number of positive samples (%) | Entamoeba species identified (n) | References |
|--------------|-----------------------------------|-------------------------|--------------------------|-------------------------------|---------------------------------|------------|
| Ethiopia     | Sediment smear under light microscope | Tomatoes            | 45                       | 1 (2.2)                       | E. histolytica/E. dispar (1)    | [43]       |
|              |                                    | Lettuce                | 45                       | 4 (8.8)                       | E. histolytica/E. dispar (4)    |            |
|              |                                    | Carrot                 | 45                       | 6 (13.3)                      | E. histolytica/E. dispar (6)    |            |
|              |                                    | Cabbage                | 45                       | 7 (15.6)                      | E. histolytica/E. dispar (7)    |            |
|              |                                    | Green pepper           | 45                       | 5 (11.1)                      | E. histolytica/E. dispar (5)    |            |
|              |                                    | Avocado                | 45                       | 5 (11.1)                      | E. histolytica/E. dispar (5)    |            |
|              | Lugol's iodine stain               | Cabbage                | 90                       | 5 (5.6)                       | Entamoeba coli (5)              | [12]       |
|              |                                    | Green pepper           | 55                       | 4 (7.3)                       | Entamoeba coli (4)              |            |
|              |                                    | Onion                  | 70                       | 2 (2.9)                       | Entamoeba coli (2)              |            |
|              |                                    | Tomato                 | 31                       | 2 (6.5)                       | Entamoeba coli (2)              |            |
|              |                                    | Lettuce                | 102                      | 4 (3.9)                       | Entamoeba coli (4)              |            |
| Ghana        | Lugol's iodine stain               | Cabbage                | 90                       | 11 (12.2)                     | Entamoeba histolytica (11)      |            |
|              |                                    | Carrot                 | 47                       | 4 (8.5)                       | Entamoeba histolytica (4)       |            |
|              |                                    | Onion                  | 70                       | 2 (2.9)                       | Entamoeba histolytica (2)       |            |
|              |                                    | Tomato                 | 31                       | 4 (12.9)                      | Entamoeba histolytica (4)       |            |
|              |                                    | Lettuce                | 102                      | 6 (5.9)                       | Entamoeba histolytica (6)       |            |
| Iran         | Lugol's iodine stain               | Vegetables             | 141                      | 18 (12.8)                     | Entamoeba coli (18)             | [84]       |
| Iran         | Sediment smear under light microscope | Leek                  | 30                       | 0                             |                                 | [42]       |
|              |                                    | Spring onion           | 22                       | 2 (9.1)                       | Entamoeba coli (2)              |            |
|              |                                    | Basil                  | 15                       | 0                             |                                 |            |
|              |                                    | Parsley                | 21                       | 0                             |                                 |            |
|              |                                    | Lettuce                | 23                       | 0                             |                                 |            |
|              |                                    | Cress                  | 17                       | 1 (5.9)                       | Entamoeba coli (1)              |            |
|              |                                    | Spearmint              | 18                       | 0                             |                                 |            |
|              |                                    | Tarragon               | 19                       | 1 (5.3)                       | Entamoeba coli (1)              |            |
|              |                                    | Coriander              | 24                       | 2 (8.3)                       | Entamoeba coli (2)              |            |
|              |                                    | Radish                 | 29                       | 0                             |                                 |            |
| Iran         | Sediment smear under light microscope | Leek                  | 30                       | 2 (6.7)                       | Entamoeba histolytica (2)       | [42]       |
|              |                                    | Spring onion           | 22                       | 0                             |                                 |            |
|              |                                    | Basil                  | 15                       | 0                             |                                 |            |
|              |                                    | Parsley                | 21                       | 0                             |                                 |            |
|              |                                    | Lettuce                | 23                       | 0                             |                                 |            |
|              |                                    | Cress                  | 17                       | 0                             |                                 |            |
|              |                                    | Spearmint              | 18                       | 1 (5.6)                       | Entamoeba histolytica (1)       |            |
|              |                                    | Tarragon               | 19                       | 0                             |                                 |            |
|              |                                    | Coriander              | 24                       | 0                             |                                 |            |
|              |                                    | Radish                 | 29                       | 0                             |                                 |            |
| Iran         | Lugol's iodine stain               | Vegetables             | 34                       | 1 (2.9)                       | Entamoeba coli (1)              | [72]       |
| Jordan       | Lugol's iodine stain               | Lettuce                | 30                       | 3 (10.0)                      | Entamoeba histolytica (3)       | [20]       |
|              |                                    | Tomato                 | 33                       | 2 (6.1)                       | Entamoeba histolytica (2)       |            |
|              |                                    | Parsley                | 42                       | 0                             |                                 |            |
|              |                                    | Cucumber               | 28                       | 0                             |                                 |            |
| Location  | Detection method | Vegetable or fruit item           | Number of samples tested | Number of positive samples (%) | Entamoeba species identified (n) | References |
|-----------|------------------|-----------------------------------|--------------------------|-------------------------------|---------------------------------|------------|
| Saudi Arabia | Lugol's iodine stain | Green onion                       | 50                       | 6 (12.0)                      | Entamoeba spp. (6)              | [17]       |
|            |                   | Watercress                        | 50                       | 8 (16.0)                      | Entamoeba spp. (8)              |            |
|            |                   | Lettuce                           | 50                       | 6 (12.0)                      | Entamoeba spp. (6)              |            |
|            |                   | Cucumber                          | 50                       | 7 (14.0)                      | Entamoeba spp. (7)              |            |
|            |                   | Cabbage                           | 50                       | 6 (12.0)                      | Entamoeba spp. (6)              |            |
|            |                   | Pea                               | 50                       | 5 (10.0)                      | Entamoeba spp. (5)              |            |
|            |                   | Tomato                            | 50                       | 0                             |                                 |            |
|            |                   | Carrot                            | 50                       | 6 (12.0)                      | Entamoeba spp. (6)              |            |
| Saudi Arabia | Lugol's iodine stain | Green onion                       | 50                       | 3 (6.0)                       | Entamoeba coli (3)              |            |
|            |                   | Watercress                        | 50                       | 4 (8.0)                       | Entamoeba coli (4)              |            |
|            |                   | Lettuce                           | 50                       | 2 (4.0)                       | Entamoeba coli (2)              |            |
|            |                   | Cucumber                          | 50                       | 2 (4.0)                       | Entamoeba coli (2)              |            |
|            |                   | Cabbage                           | 50                       | 4 (8.0)                       | Entamoeba coli (4)              |            |
|            |                   | Pea                               | 50                       | 3 (6.0)                       | Entamoeba coli (3)              |            |
|            |                   | Tomato                            | 50                       | 2 (4.0)                       | Entamoeba coli (2)              |            |
|            |                   | Carrot                            | 50                       | 3 (6.0)                       | Entamoeba coli (3)              |            |
| Sudan     | Lugol's iodine stain | Tomatoes                          | 36                       | 1 (2.8)                       | Entamoeba coli (1)              | [29]       |
|            |                   | Cucumber                          | 12                       | 0                             |                                 |            |
|            |                   | Armenian cucumber                 | 16                       | 0                             |                                 |            |
|            |                   | Green pepper                      | 25                       | 0                             |                                 |            |
|            |                   | Cayenne pepper                    | 7                        | 0                             |                                 |            |
|            |                   | Radish                            | 24                       | 1 (4.2)                       | Entamoeba coli (1)              |            |
|            |                   | Beet                              | 19                       | 1 (5.3)                       | Entamoeba coli (1)              |            |
|            |                   | Watercress                        | 23                       | 1 (4.3)                       | Entamoeba coli (1)              |            |
|            |                   | Lettuce                           | 11                       | 1 (9.1)                       | Entamoeba coli (1)              |            |
|            |                   | Green onion                       | 36                       | 0                             |                                 |            |
|            |                   | Carrot                            | 50                       | 0                             |                                 |            |
| Sudan     | Lugol's iodine stain | Tomatoes                          | 36                       | 1 (2.8)                       | Entamoeba spp. (1)              | [29]       |
|            |                   | Cucumber                          | 12                       | 0                             |                                 |            |
|            |                   | Armenian cucumber                 | 16                       | 2 (12.5)                      | Entamoeba spp. (2)              |            |
|            |                   | Green pepper                      | 25                       | 1 (4.0)                       | Entamoeba spp. (1)              |            |
|            |                   | Cayenne pepper                    | 7                        | 0                             |                                 |            |
|            |                   | Radish                            | 24                       | 0                             |                                 |            |
|            |                   | Beet                              | 19                       | 1 (5.3)                       | Entamoeba spp. (1)              |            |
|            |                   | Watercress                        | 23                       | 1 (4.3)                       | Entamoeba spp. (1)              |            |
|            |                   | Lettuce                           | 11                       | 2 (18.2)                      | Entamoeba spp. (2)              |            |
|            |                   | Green onion                       | 36                       | 4 (11.1)                      | Entamoeba spp. (4)              |            |
|            |                   | Carrot                            | 50                       | 3 (6.0)                       | Entamoeba spp. (3)              |            |
| Total     |                   |                                   | 5647                     | 199 (3.5)                     |                                 |            |

* Single sample in a case report
health problems. On many occasions, the contamination of vegetables and fruits results in outbreaks of the parasitic diseases. Globally, the occurrence of protozoan parasitic contamination in vegetables and fruits ranges from 1.9% to 9.3%. However, contamination with protozoans may be grossly underestimated, especially in regions with poor sanitation. Contamination of vegetables and fruits with parasites can occur in many ways. The common stages between farm and fork at which vegetables and fruits are contaminated include production, processing, storage and selling. Therefore, the implementation of hygienic practices at every step between production and consumption may eliminate the contamination. The appropriate local public health authority is recommended to establish a system for continuous monitoring of contamination of vegetables and fruits sold at local markets.

| Location       | Detection method | Vegetable or fruit item          | No. of samples tested | No. of positive samples (%) | Toxoplasma gondii genotypes identified (n) | References |
|----------------|------------------|----------------------------------|-----------------------|----------------------------|--------------------------------------------|------------|
| Brazil         | PCR              | Smooth lettuce                   | 62                    | 1 (0.6)                   | Toxo4-5 D (1)                              | [62]       |
|                |                  | Crisp head lettuce               | 106                   | 4 (3.7)                   | B22-23 D (4)                               |            |
|                |                  | Chicory                          | 40                    | 2 (5.0)                   | B22-23 D (1); Toxo4-5 D (1)                |            |
|                |                  | Rocket                           | 7                     | 1 (14.3)                  | B22-23 D (1)                               |            |
|                |                  | Parsley                          | 5                     | 1 (20.0)                  | B22-23 D (1)                               |            |
| Brazil         | PCR              | Vegetables                       | 21                    | 3 (14.3)                  | N/A (3)                                    | [45]       |
| China          | Quantitative real-time PCR (qPCR) | Lettuce                   | 71                    | 5 (7.0)                   | Type I (4); Type II (1)                     | [63]       |
|                |                  | Spinach                          | 50                    | 2 (4.0)                   | Type I (2)                                 |            |
|                |                  | Pak choi                         | 34                    | 1 (2.9)                   | Type I (1)                                 |            |
|                |                  | Chinese cabbage                  | 26                    | 0                         |                                            |            |
|                |                  | Rape                             | 22                    | 1 (4.5)                   | Type II (1)                                |            |
|                |                  | Asparagus                        | 18                    | 0                         |                                            |            |
|                |                  | Chrysanthemum coronarium         | 16                    | 0                         |                                            |            |
|                |                  | Endive                           | 14                    | 0                         |                                            |            |
|                |                  | Chinese chives                  | 11                    | 0                         |                                            |            |
|                |                  | Cabbage                          | 9                     | 0                         |                                            |            |
|                |                  | Red cabbage                      | 8                     | 1 (12.5)                  | Type II (1)                                |            |
| Czech Republic | Triplex real time PCR          | Carrots                         | 93                    | 7 (7.5)                   |                                            | [64]       |
|                |                  | Cucumbers                        | 109                   | 13 (11.9)                 | Type II (5)                                |            |
|                |                  | Salads                           | 90                    | 8 (8.9)                   | Type II (2)                                |            |
| Italy          | qPCR              | Ready-to-eat packaged salad      | 648                   | 5 (0.8)                   | Type I (5)                                 | [23]       |
| Poland         | qPCR              | Strawberries                     | 60                    | 0                         |                                            | [61]       |
|                |                  | Radish                           | 60                    | 3 (5.0)                   | Type I (2); Type II (1)                     |            |
|                |                  | Carrot                           | 46                    | 9 (19.6)                  | Type I (3); Type II (1)                     |            |
|                |                  | Lettuce                          | 50                    | 9 (18.0)                  | Type I (1)                                 |            |
| Total          |                  |                                  | 1676                  | 63 (3.8)                  |                                            |            |
Table 6  Contamination of vegetables and fruits with *Balantidium coli*, *Cystoisospora belli*, *Blastocystis* sp. and *Enterocytozoon bieneusi*

| Location | Detection method | Vegetable or fruit item | No. of samples tested | No. of positive samples (%) | Identified species or genotypes (n) | References |
|----------|------------------|-------------------------|-----------------------|----------------------------|------------------------------------|------------|
| **Balantidium coli** | | | | | | |
| Bangladesh | Sediment smears, followed by light microscopy | Vegetables | 200 | 8 (4.0) | *B. coli* | [52] |
| Brazil | Sediment smears, followed by light microscopy | Loose leaf lettuce<sup>a</sup> | 1 | 1 | *B. coli* | [30] |
|  |  | Red lettuce<sup>a</sup> | 1 | 1 | *B. coli* | |
|  |  | Curly lettuce<sup>a</sup> | 1 | 1 | *B. coli* | |
|  |  | Iceberg lettuce<sup>a</sup> | 1 | 1 | *B. coli* | |
|  |  | Parsley<sup>a</sup> | 1 | 1 | *B. coli* | |
|  |  | Chive<sup>a</sup> | 1 | 1 | *B. coli* | |
| Cameroon | Sediment smears, followed by light microscopy | Green cabbage | 30 | 3 (10.0) | *B. coli* (3) | [66] |
|  |  | Red cabbage | 30 | 7 (23.3) | *B. coli* (7) | |
|  |  | Lettuce | 30 | 8 (26.7) | *B. coli* (8) | |
|  |  | Cucumber | 30 | 5 (16.7) | *B. coli* (5) | |
|  |  | Carrots | 30 | 4 (13.3) | *B. coli* (4) | |
|  |  | Green pepper | 30 | 2 (6.7) | *B. coli* (2) | |
| Ethiopia | Sediment smears, followed by light microscopy | Tomato | 100 | 0 | | [14] |
|  |  | Cabbage | 96 | 4 (4.2) | *B. coli*-like (4) | |
|  |  | Green pepper | 66 | 6 (9.1) | *B. coli*-like (6) | |
|  |  | Carrot | 62 | 4 (6.5) | *B. coli*-like (4) | |
|  |  | Salad | 23 | 1 (4.3) | *B. coli*-like (1) | |
| Ghana | Sediment smears, followed by light microscopy | Cabbage | 72 | 21 (29.2) | *B. coli* (21) | [67] |
|  |  | Lettuce | 72 | 3 (4.2) | *B. coli* (3) | |
|  |  | Carrot | 72 | 2 (2.8) | *B. coli* (2) | |
|  |  | Spring onion | 72 | 1 (1.4) | *B. coli* (1) | |
|  |  | Tomatoes | 72 | 22 (30.6) | *B. coli* (22) | |
| **Subtotal** | | | 1087 | 101 (9.3) | | |
| **Cystoisospora belli** | | | | | | |
| Ethiopia | Modified Ziehl-Neelsen stain | Fruits and vegetables | 360 | 11 (3.1) | *I. belli* (11) | [32] |
| Ethiopia | Modified Ziehl-Neelsen stain | Tomatoes | 45 | 0 | | [43] |
|  |  | Lettuce | 45 | 1 (2.2) | *C. belli* (1) | |
|  |  | Carrot | 45 | 2 (4.4) | *C. belli* (2) | |
|  |  | Cabbage | 45 | 4 (8.8) | *C. belli* (4) | |
|  |  | Green pepper | 45 | 0 | | |
|  |  | Avocado | 45 | 0 | | |
| Ghana | Ziehl-Neelsen stain | Cabbage | 90 | 0 | | [12] |
|  |  | Green pepper | 55 | 0 | | |
|  |  | Carrot | 47 | 0 | | |
|  |  | Onion | 70 | 0 | | |
|  |  | Tomato | 31 | 1 (3.2) | *I. belli* (1) | |
|  |  | Lettuce | 102 | 0 | | |
| **Subtotal** | | | 1025 | 19 (1.9) | | |
| **Blastocystis sp.** | | | | | | |
| Brazil | Sediment being stained in Lugol’s solution | Lettuce | 100 | 15 (15.0) | *B. hominis* (15) | [15] |
|  |  | Coriander | 100 | 19 (19.0) | *B. hominis* (19) | |
| Italy | Lugol’s stain, Giemsa Stain, and PCR | Ready-to-eat packaged salad | 648 | 3 (0.5) | *B. hominis* (3) | [23] |
| **Subtotal** | | | 848 | 37 (4.4) | | |
Abbreviations
CI: confidence interval; ITS: internal transcribed spacer; PCR: polymerase chain reaction; SNP: single-nucleotide polymorphism.

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Authors’ contributions
LZ and JL conceived and designed the review. JL, ZW and MRK analyzed the data and wrote the original draft of the manuscript. LZ and JL revised the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Table 6 (continued)

| Location     | Detection method          | Vegetable or fruit item                  | No. of samples tested | No. of positive samples (%) | Identified species or genotypes (n) | References |
|--------------|---------------------------|------------------------------------------|-----------------------|----------------------------|-------------------------------------|------------|
| Enterocytozoon bieneusi |                           |                                          |                       |                            |                                     |            |
| China        | PCR                       | Lettuce                                  | 200                   | 14 (7.0)                   | *E. bieneusi* genotype CM8 (2); CD6 (7); EbpA (3); Henan-IV (1) | [36]       |
|              |                           | Coriander                                | 152                   | 1 (0.7)                    | *E. bieneusi* genotype CM8 (1)    |            |
|              |                           | Celery                                   | 70                    | 1 (1.4)                    | *E. bieneusi* genotype EbpA (1)   |            |
|              |                           | Baby bok choy                            | 59                    | 1 (1.7)                    | *E. bieneusi* genotype CHV3 (1)   |            |
|              |                           | Chinese cabbage                          | 47                    | 0                          |                                      |            |
|              |                           | Leaf lettuce                             | 44                    | 2 (4.5)                    | *E. bieneusi* genotype CHG19 (1)  |            |
|              |                           | Water spinach                            | 28                    | 3 (10.7)                   | *E. bieneusi* genotype CD6 (1), BEB8 (1), CTS3 (1) |            |
|              |                           | Crown daisy                              | 27                    | 0                          |                                      |            |
|              |                           | Fennel plant                             | 26                    | 1 (3.9)                    | *E. bieneusi* genotype EbpC (1)   |            |
|              |                           | Endive                                   | 25                    | 1 (4.0)                    | *E. bieneusi* genotype Henan-IV (1) |            |
|              |                           | Spinach                                  | 20                    | 0                          |                                      |            |
|              |                           | Schizonepeta                             | 20                    | 0                          |                                      |            |
|              |                           | Cabbage                                  | 18                    | 0                          |                                      |            |
|              |                           | Leaf mustard                             | 11                    | 0                          |                                      |            |
|              |                           | Chinese chive                            | 132                   | 6 (4.5)                    | *E. bieneusi* genotype CD6 (1), EbpA (2), EbpC (1), CHV1 (1) |            |
|              |                           | Chive                                    | 128                   | 4 (1.4)                    | *E. bieneusi* genotype CD6 (2), CHV2 (1), CTS3 (1) |            |
|              |                           | Cucumber                                 | 41                    | 1 (2.4)                    | *E. bieneusi* genotype CD6 (1)    |            |
|              |                           | Watermelon                               | 15                    | 1 (6.7)                    | *E. bieneusi* genotype CD6 (1)    |            |
|              |                           | Potato                                   | 3                     | 1 (33.3)                   | *E. bieneusi* genotype CHV4 (1)   |            |
|              |                           | Bean (kidney/French bean)                | 28                    | 4 (14.3)                   | *E. bieneusi* genotype CD6 (4)    |            |
|              |                           | Green chili                              | 5                     | 0                          |                                      |            |
| Costa Rica   | Zielh-Nielsen stain       | Lettuce                                  | 50                    | 16 (32.0)                  | *E. bieneusi* (16)                 | [71]       |
|              |                           | Parsley                                  | 50                    | 0                          |                                      |            |
|              |                           | Cilantro                                 | 50                    | 2 (4.0)                    | *E. bieneusi* (2)                  |            |
|              |                           | Strawberries                             | 50                    | 1 (2.0)                    | *E. bieneusi* (1)                  |            |
|              |                           | Blackberries                             | 50                    | 0                          |                                      |            |
| Poland       | Conventional stain and FISH| Berries                                  | 25                    | 6 (24.0)                   | *E. intestinalis* (4); *E. bieneusi* (2) | [21]       |
|              |                           | Sprouts                                  | 20                    | 1 (5.0)                    | *E. bieneusi* (1)                  |            |
|              |                           | Vegetables                               | 35                    | 2 (5.7)                    | *E. cuniculi* (1); *E. bieneusi* (1) |            |
|             |                           | Sub-total                                | 1429                  | 52 (3.6)                   |                                      |            |

* Single sample in a case report
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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