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Review Article

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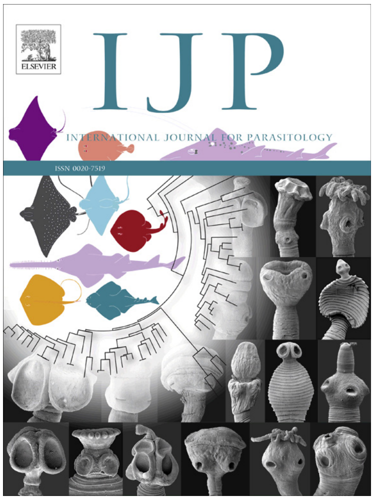

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Review Article

Giardia: an under-reported foodborne parasite

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Abstract

Foodborne zoonotic pathogens are a serious public health issue and result in significant global economic losses. Despite their importance to public health, epidemiological data on foodborne diseases including giardiasis caused by the enteric parasite, *Giardia duodenalis,* are lacking. This parasite is estimated to cause ~28.2 million cases of diarrhoea each year due to contamination of food, but very few foodborne outbreaks have been documented due to the limitations of current detection as well as surveillance methods. The current method for the recovery of *Giardia* cysts from food matrices using immunomagnetic separation requires further standardisation and cost reduction before it can be widely used. It also should incorporate downstream molecular procedures for genotyping, and traceback and viability analyses. Foodborne giardiasis can be potentially controlled through improvements in national disease surveillance systems and the establishment of Hazard Analysis and Critical Control Point (HACCP) interventions across the food chain. Studies are needed to assess the true prevalence and public health impact of foodborne giardiasis.

Keywords: *Giardia*; Giardiasis; Foodborne; Transmission; Outbreaks; Prevention

1. Introduction

Foodborne diseases can result in serious health and economic consequences globally (Havelaar et al., 2015; WHO, 2015; Sekse et al., 2017). Diarrheal diseases account for the majority of foodborne diseases, with their most severe impacts on children (Pires et al., 2015). Despite the important public health impacts of foodborne diseases, relatively little information is available, particularly in developing countries, and frequently outbreaks are not clearly identified or researched (Pires et al., 2015; Havelaar et al., 2015).

The protozoan parasite *Giardia* is extremely common and is responsible for ~280 million human cases of diarrhoea every year (total giardiasis acquired by all transmission routes) (Einarsson et al., 2016) and infects >40 animal species (Horlock-Roberts et al., 2017). The role of contaminated food in the spread of giardiasis is not well understood, but in the US, it is thought that 7-15% of *Giardia* infections are acquired by foodborne transmission (Torgerson et al., 2015). In 2010, the World Health Organization (WHO) reported that *Giardia* caused 28.2 million cases of foodborne disease and 26,270 disability-adjusted life years (DALYs) (Havelaar et al., 2015; WHO, 2015). The United Nations Food and Agriculture Organization (FAO) and WHO jointly ranked *Giardia* 11th out of 24 foodborne parasites in 2014 (FAO/WHO 2014) (compared with 5th for *Cryptosporidium*). However, due to inadequate detection and surveillance systems in many countries, it is likely that the real prevalence and impact of foodborne infections caused by *Giardia* is much higher (see below).

Currently eight species of *Giardia* are accepted as valid, including the recently described *Giardia cricetidarum* in hamsters and *Giardia peramelis* in bandicoots (Hillman et al., 2016; Lyu et al., 2018). *Giardia duodenalis* infects humans and is a species complex consisting of eight assemblages (A-H) (Ryan and Cacciò, 2013). Assemblages A and B are the predominant assemblages in humans, but assemblages C, D, E and F have also been identified (Table 1; Cacciò et al., 2017). Within Assemblage A, sub-assemblages AI, AII and AIII have been identified and of these AI and AII are commonly reported in humans and animals with sub-assemblage AIII reported in wild ruminants (Feng and Xiao, 2011).

Symptoms of giardiasis include diarrhoea, abdominal bloating and cramps, malabsorption and weight loss (Feng and Xiao, 2011; Einarsson et al., 2016). Infections may frequently be asymptomatic or cause mild illness, which usually resolve without treatment. However, some individuals can experience chronic and sometimes severe disease that is unresponsive to treatment (Bartelt and Sartor, 2015). Chronic *Giardia* infections are also associated with food allergies, irritable bowel syndrome (IBS), chronic fatigue syndrome and arthritis (Bartelt and Sartor, 2015), and in paediatric populations giardiasis is associated growth shortfalls (Bartelt and Platts-Mills, 2015). There is considerable controversy as to whether *Giardia* is associated with diarrhea, as it is frequently identified in asymptomatic individuals, particularly in developing countries (Bartelt and Platts-Mills, 2015). The Global Enteric Multicenter Study (GEMS) reported that *Giardia* was not associated with severe diarrhoea (Kotloff et al., 2013). However, another study linked *G. duodenalis* assemblage A with vomiting and abdominal pain in children (Ignatius et al., 2012). In addition, volunteer cyst challenge studies have shown that *G. duodenalis* is capable of causing diarrhoea in immunocompetent adults (Rendtorff and Holt, 1954; Nash et al., 1987), and a meta-analysis of giardiasis in children indicated that while giardiasis in these populations appeared to offer protection from acute diarrhoea, the risk of persistent diarrhea was increased (Muhsen and Levine, 2012).

A vaccine for human giardiasis is not commercially available and current treatments include nitazoxanide and 5-nitroimidazole compounds such as metronidazole and tinidazole (Einarsson et al., 2016). Albendazole is also used to treat giardiasis but exhibits variable efficacy (25–90%) (Miyamoto and Eckmann, 2015) and resistance has been reported to most anti-giardial drugs (Ansell et al., 2015). Currently, auranofin (Ridaura), a US Food and Drug Administration (FDA) approved drug for the treatment of rheumatoid arthritis, is in clinical trials as an anti-parasitic drug against *Giardia* (and *Entamoeba histolytica*) and shows potential as a broad spectrum anti-parasitic drug (Capparelli et al., 2017).

2. Limitations of current detection methods

Diagnosis of giardiasis has traditionally been based predominantly upon the identification of cysts in faeces via microscopy. While this method is economical and rapid, it is labour intensive and lacks specificity and sensitivity (due to sporadic shedding of *Giardia* cysts and/or by the presence of low numbers of cysts) (Soares and Tasca, 2016; Adeyemo et al., 2018). Immunoassays such as enzymatic immunoassays (EIAs) and rapid tests (immunochromatographic tests) are available for detecting *Giardia* in faeces and on food, but their performance can be very variable; specificity can be reduced due to antibody cross-reactions and sensitivity can be as low at 44.4% (Johnston et al., 2003; Soares and Tasca, 2016).

The detection of *Giardia* on food has been improved by the use of immunomagnetic separation (IMS) methods to isolate cysts (Cook et al., 2007), and IMS methods for the elution of *Giardia* cysts (as well as *Toxoplasma* and *Cryptosporidium*) from the same food sample have been developed (Hohweyer et al., 2016). While standardised methods for the detection of *Giardia* from water such as US EPA 1623 have been widely available for decades, a standardised method for the detection and enumeration of *Giardia* cysts on or in berry fruits and fresh leafy green vegetables based on IMS only became available in 2016 (ISO 18744:2016 www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=63252). A limitation of implementation of this method, particularly in developing countries, is the cost of IMS beads. A modified version of this method which uses smaller quantities of the expensive IMS beads was evaluated in 10 different experienced microbiology laboratories, with mean recovery rates of 33% for *Giardia* (Utaaker et al., 2015). Therefore, this revised method may be useful in countries and laboratories where using the standard ISO method is too costly.

PCR detection is increasingly being used for the identification of *Giardia* on food as it offers improved sensitivity and specificity compared with microscopy and immunology-based detection methods (Dixon et al., 2013; Ramirez-Martinez et al., 2015; Hohweyer et al., 2016). For example, a study in Brazil which screened 128 samples of leafy greens by direct immunofluorescence and PCR detected *Giardia* in 12.5% (16/128) of samples by PCR, compared with 0.8% (1/128) by immunofluorescence (Tiyo et al., 2016). It is important to note, however, that while PCR is more sensitive, it may sometimes detect parasite DNA and not intact cysts, which may therefore represent a low infection risk. Conversely, the relatively low sensitivity of microscopy may fail to detect cyst levels that would be more than sufficient to constitute an infectious dose (25 - 100 cysts), which can be as low as 10 cysts (Rendtorff, 1954, 1979).

Widely used loci for the detection of *Giardia* include glutamate dehydrogenase (*gdh*), triose phosphate isomerase (*tp*i), beta-giardin (*bg*) and 18S rRNA (Feng and Xiao, 2011; Koehler et al., 2014). Data from foodborne giardiasis studies suggests that different food matrices display specific characteristics (e.g. some foods more effectively trap cysts and/or are more “sticky”), which may interfere with *Giardia* extraction/elution and therefore recovery of *Giardia* cysts from food matrices (Hohweyer et al., 2016). A major limitation of the IMS-based ISO 18744:2016 method for detecting *Giardia* on food is that because the method involves determining recovery rates by spiking cysts into the samples, molecular detection and genetic characterisation steps cannot currently be included. Therefore, the method needs to be refined to allow it to be combined with molecular testing, as this is essential in tracking the contamination of *G. duodenalis* on food and transmission of foodborne giardiasis in humans.

The ability to discriminate between infectious and non-infectious cysts is essential for determining if *Giardia* cysts in foods across the food chain are still viable, however viability is not part of the current ISO method. Infectivity can be partially accessed by species and assemblage identification, as *G. duodenalis* assemblages A and B cause most human infections (Feng and Xiao, 2011). Current in vitro and in vivo methods to determine the viability and infectivity of the *Giardia* cysts are not reliable enough or suitable for routine application in the water and food industries. For example, in vitro methods such as amplification of *hsp70* and *β-giardin* mRNAs have been applied to the detection of viable *Giardia* cysts (on the basis that only infectious organisms will be expressing mRNA), however heat-inactivated cysts have been shown to produce *β-giardin* mRNA amplicons in reverse transcriptase PCR assays (Rousseau et al., 2018). Other in vitro methods including fluorescence in situ hybridization (FISH) and vital dyes combined with DNA amplification also do not correlate well with infectivity assays (Rousseau et al., 2018). In vivo infectivity assays require the use of laboratory animals, which are usually incompatible with assemblages A and B (see Rousseau et al., 2018 for an in-depth review on the subject).

Giardia cysts have been detected on various types of foods including dairy products, meat, shellfish, fruit and vegetables (Robertson, 2013; Dixon, 2015), with overall contamination rates of 0.6% to 52.6% on different vegetables and salad products (Table 2). Understanding the potential public health risks from these studies is difficult as few studies examined washed and “ready-to-eat” vegetables and salads at supermarkets, and different detection methods with varying sensitivities were used (Table 2). Generally, the numbers of cysts recovered from fruit and vegetables were low (Table 2), however, contamination rates are likely to be higher, as recoveries of *Giardia* cysts from foodstuffs are highly variable with recoveries of 16.7% -~83% reported (Amorós et al., 2010; Utaaker et al., 2015). Washing fruit and vegetables can reduce the risk of contamination but the source of water for washing is also very important (Shrestha et al., 2017). For example, a study in Nepal, which examined the effect of washing vegetables with water from different sources, reported that even when treated water was used for washing vegetables, the risk of infection was still greater than the US Environmental Protection Agency (EPA) recommendation of less than 10^{-4} infection per person per year (Shrestha et al., 2017).

There have been very few reports of studies which have genotyped *Giardia* cysts recovered from food matrices, but assemblages A and B as well as D and E have been identified, predominantly using analysis at two loci but in some cases only one locus (Dixon et al., 2013; Giangaspero et al., 2014; Colli et al., 2015a; Tiyo et al., 2016; Utaaker et al., 2017a; Rafael et al., 2018). A study in an urban area of southern Brazil, which screened humans (*n*=380), animals (*n*=34), water samples (*n*=44) and vegetables (*n*=11) for *Giardia*, identified the same genotype of assemblage B in humans (*n*=19) (through PCR-RFLP of *bd* and *gdh* genes and sequencing of *gdh*), one dog and two lettuce samples with 100% similarity, suggesting a linkage in *G. duodenalis* contamination (Colli et al., 2015a). In that study, the lettuce was irrigated with water originating from a poorly maintained shallow well near septic tanks, which was the likely source of contamination (Colli et al., 2015a).

Recently, several commercially available multiplex PCR assays for the detection of *Giardia* and *Cryptosporidium* (as well as bacteria and/or viruses) have been approved by the FDA, which will improve detection of foodborne giardiasis and will also be able to detect foodborne infections caused by multiple pathogens (see Ryan et al., 2017), however further testing on different food types is required.

A limitation of current diagnostics, however, is that the diagnostic test will only detect the pathogen that it is designed to screen for. In many studies however, the causative agent cannot be determined, with one study by Vernacchio et al. (2006), reporting that in ~80% of diarrheal faecal samples from humans, the causative agent could not be identified. Next generation sequencing (NGS) methods or "high through-put sequencing" can generate millions of sequences per sequencing run and are increasingly used in the investigation of foodborne outbreaks, particularly for bacterial pathogens (Sekse et al., 2017), but are in their infancy for parasites. One study used metagenomics sequencing to identify food poisoning due to consumption of raw fish contaminated by the myxozoan parasite *Kudoa septempunctata* (Kawai et al., 2012). More recently, whole-genome sequencing (WGS) was used to characterise *G. duodenalis* isolates (*n*=89) and link *Giardia* from beavers as the cause of two small community waterborne outbreaks (Tsui et al., 2018). In addition, an assemblage A-specific multilocus sequence typing (MLST) tool, based on six previously unidentified genetic loci from assemblage A genomes, has been developed, which has provided increased levels of polymorphism for differentiation of assemblage A isolates (Ankarklev et al., 2018), facilitating source tracking for foodborne outbreaks.

3. Foodborne transmission of *G. duodenalis*

Transmission of *Giardia* to humans can occur via direct contact with infected humans and animals as well as through consumption of water and/or food contaminated with cysts (Feng and Xiao, 2011). The simple direct life cycle of *G. duodenalis* facilitates its transmission (Horlock-Roberts et al., 2017). This consists of two main stages: the pathogenic trophozoite which infects the intestine and the hardy cyst stage shed in the faeces. The partial resistance of the cyst stage to chlorine disinfection of water and its ability to persist for long periods of time in the environment and still remain viable, further enhances foodborne transmission of *Giardia* (DeRegnier et al., 1989; Tonani et al., 2013). The large numbers of infective cysts that can be shed by infected individuals into the environment also contributes to the spread of giardiasis. For example, one study reported that ~3.8×10^{14} *Giardia* cysts were shed annually in the Netherlands alone (2.5×10^{7} cysts per inhabitant/year) (Medema and Schijven, 2001). Another factor which facilitates the transmission of giardiasis is the low infectious dose (Rendtorff, 1954, 1979), with even ingestion of one single cyst having a 2% probability of causing giardiasis (Teunis et al., 1996). Furthermore, organic faecal matter enhancement of the survival of *Giardia* cysts (Alum et al., 2014), their small size (8–12 µm in length), which allows them to penetrate and survive water filters such as sand filters, which are commonly used by the water industry, and the ability of *Giardia* to survive at low temperatures, indicate that cysts on the surface of salads or herbs may still be viable even after a few days in a household refrigerator (Hohweyer et al., 2016). The potential for environmental spread and contamination is also increased because *G. duodenalis* has a very wide host range, with livestock and wildlife shedding zoonotic *G. duodenalis* assemblages in the environment (Feng and Xiao, 2011). For example, cattle can shed 7.6×10^{6} cysts individual^{-1} day^{-1}; (Hoar et al., 2009; Oates et al., 2012), while wild canids can shed up to 1.0×10^{6} cysts individual^{-1} day^{-1} (Oates et al., 2012). Insects can also disseminate *Giardia* cysts in the environment (Conn et al., 2007; Zhao et al., 2014).

Fresh produce, particularly produce that is consumed raw, is also a source of transmission, evidenced by investigations of foodborne outbreaks in the US during 1973-2011 (Adam et al., 2016) and a study of giardiasis in England, which reported that eating lettuce was associated with an increased risk for sporadic giardiasis (Stuart et al., 2003). The agricultural sector accounts for approximately 70% of water use globally (FAO, 2011) and therefore, fruit and vegetables can become contaminated with *Giardia* cysts when water contaminated with human or animal faeces is used for irrigation and washing of produce

(Budu-Amoako et al., 2011; Rafael et al., 2018). Infected farm workers can also contaminate fruit and vegetables during harvesting and packaging or during transport (Budu-Amoako et al., 2011).

The demand for fresh produce is increasing due to advice from the medical community for members of the public to increase their intake of fruits and fiber-rich vegetables and consume more raw food to reduce the incidence of chronic diseases (Dixon, 2015). There is also a growing demand for organically farmed produce using animal manure, which is likely to increase foodborne transmission of *G. duodenalis*. Other factors contributing to foodborne transmission of *G. duodenalis* include (i) the global trade in food, (ii) increased consumption of food outside the home such as in restaurants (Dixon, 2015; Utaaker et al., 2017b), (iii) increased production of free-range and organic animals as a result of animal welfare concerns and (iv) higher proportions of the population that are immunologically compromised due to an increase in individuals with immunosuppressive diseases and/or treatments and an increasingly elderly population (Newell et al., 2010).

Temperature and humidity critically affect the survival and transmission of *Giardia* cysts on food matrices. A recent study reported that while both *Giardia* and *Cryptosporidium* oo/cysts survive well in moist and refrigerated conditions; when lettuce was stored at room temperature, ~ 50% of cysts lost viability within the first 24 h (Utaaker et al., 2017b). If transported under appropriately cool and moist conditions, however, *Giardia* cysts can survive for long periods. For example, a temperature around 0 °C and a relative humidity of 98–100% is recommended for storage and transport of lettuce (Saltveit, 2014), which is also suitable for *Giardia* cyst survival. Most fresh produce (particularly in developing countries), however, are not transported under these conditions (Vigneault et al., 2009) and although this is improving (Rodrigue and Notteboom, 2013), lack of appropriate transport conditions and storage may decrease the survival of *Giardia* cysts (Utaaker et al., 2017b). Climate change may also increase the transmission of *Giardia* cysts worldwide. For example, an increase in the number and force of extreme precipitation events will likely increase surface runoff of animal faecal samples containing *Giardia* cysts into waterways used for irrigating fresh fruit and vegetables (Semenza et al., 2012). Higher ambient temperatures may also result in *Giardia* cysts surviving in water bodies that had previously become frozen in winter but conversely, higher temperatures may also reduce the viability of *Giardia* cysts on produce.

Infected food handlers (both the ill or asymptomatic) with poor personal hygiene are a major source of transmission of foodborne giardiasis (Greig et al., 2007) and the parasite is frequently identified in food handler faecal samples and under their nails (Baswaid and Al-Haddad, 2008; Takizawa et al., 2009; Saeed and Hamid, 2010; Zaglool et al., 2011; Abdel-Dayem et al., 2014; Kheirandish et al., 2014; Colli et al., 2015b; Beiromvand et al., 2017). For example, *G. duodenalis* was the most common foodborne pathogen (19%; 5/27) identified amongst food handlers in public schools in Angulo, Brazil, with sub-assemblages AII and BIV detected (Colli et al., 2015b). These were the same sub-assemblages detected in students attending these same schools in a previous study (Colli et al., 2015a). In an outbreak of foodborne giardiasis in 2015, 20 giardiasis cases were identified, which were epidemiologically linked to a grocery store chain on Long Island, New York, US (Figgatt et al., 2017). Typing of faecal samples from three asymptomatic food handlers who worked at the store and two outbreak cases, identified sub-assemblage BIII, supporting the conclusion that the infected food handlers transmitted the parasite via the handling of ready-to-eat food (Figgatt et al., 2017).

Shellfish can filter large amounts of water and in doing so accumulate and concentrate *Giardia* cysts, and are thus a source of foodborne giardiasis. Shellfish are commonly found in coastal areas, and can acquire and concentrate *Giardia* cysts via contact with run-off from land contaminated with *Giardia* cysts or wastewater discharged from treatment plants

(Robertson, 2007, 2013). *Giardia* has frequently been reported in shellfish (Graczyk et al., 2003; Gómez-Couso et al., 2004, 2005a,b; Lévesque et al., 2006; Lucy et al., 2008; Schets et al., 2007; Robertson and Gjerde 2008; Lévesque et al., 2010; Gómez-Couso and Ares-Mazás, 2012; Leal Diego et al., 2013; Robertson, 2013) and genotyping has identified assemblages A, B, C and D in haemolymph (Adell et al., 2014; Giangaspero et al., 2014). Transmission of *Giardia* cysts from shellfish to humans can occur when they are eaten raw or under-cooked. To date, two outbreaks of giardiasis associated with shellfish have been reported (Table 3).

3. Foodborne outbreaks of giardiasis

Very few outbreaks of foodborne giardiasis have been identified and investigated (Table 3), although there have been many published outbreaks of waterborne giardiasis (Karanis et al., 2007; Baldursson and Karanis, 2011; Painter et al., 2015; Efstratiou et al., 2017; McClung et al., 2017). This is likely due to better national and international standards for monitoring drinking water (Painter et al., 2015). The largest drinking water outbreak of giardiasis was reported in Portland, Oregon, USA in 1955, with ~50,000 infected individuals (Veazie, 1969; Meyer, 1973). More recently in 2004, in Bergen, Norway, ~2,500 individuals became infected with *Giardia* due to drinking contaminated water (Nygård et al., 2006).

Currently, only 38 foodborne outbreaks of giardiasis have been reported, all in the US (Adam et al., 2016). Table 3 lists 27 of these, only two of which were investigated by genotyping. In many of the outbreak investigations, the food type or source of outbreak was frequently undetermined. However, a variety of foods have been implicated, with fresh produce the most common food type and infected food handlers the most common source (Adam et al., 2016; Table 3). Given the potential for foodborne transmission, the total number of outbreaks is likely greatly underestimated.

Under-reporting of giardiasis outbreaks is also likely due to the fact that many countries lack a system for reporting cases and outbreaks of foodborne diseases, and for countries that do, many surveillance systems do not include giardiasis. In the USA, the Centers for Disease Control and Prevention (CDC) has a Foodborne Disease Outbreak Surveillance System (FDOSS) through the online National Outbreak Reporting System (NORS) (www.cdc.gov/nors/index.html), which can be readily analysed for foodborne giardiasis outbreaks. The European Union (EU) regulatory bodies include the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). In the EU, the Zoonoses Directive 2003/99/EC system,requires EU Member States to collect data on zoonoses and foodborne outbreaks (Anon, 2003). However, *Giardia* is not one of the notifiable foodborne agents (Anon, 2009; EFSA, 2017) and while there were 18,985 cases of giardiasis reported to EFSA and ECDC in 2016 (https://ecdc.europa.eu/en/giardiasis/surveillance/atlas), information on the number of cases of giardiasis due to food contamination is not available. In the USA and Australia, the National Notifiable Diseases Surveillance System (NNDSS) collects national surveillance data on notifiable diseases (www9.health.gov.au/cda/source/cda-index.cfm; https://wwwn.cdc.gov/nndss/). However, while giardiasis is notifiable in the USA and in some Australian states, it is not a nationally notifiable disease in Australia and therefore specific information on foodborne giardiasis cases is not readily available in Australia. There is also the WHO Global Foodborne Infections Network (GFN) (www.who.int/gfn/en/), which aims to improve laboratory-based surveillance of foodborne infections by conducting international training courses and has 1,500 individual members in 177 Member States and territories. In the US, FoodNet (Foodborne Diseases Active Surveillance Network), has been tracking infections commonly transmitted through food in 10 US states/sites since 1996 (https://www.cdc.gov/foodnet/index.html). In addition, FoodCORE (Foodborne Diseases

Centers for Outbreak Response Enhancement), supported by CDC, develops improved methods to detect, investigate and control foodborne outbreaks of disease (https://www.cdc.gov/foodcore/). Although they target mostly major foodborne bacterial pathogens, the expertise and infrastructure provided by FoodNet and FoodCORE are valuable to the surveillance and investigation of foodborne giardiasis outbreaks in the US.

Even in those countries that do have a system for reporting giardiasis as a foodborne disease, under-diagnosis and under-reporting are very common, largely because illness due to foodborne diseases frequently involve a single household or a few individuals and the contaminated food is usually no longer available for analysis (Robertson, 2007). For example, in the US, the CDC estimates 1.3% and 46.3%, respectively, for under-diagnosis and under-reporting of giardiasis (Scallan et al., 2011). In addition, only around 10% of people with diarrhoea will visit their general practioner (GP) and only ~10% of these will have a faecal sample collected and screened for *Giardia,* and therefore most cases of giardiasis will not be detected (Budu-Amoako et al., 2011; Tam et al., 2012; McHardy et al., 2014; Ryan et al., 2017). Lack of access to transport to medical facilities, unavailable or inadequate laboratory diagnostic methods and communication infrastructures compound this problem in developing countries (WHO, 2015).

Under-reporting is also due to technical challenges in detecting the environmentally resistant stage (cysts). The low numbers of cysts that may be present in foodstuffs and the wide differences in food matrices require the development of food-specific detection methods (Caccio and Lalle, 2015). In addition, the long incubation period for *Giardia* infection (1-3 weeks) (Katz et al., 2006; Caccio and Lalle, 2015) results in a significant time delay between consumption of food contaminated with *Giardia* cysts and an outbreak, which decreases the ability to detect and trace infections back to the source (Gajadhar and Allen, 2004). Early detection of foodborne disease outbreaks is essential for limiting the number of infected individuals and limiting their spread. This time delay associated with foodborne giardiasis outbreaks makes it difficult for individuals to remember which food they had consumed during the infection incubation period and delays identification of other linked cases (van de Venter et al., 2015).

ZOOPNET (the ZOOnotic Protozoa NETwork) was recently established as a European network of public and veterinary health institutions from nine European countries to study *Giardia* (and *Cryptosporidium*) isolates and conduct epidemiological traceback in outbreaks of giardiasis. ZOOPNET aims to standardise detection and control methods for *Giardia* (and *Cryptosporidium*) and conduct molecular epidemiological investigations of outbreaks (Sprong et al., 2009). The database is not yet publically available but will be useful in typing foodborne outbreaks in the future.

4. Prevention of foodborne outbreaks

The increasing globalisation of the sale of food has increased the risk of foodborne disease. Therefore, effective control and prevention of foodborne diseases requires international co-operation for foodborne disease surveillance and interventions targeting the food production industry, food services, and consumers. A major component of this is the establishment of autonomous, proficient food safety authorities and co-ordination of food surveillance programmes such as the International Food Safety Authorities Network (INFOSAN), established by the FAO and WHO, with 186 member states globally (www.who.int/foodsafety/areas_work/infosan/en/). The role of INFOSAN is to provide assistance in information sharing between member states, particularly during foodborne outbreaks, in order to limit the transport of contaminated food between countries as well as providing rapid and reliable information on the prevalence and emergence of foodborne diseases.

The most common form of foodborne disease surveillance is event-based surveillance, which involves detection and analysis of a foodborne event. A more reliable form of surveillance is indicator-based surveillance, which involves monitoring long-term trends in notifiable diseases. A much more complete and effective form of surveillance is integrated food-chain surveillance that monitors data from each point across the food chain, but this is expensive and requires strong collaboration and communication between academics, microbiology laboratories, food safety laboratories and animal health and food safety departments (Ford et al., 2015). WHO also provides specific guidance on strengthening surveillance response to foodborne diseases (WHO, 2008) (www.who.int/foodsafety/publications/foodborne_disease/surveillancemanual/en/).

In addition to surveillance, prevention of foodborne outbreaks also requires better regulation and enforcement of food safety legislation, development of better outbreak tracing and contaminated food recall systems as well as rapid detection, investigation and control of food safety outbreaks as per the 2007 Beijing Declaration on Food Safety (WHO, 2007). In developed countries which utilise integrated food chain surveillance, Good Agricultural Practices (GAP) and Good Handling Practices (GHP) (which are voluntary audits to prove adherence to FDA regulations), are some of the food safety practices used to minimise the risk of microbial contamination during the production, packaging, shipment, and storage of fruits and vegetables (www.fda.gov/downloads/Food/GuidanceRegulation/UCM169112.pdf) (Sant'Ana et al., 2014). Food chain surveillance in the US is complemented by the FDA Food Safety Modernisation Act (FSMA) (https://www.fda.gov/Food/GuidanceRegulation/FSMA/), which monitors many different points in the global supply chain for both human and animal food, and requires that specific actions that must be taken at each of these points to prevent contamination, with foreign suppliers also required to meet the same standards as domestic producers.

Quantitative microbial risk assessment (QMRA) and Hazard Analysis and Critical Control Points (HACCP) (www.fda.gov/Food/GuidanceRegulation/HACCP/) are widely used to identify foodborne disease risks and reduce the diseases they cause (Dawson, 2005; Gale, 2005; Hamilton et al., 2006; Mota et al., 2009; Kouamé et al., 2017; Shrestha et al., 2017). QMRA is a modelling process that estimates the potential risk of infection from microorganism exposure (Hamilton et al., 2006). A recent QMRA study conducted on wastewater used for irrigation of urban agricultural areas in Côte d'Ivoire, West Africa, estimated the annual risk of infection at 0.36 and the probability to become ill (P_{ill}) from eating salad vegetables grown in these areas, at 1.0% for *Giardia* (Kouamé et al., 2017). Another study in Thailand reported a 100% risk of giardiasis from eating vegetables irrigated with wastewater (Ferrer et al., 2012). In many less developed countries, the annual risks of infection from consuming raw vegetables is higher than the acceptable risk, which the WHO has defined as 10^{-4} for water used for irrigating produce (WHO, 2006) and 10^{-6} for foods consumed (WHO, 2006; Asano, 2007). The ECDC is currently developing a "QMRA-based climate change decision-making tool for food and waterborne diseases" to ensure appropriate surveillance and control of climate change impacts on foodborne diseases (http://climate-adapt.eea.europa.eu/ecdc-tool). An "adjusted likelihood ratio" statistical tool has also been developed to improve the identification of which food products should be analysed for *Giardia* cysts, thereby expediting foodborne giardiasis outbreak investigations. The tool examines the association between outbreak cases and food distribution, which will assist in identifying the source of future foodborne outbreaks (Norström et al., 2015), particularly where the traditional epidemiological approaches fail to identify the source of infection.

In developing countries, farmers need to be educated about the potential food threat when irrigating fruit and vegetables using wastewater, and the importance of washing raw vegetables prior to consumption. Access to programs such as Water Sanitation and Hygiene (WASH) (Freeman et al., 2013) are central to reducing foodborne transmission. However, clean drinking water sources are still unavailable to ~ 663 million people and 2.4 billion people lack access to appropriate sanitation (UNICEF, 2015).

5. Conclusions

Foodborne giardiasis is a neglected but important public health issue and serious social and economic burden worldwide. The lack of targeted surveillance systems has resulted in a lack of awareness of the importance of foodborne transmission routes in disease epidemiology, despite the fact that *G. duodenalis* is one of the most common enteric pathogens in humans. This is especially the case in developing countries, where hygiene is poor, sanitation facilities are not widely available, and wastewater is widely used in growing vegetables. Understanding the disease burden and epidemiology of foodborne giardiasis in both industrialised nations and developing countries can be improved by field investigations of the disease through case-control studies or multivariate analysis of risk factors. They can be coupled with molecular tools and analysis of fresh produce and irrigation water for *Giardia* cysts, as well as identification of infection sources and contamination trace-back.

For both outbreak investigations and research studies, the current IMS-based methods for recovery of *Giardia* cysts from different food products needs considerable improvement and needs to be combined with molecular detection methods to more effectively prevent future foodborne outbreaks, as molecular techniques can more sensitively detect the prevalence, numbers, source and transmission routes for *Giardia* cysts.

Detection of the proportion of *Giardia* cysts that are viable and infectious on produce is a key area that needs considerable research. To determine inactivation efficiency (log10 viability reduction) of *Giardia* on produce, quantitative reverse transcription PCR-based assays could be used initially until more robust viability measures can be developed, even though they will likely overestimate the numbers of viable cysts (Rousseau et al., 2018).

In the absence of effective surveillance systems and trace-back methods, the application of QMRA and HACCP are central to the reduction and control of food contamination with *G. duodenalis* and to minimise foodborne outbreaks of giardiasis. To date, the effectiveness of these intervention strategies against foodborne giardiasis has remained largely unproven. Similarly, WASH used in the control and prevention of other enteric diseases has rarely been adopted specifically to reduce foodborne transmission of *G. duodenalis* in endemic settings. Research on the effectiveness of risk management and intervention strategies against *G. duodenalis* contamination and infections is urgently needed for the implementation of effective control programs against foodborne giardiasis.

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Highlights

- *Giardia* is an under-reported foodborne parasite
- Contributing factors were reviewed
- Documented outbreaks were analysed
- Strategies to prevent foodborne transmission are discussed

Table 1. *Giardia duodenalis* assemblages.

Assemblage	Main Host	Reports in humans	References
A	Humans and a range of other mammals	Commonly reported	Cacciò et al., 2017
B	Humans and a range of other mammals	Commonly reported	Cacciò et al., 2017
C	Dogs and wild canids	Reports in humans in China and Slovakia	Liu et al. 2014; Štrkolcová et al. 2015
D	Dogs and wild canids	One report in German travelers	Broglia et al. 2013
E	Ungulates		Foronda et al., 2008; Helmy et al., 2014; Abdel-Moein and Saeed, 2016; Fantinatti et al., 2016; Scalia et al., 2016; Zahedi et al., 2017
F	Cats	One report in humans in Ethiopia	Gelanew et al., 2007
G	Rodents	No reports	Cacciò et al., 2017
H	Pinnipeds	No reports	Cacciò et al., 2017

Table 2. Occurrence of *Giardia* cysts on fresh produce.

Country	Location	Food	% of	No of	Suspect	Detection	Assem	Referen

		analysed	samples contaminated with *Giardia* cysts	*Giardia* cysts detected	ed source	method used	blage detected	ce
Brazil	Street markets and community vegetable gardens	Lettuce, kale, chicory and rocket	7.3% (19/260)	-	Unknown	PCR-RFLP of *gdh* gene	AI (90%), B and E	Rafael et al., 2018
Chandigarh, Northern India	Public markets and supermarkets	Vegetables	5% (13/ 284)	<5 (per 30g)	Unknown	IMS & Fluorescent antibody + PCR at *gdh, tpi* and 18S	A & D (18S only)	Utaaker et al., 2017a;
Italy	Supermarkets	Ready-to-eat salads	0.6% (4/72 pooled samples) (9/pool)	-	Unknown	IMS and Iodine staining and PCR at *tpi* locus	A	Caradonna et al., 2017
Brazil	Public market	Raw leafy vegetables	12.5% (16/128) (PCR); 0.8 (1/128) microscopy	-	Unknown	Direct immunofluorescence PCR at *gdh, tpi* and 18S	AII	Tiyo et al., 2016
Sudan, Khartoum state	Public market	Vegetables	3% (8/260)	-	Contaminated water	Iodine staining of vegetables washings	-	Mohamed et al., 2016;
Southern Brazil	Local producers	Lettuce and wild chicory	18.2% (2/11)	-	Unknown but humans in the area has the same assemblage	PCR of *gdh* gene	BIV	Colli et al., 2015a
Southwest Ethiopia	Public markets	Fruit and vegetables	7.5% (27/360)	-	Unknown	Microscopy	-	Tefera et al., 2014
Egypt (Benha)	Public markets	Leafy vegeta	8.8% (47/530)	-	Unknown	Zinc sulphate flotation	-	Eraky et al.,

		bles				combined with Iodine staining		2014
Canada (Ontario)	Grocery stores	Packaged leafy greens	1.8% (10/544) 10 by PCR positive, 2 by IMS	-	Unknown	IMS (ISO 18744:2016) + PCR (18S)	B	Dixon et al., 2013
Iran (Ilam city)	Grocery stores	Vegetables	55% (11/20)	-	Unknown	Microscopy	-	Hafez et al., 2013
Norway	Imported and locally produced produce	Fruit and vegetables	10% (1/10) for mangetout. All others negative	1 cysts/ 50g mangetout	Unknown	IMS (ISO 18744:2016)	-	Johannessen et al., 2013
Thailand, (Pathumthani Province)	Salad from field irrigation water systems	Freshly harvested lettuce and water spinach	-	50 cysts per 200 g of salad	Irrigation water	IMS + Flow Cytometry + qPCR	-	Keserue et al., 2012
Iran (Shahrekord)	Public markets	Vegetables	8.2% (25/304)	-	Unknown	Iodine staining	-	Fallaha et al., 2012
Iran (Qazvin Province)	Wholesalers	Vegetables	1.3% (3.218)	3-4 cysts per 200g of vegetable	Unknown	Microscopy	-	Shahnazi and Jafari-Sabet, 201
Spain (Valencia)	Field collection from agricultural areas	Vegetables	52.6% (10/19)	1-9 cysts/ 50g of produce	Giardia cysts recovered from irrigation water	IMS (ISO 18744:2016)	-	Amorós et al., 2010
Italy, Palermo	Supermarkets	Ready-to-salad/ vegetable mixes	0.5% (1/20)	12 cysts per 50g produce	Unknown	IMS (ISO 18744:2016)	-	Di Benedetto et al., 2007
UK (York)	Wholesa	Lettuc	0.5%	1	Unknow	IMS (ISO	-	Cook et

	lers	e	(1/20)	cyst/50g of produce	n	18744:2016)		al., 2007
Turkey (Kahramanmara)	Irrigation canals	Fruit and vegetables	9.1% (5/55)	3-9 cysts per 100g produce	Unknown	Microscopy	-	Erdogrul and Sener, 2005
Norway	Norway producers	Vegetables	2.3% (8/342)	1 cyst per 100g produce	*Giardia* cysts isolated from spent irrigation water	Microscopy based on USEPA method 1623[a]	-	Robertson et al., 2002
Brazil (Ribeirão Preto)	Vegetable gardens	Lettuce	0.7% (1/129)	-	Unknown	Microscopy	-	Takayanagui et al., 2000
Norway	Retail – multiple countries of origin	Fruit and Vegetables	2.1% (10/475)	1-8 cysts/100g produce	Possibly irrigation water	IMS (ISO 18744:2016)	-	Robertson and Gjerde, 2001
Morocco (Marrakech)	Field crops	Vegetables	25% (15/58)	5.1 cysts/kg of produce	Irrigation water	Microscopy	-	Amahmid et al., 1999
Costa Rica	Public market	Cilantro	5.2% (4/80) of cilantro leaves and in 2.5% (2/80) of cilantro roots.	-	Unknown	Microscopy	-	Monge and Arias, 1996
Philippines	Supermarkets and a public market	Lettuce and other leafy vegetables	2.5% (1/40)	-	Unknown	Microscopy	-	de Leon et al., 1992

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[a] Anonymous. 1999. Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC 20460, EPA-821-R-99-006. Available at: https://www.epa.gov/homeland-security-research/method-1623-cryptosporidium-and-giardia-water-filtrationimsfa.

gdh, glutamate dehydrogenase; *tpi,* triose phosphate isomerase; 18S, 18S rRNA; IMS, immunomagnetic separation.

Table 3. Reported foodborne giardiasis outbreaks.

Country	**Associated food**	**Attributed source**	**No. of confirmed cases**	**Year**
Pennsylvania, USA	Mixed green salad	Unknown	25	2016
New York State, USA	No specific food type identified but 70% (14/20) of cases reported shopping at or consuming food from a local grocery store.	Asymptomatic food handlers	20	2015
Wisconsin, USA	Unpasturised milk	Unknown but outbreak occurred at a private home/residence and *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli* also detected	38	2014
Idaho, USA	Raw oysters	Unknown but purchased from the same grocery store	4	2012
Idaho, USA	Unknown	Unknown	3	2012
Virginia, USA	Unknown but all ate at a restaurant	Infected food handler	6	2010
Wyoming, USA	No specific food type identified	Unknown	8	2008
Missouri, USA	People who ate chicken parmesan and lettuce-based salads while in an office setting	Unknown thought to have been food caterer	15	2007
Vermont, USA	All were attending a camp but no specific food type identified	Unknown	36	2007
New York, USA	All ate at a restaurant catered lunch while at a school in New York state, but no specific food type identified	Unknown	8	2006
Florida, USA	All ate at a restaurant, but no specific food type identified	Unknown	4	2006
California, USA	Outbreak occurred at a religious facility, but no specific food type identified	Unknown	48	2006
New Jersey, USA	Fresh fruit and vegetables served at a camp	Fresh fruit and vegetables sourced from garden where the camp's goats	50	2005

		had access. Goat manure tested positive for *Giardia*.		
Pennsylvania, USA	Unknown	Unknown	2	2004
Pennsylvania, USA	Unknown but all had lunch at the same restaurant	Unknown	7	2004
Pennsylvania, USA	Unknown	Unknown	4	2004
Tennessee, USA	Unknown but outbreak occurred at a private home/residence	Chicken salad	6	2004
New York, USA	Unknown but all had lunch at the same restaurant	Unknown	20	2004
Washington, USA	Unknown but all had lunch at the same restaurant	Possibly contaminated ice	19	2004
New York, USA	Multiple foods implicated	Unknown	82	2000
San Francisco, USA	Unknown but all had lunch at the same restaurant	Infected food handler	34	2001
Washington, USA	Oysters	Unknown	3	1998
USA	Raw sliced vegetables served in a corporate office employee cafeteria	Infected food handler	18 (and 9 suspected cases)	1990
Washington state, USA	All ate all items on a fixed menu at a restaurant but thought to be contaminated ice.	Infected food handler	27	1990
New Mexico, USA	Lettuce, onions, tomatoes	Infected food handler	21	1989
Albuquerque, USA	Lettuce and taco ingredients	Infected food handler	10	1988
	Trip soup	Infected sheep?	-	
New Jersey, USA	Fruit salad	Infected food handler	10	1986
Minnesota, USA	Sandwiches	Infected food handler	88	1986
Connecticut, USA	Noodle salad	Infected food handler	13	1985
Minnesota, USA	Home-canned salmon	Infected food handler	29	1979
-	Christmas pudding	Rodent faeces	3	1960

[a] https://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx
[b] http://www.vdh.virginia.gov/content/uploads/sites/3/2016/02/Giardiasis2010.pdf
[c] https://www.health.ny.gov/statistics/diseases/foodborne/outbreaks/2005/docs/report.pdf
CDC NORS, US Centers for Disease Control and Prevention National Outbreak Reporting System https://wwwn.cdc.gov/norsdashboard/