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Review Article

Foodborne cryptosporidiosis

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ABSTRACT

Foodborne illness, the majority of which is caused by enteric infectious agents, costs global economies billions of dollars each year. The protozoan parasite *Cryptosporidium* is particularly suited to foodborne transmission and is responsible for >8 million cases of foodborne illness annually. Procedures have been developed for sensitive detection of *Cryptosporidium* oocysts on fresh produce and molecular diagnostic assays have been widely used in case linkages and infection source tracking, especially during outbreak investigations. The integrated use of advanced diagnostic techniques with conventional epidemiological studies is essential to improve our understanding of the occurrence, source and epidemiology of foodborne cryptosporidiosis. The implementation of food safety management tools such as Good Hygienic Practices (GHP), Hazard Analysis and Critical Control Points (HACCP), and Quantitative Microbial Risk Assessment (QMRA) in industrialised nations and Water, Sanitation, and Hygiene (WASH) in developing countries is central for prevention and control and foodborne cryptosporidiosis in the future.

1. Introduction

The global burden of foodborne illness caused by microbial, parasitic and chemical contamination of food is considerable, and affects individuals of all ages, particularly children <5 years of age and persons living in low-income regions of the world (CDC, 2016a). Of the approximately 600 million cases of foodborne illness in 2010, enteric infectious agents accounted for the vast majority (550 million) and were responsible for 230,000 deaths and 18 million disability-adjusted life years (DALYs) (WHO, 2015a,b). Foodborne illness costs have not been extensively studied but were estimated at US $10–83 billion per year in the United States (Nyachua, 2010). In New Zealand and Australia, the cost of foodborne illness has been estimated at US $86 million and US $1.289 billion respectively, per year (Lake et al., 2010; McPherson et al., 2011), and US $171 million in Sweden (Toljander et al., 2012).

While norovirus and *Campylobacter* spp. are among the most frequent causes of foodborne diarrhoeal disease, parasites are also a major contributor, responsible for ~104 million cases of diarrhoea in 2010. Of these, protozoa comprised the majority of cases (77.5 million), followed by nematodes, (12.3 million), cestodes (0.43 million) and trematodes (0.22 million) (WHO, 2015a,b). Of the protozoa, cryptosporidiosis caused by *Cryptosporidium* was responsible for 8.6 million cases of foodborne illness in 2010, 3,759 deaths and 296,156 DALYs (WHO, 2015a,b). The recent Global Enteric Multicenter Study (GEMS) and other studies such as the Global Burden of Disease 2015 Study, to identify the aetiology and population-based burden of paediatric diarrheal disease, revealed that *Cryptosporidium* is the number two cause of diarrhoea-associated mortality in children under 5 years of age, responsible for 60,4000 deaths in 2015 (Kotloff et al., 2013; Sow et al., 2016; G.B.D. Diarrhoeal Diseases Collaborators, 2017).

In a recent joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organisation risk ranking of foodborne parasites, which ranked parasites across five categories (public health, microbial ecology, animal health, agribusiness and trade, and socio-economic impact), *Cryptosporidium* was ranked fifth out of 24 potentially foodborne parasites in terms of importance as a foodborne pathogen (FAO/WHO, 2014), exceeded only in importance by *Taenia solium*, *Echinococcus granulosus, Echinococcus multilocularis* and *Toxoplasma gondii*. *Cryptosporidium* is among the 10 foodborne pathogens monitored by the FoodNet surveillance system in the United States (Crim et al., 2014). It is likely, however, that the number of cases of diarrhoea and the global burden of foodborne parasites are under-reported due to the lack of adequate detection and surveillance systems, particularly in developing countries, and due to the fact that most parasitic diseases are not notifiable and routine surveillance systems detect only a small

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fraction of the pathogenic infections that occur in the community. For example, a recent epidemiological study in the UK found that the overall ratio between identified enteric pathogenic cases reported to the national surveillance system and symptomatic cases of gastroenteritis in the community was one reported pathogen per 147 community cases (Tam et al., 2012). In Australia, a ratio of approximately 500 community cases to one notified case of gastroenteritis has been suggested (Hall et al., 2006). This is despite the fact that surveillance for *Cryptosporidium* is mandatory in the European Union (EU) and European Economic Area (EEA) countries and is a notifiable disease in many countries including Australia (Blumer et al., 2003; Cacciò and Chalmers, 2016). In addition, the infective stages of most foodborne parasites are environmentally resistant and can survive for long periods on the moist and sheltered surfaces of fruit and vegetables, and this coupled with prolonged incubation periods for many parasites results in a long lag time between contamination of a food item and an outbreak, thus increasing the difficulty of detection and trace-back (Gajadhar and Allen, 2004). Trace-back of outbreaks to food items is also complicated by the fact that in the majority of cases, the food item has been consumed and is therefore unavailable for testing.

The general symptoms associated with cryptosporidiosis, in addition to diarrhoea, include vomiting, nausea, lack of appetite and cramps (Chalmers and Davies, 2010) and cryptosporidiosis is associated with retarded cognitive and functional development in children in developing countries (Kirkpatrick et al., 2002; Valenzuela et al., 2014). In immunocompetent individuals, the symptoms of cryptosporidiosis are usually self-limiting but can be chronic in compromised immune systems, such as in children under 5 years of age, HIV patients and cancer patients (Chalmers and Davies, 2010). There is no effective treatment for cryptosporidiosis in all populations and no vaccine is available (Ryan et al., 2016). In the USA, approximately 8% of domestically acquired cases of cryptosporidiosis are foodborne (Scallan et al., 2011). Humans are susceptible to a wide range of *Cryptosporidium* spp. (~17) and genotypes (Table 1), with *Cryptosporidium hominis* and *Cryptosporidium parvum* the main species infecting humans globally (Xiao, 2010; Ryan et al., 2016). The former infects mostly humans and non-human primates, thus is predominately transmitted anthroponotically, whereas the latter infects humans as well as ruminants and some other animals, therefore can be transmitted both anthroponotically and zoonotically.

2. Detection of *Cryptosporidium* on food and in faecal samples

Prior to the advent of molecular techniques, the only way to identify *Cryptosporidium* spp. was microscopic examination of purified or unpurified oocysts (Ryan et al., 2016). This technique however lacks sensitivity, is labour intensive and is prone to human error (McHardy et al., 2014). Immunologically based detection methods have been developed, however cross-reactivity with other microorganisms can occur due to the non-specific nature of antibody-based methods and this can limit their use (Roellig et al., 2017). Although immunological methods are usually more sensitive than microscopy, they lack the sensitivity of molecular methods. For example, the sensitivity of modified Ziehl-Neelson staining has been reported as low as 70–87.5%, compared with immunofluorescent antibody stains (Chalmers et al., 2011b; Shimelis and Tadesse, 2014; Aghamolaie et al., 2016), but could miss more than half of cases compared with molecular methods (Checkley et al., 2015). The use of immunomagnetic separation (IMS) methods has significantly improved the specific detection of *Cryptosporidium* on food and standardised methods to detect *Cryptosporidium* oocysts using IMS and IFA staining on lettuce and raspberries have been developed with an overall sensitivity of 89.6% and 95.8%, and a specificity of 85.4% and 83.3% respectively, for lettuce and raspberries (Cook et al., 2006a, b). Despite this, in practice the specificities and particularly the sensitivities of these tests are highly variable, and some *Cryptosporidium* immunochromatographic assays have such low sensitivity and specificity that the US Council of State and Territorial Epidemiologists (CSTE) now exclude cases diagnosed as positive by immunochromatographic assays from cryptosporidiosis surveillance data, and specifies that cases diagnosed with these laboratory tests be considered probable rather than confirmed (Robinson et al., 2010a; Roelling et al., 2017) (http://c.ymcdn.com/sites/www.cste.org/resource/resmgr/PS/11-ID-14.pdf).

The application of molecular techniques such as PCR is increasingly used for the detection of *Cryptosporidium* on food in research laboratories and offers high sensitivity and specificity (Checkley et al., 2015; Hohweyer et al., 2016). Recently, several US Food and Drug Administration (FDA)-cleared molecular assays, based on multiplex PCR assays, have become available for simultaneous detection and identification of *Cryptosporidium* and other common enteric protozoan parasites (as well as bacteria and/or viruses). These include the BD MAX™ Enteric Parasite Panel (EPP) (Becton, Dickinson and Company, USA), the Luminex xTAG® Gastrointestinal Pathogen Panel (Luminex Corporation, Toronto, Canada), the NanoCHIP® GIP (Savyon® Diagnostics Ltd, Israel), the Biofire FilmArray™ Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT), the Verigene Enteric Pathogens (EP) test (Luminex) and the EasyScreen™ Enteric Parasite Detection Kit (Genetic Signatures, Sydney, Australia). These enteric panel assays offer enhanced sensitivity and specificity as well as the ability to detect mixed infections with multiple pathogens, and will play an important role in future foodborne outbreak investigations (Ryan et al., 2017a). However more extensive evaluation of these tests in different settings and sample types is essential.

The 18S rRNA locus is considered the most reliable locus for detection and identification of all *Cryptosporidium* spp. and genotypes (Xiao, 2010; Ryan et al., 2014). It is multi-copy and has both hypervariable and semi-conserved regions which facilitate specific and sensitive detection (Xiao, 2010). Subtyping tools are essential to better understand the transmission dynamics of *Cryptosporidium* spp. and particularly foodborne outbreaks. One of the most common subtyping tools is the DNA sequence analysis of the 60 kDa glycoprotein (*gp60*, also known as gp40/15) gene. IMS coupled with molecular subtyping tools for *Cryptosporidium*, particularly sequence analysis of the *gp60* gene and multilocus typing tools (Xiao, 2010; Hunter et al., 2008; Pérez-Cordón et al., 2016; Roelfsema et al., 2016; Sikora et al., 2017), have informed our understanding of the transmission dynamics of cryptosporidiosis in foodborne outbreaks, and are important tools in identifying clusters of cases and linking them to food items. Whole genome sequencing and comparative genomic analysis (Guo et al., 2015a), are now increasingly used in the characterisation of *Cryptosporidium* transmission in industrialised nations.

Molecular detection methods have been used to report prevalences of 0.6–12.5% for *Cryptosporidium* oocysts on different vegetables (Bohaychuk et al., 2009; Dixon et al., 2013; Hong et al., 2014). Recently an aptamer-based electrochemical biosensor has been developed for detecting *C. parvum* oocysts on fresh produce (Iqbal et al., 2015). In that study, aptamers (synthetic nucleic acids) specific for *C. parvum* were selected from an aptamer library following 10 rounds of selection, incorporated into a biosensor and used to detect oocysts on seeded pineapple and mango with a detection limit of 100 oocysts (Iqbal et al., 2015). However, detection limits of ~10 oocysts (approximately the lowest infectious dose of *C. parvum* for healthy adult human volunteers; Okhuysen et al., 1999) are required for detection of *Cryptosporidium* on food

Table 1
Cryptosporidium spp. and genotypes reported in humans.

Species/genotype name	Author(s)	Type host(s)	Major host(s)	Reports in humans
C. hominis	Morgan Ryan et al. (2002)	*Homo sapiens* (Human)	Humans	Most common species in humans
C. parvum	Tyzzer (1912)	*Bos taurus* (Cattle)	Ruminants	Commonly reported in humans
C. meleagridis	Slavin (1955)	*Meleagris gallopavo* (Turkey)	Birds and humans	Commonly reported in humans
C. felis	Iseki (1979)	*Felis catis* (Cat)	Cats	Many reports (cf. Lucio-Forster et al. 2010)
C. canis	Fayer et al. (2001)	*Canis familiaris* (Dog)	Dogs	Many reports (cf. Lucio-Forster et al., 2010)
C. ubiquitum	Fayer et al. (2010)	*Bos taurus* (Cattle)	Ruminants, rodents, primates	Commonly reported (cf. Li et al., 2014)
C. muris	Tyzzer (1907, 1910)	*Mus musculus* (House mouse)	Rodents	Many reports – Gatei et al. (2002), Tiangtip and Jongwutiwes (2002), Gatei et al. (2003, 2006), Palmer et al. (2003), Leoni et al. (2006), Muthusamy et al. (2006), Azami et al. (2007), Al Brikan et al. (2008), Neira et al. (2012), Hasajová et al. (2014), Chappell et al. (2015), Petrincová et al. (2015), Spanakos et al. (2015)
C. viatorum	Elwin et al. (2012a)	*Homo sapiens* (Human)	Humans	Only reported in humans to date. Elwin et al. (2012a), Insulander et al. (2013), Lebbad et al. (2013), Adamu et al. (2014), Ayinmode et al. (2014), Stensvold et al. (2015), de Lucio et al. (2016), Sanchez et al. (2017), Ukwah et al. (2017).
C. cuniculus	Robinson et al. (2010a)	*Oryctolagus cuniculus* (European rabbit)	Rabbits	Chalmers et al. (2009a, 2011a), Molloy et al. (2010), Elwin et al. (2012b), Koehler et al. (2014)
C. andersoni	Lindsay et al. (2000)	*Bos taurus* (Cattle)	Cattle	Guyot et al. (2001), Leoni et al. (2006), Morse et al. (2007), Waldron et al. (2011), Agholi et al. (2013), Jiang et al. (2014), Liu et al. (2014), Hussain et al. (2017)
C. suis	Ryan et al. (2004)	*Sus scrofa* (Pig)	Pigs	Xiao et al. (2002a), Leoni et al. (2006), Cama et al. (2007), Wang et al. (2013), Bodager et al. (2015)
Chipmunk genotype I	Jiang et al. (2005)	Chipmunk sp. (*Tamias* sp.)	Eastern grey squirrel (*Sciurus carolinensis*), Deer mice (*Peromyscus maniculatus*)	Considered an emerging human pathogen (cf. Feltus et al., 2006; Network, 2010; Lebbad et al., 2013; Guo et al., 2015b).
C. bovis	Fayer et al. (2005)	*Bos taurus* (Cattle)	Cattle	Khan et al. (2010), Ng et al. (2012), Helmy et al. (2013)
Skunk genotype	Xiao et al. (2002b)	Shunk (*Mephitis mephitis*)	Rodents	Robinson et al. (2008), Chalmers et al. (2009b)
Mink genotype	Wang et al. (2008)	American minks (*Mustela vison*)	River otter (*Lontra canadensis*), Ermine (*Mustela ermine*)	Ng-Hublin et al. (2013), Ebner et al. (2015)
Horse genotype	Ryan et al. (2003)	Przewalski's wild horse (*Equus przewalski*)	Four-toed hedgehog (*Atelerix albiventris*)	Robinson et al. (2008), Chalmers et al. (2009b), Xiao et al. (2009), Elwin et al. (2012b)
C. erinacei	Kváč et al. (2014)	*Erinaceus europaeus* (European hedgehog)	Hedgehogs, horses	Kváč et al. (2014)
C. scrofarum	Kváč et al. (2013)	*Sus scrofa* (Pig)	Pigs	Kváč et al. (2009)
C. tyzzeri	Tyzzer, 1912; Ren et al. (2012)	*Mus musculus* (Mouse)	Rodents	Rasková et al. (2013)
C. xiaoi	Fayer and Santín (2009)	*Ovis aries* (Sheep)	Sheep and goats	Adamu et al. (2014)
C. fayeri	Ryan et al. (2008)	*Macropus rufus* (Kangaroo)	Marsupials	Waldron et al. (2010)

(Cook et al., 2006b) and the aptamer method needs to be validated against PCR. Despite the high sensitivity of molecular methods, sample turbidity, pH and PCR inhibitors are significant issues for molecular detection of *Cryptosporidium* on food (Frazar and Orlandi, 2007). Other molecular tools such as LAMP-PCR (Gallas-Lindemann et al., 2016) or droplet digital PCR (ddPCR) (Yang et al., 2014), which are less sensitive to inhibitors, should increase the sensitivity of detection of *Cryptosporidium* on food in the future.

3. Transmission of foodborne *Cryptosporidium*

Humans can acquire cryptosporidiosis through several transmission routes such as direct contact with infected persons (anthroponotic) or animals (zoonotic transmission), and consumption of contaminated water (drinking or recreational) or food (Chalmers and Davies, 2010). Numerous aspects of the biology of *Cryptosporidium* render the parasite particularly suited to foodborne and/or waterborne transmission. These include: (i) oocysts are shed containing fully infective sporozoites and no secondary hosts or maturation conditions are required; (ii) the parasite's resistance to disinfection including chlorine (Painter et al., 2015); (iii) its ability to be shed in very large quantities, for example, neonatal calves can excrete up to 30 billion oocysts or more over a 1–2 week period (Kuczynska and Shelton, 1999); (iv) the low infectious dose (10–100 oocysts) (DuPont et al., 1995; Okhuysen et al., 1999; Chappell et al., 2006); (v) the viability of oocysts on fruit and vegetables is not affected by low temperature and are therefore still potentially infectious for several days to weeks in a household refrigerator (Macarisin et al., 2010a; Hohweyer et al., 2016); (vi) the long incubation period of cryptosporidiosis (averaging 7 days) (Chalmers and Davies, 2010), which delays the identification of the source and implementation of interventions to prevent transmission; and (vii) the fact that oocysts may continue to be shed in faeces for up to 60 days after cessation of gastrointestinal symptoms (Jokipii and Jokipii, 1986; Stehr-Green et al., 1987), which means that infected foodhandlers can still contaminate food long after their diarrhea resolves.

Other factors which increase the potential for foodborne cryptosporidiosis include the global nature of the food trade (which

has led to the rapid and widespread international distribution of foods), international travel, changes in consumer habits including eating outside of the home and consumption of more raw and undercooked foods (Jung et al., 2014; Dixon, 2015; Torgerson et al., 2015). A considerable number of foodborne illnesses occur in restaurants or catering venues. For example, in 2014, Centers for Disease Control (CDC), USA, reported that 65% of foodborne outbreaks occurred in restaurants (CDC, 2016b). In addition, the proportions of the population who are elderly, immunosuppressed or otherwise disproportionately susceptible to severe outcomes from cryptosporidiosis are growing in many countries (WHO, 2008).

Fruit and vegetables can become contaminated due to irrigation of crops with fecally-contaminated water, the application of manure to crops as fertiliser and the use of contaminated water to mix pesticides or wash produce (Budu-Amoako et al., 2011; Iqbal et al., 2015). The contamination of fresh produce during harvest, packaging, transport, food packing or preparation can also occur via infected farm workers. Large scale industrialisation of food processing, which means that food or food ingredients are often handled by several people, can also increase the opportunities for contamination. A contaminated food-contact surface or food ingredient can result in contamination of several batches of final product and wide distribution of contaminated products within a short period of time. Insufficient washing of hands by food handlers and of produce is a major issue. The problem is compounded by the fact that even vigorous washing of the surface of produce is not fully effective in removing the oocysts, which are often sticky and/or are protected by crevasses on the surface of the produce (Ortega et al., 1997; Hohweyer et al., 2016). Transmission is also facilitated by the fact that *Cryptosporidium* oocysts can survive within, and be protected by, the stoma of fresh fruits and leafy vegetables (Macarisin et al., 2010b). Money (coins and banknotes) has also been implicated as a vehicle for transmission of *Cryptosporidium* between food-related workers (Hassan et al., 2011). In that study, 60.2% of banknotes and 56.6% of coins obtained from food-related workers in Alexandria, Egypt, were contaminated, with *Cryptosporidium* being one of the most prevalent parasite species detected. In other studies, a *Cryptosporidium* prevalence of 10% has been reported in food handlers from Venezuela (Freites et al., 2009), and *C. hominis* was detected in a foodborne outbreak at a University canteen and in a food handler with diarrhoea who had prepared the vegetables and fruits consumed by outbreak cases (Quiroz et al., 2000).

4. Foodborne outbreaks of cryptosporidiosis

Cryptosporidiosis exerts its most public health impact through outbreaks and numerous foodborne (Table 2) and waterborne outbreaks due to cryptosporidiosis have been reported (Robertson, 2014; Dixon, 2015). Waterborne outbreaks of cryptosporidiosis have been extensively documented (Moreira and Bondelind, 2017). For example, from the start of the last century until 2016, *Cryptosporidium* was responsible for 60% (524/905) of all reported waterborne outbreaks caused by protozoan parasites (Karanis et al., 2007; Baldursson and Karanis, 2011; Efstratiou et al., 2017). The largest waterborne *Cryptosporidium* outbreak occurred in Milwaukee, USA in 1993, where an estimated 403,000 individuals contracted cryptosporidiosis via contaminated drinking water (MacKenzie et al., 1995), with an estimated illness-associated cost of US $96.2 million and 100 deaths (Corso et al., 2003). In 2010, the second largest waterborne outbreak occurred in the Swedish city of Östersund with an estimated 27,000 individuals infected (Widerström et al., 2014) and in 2011, another waterborne outbreak affected approximately 18,500 individuals in Skellefteå, Sweden (Bjelkmar et al., 2017). With the implementation of more stringent treatment of drinking water, the number of drinking water-associated outbreaks is in decline in developing countries, and most outbreaks in the USA and Australia are now associated with recreational water (Painter et al., 2015; Ryan et al., 2017b).

Foodborne outbreaks of cryptosporidiosis have been increasingly reported, however most foodborne outbreaks are never recognised, and those that are recognised, frequently are poorly investigated, if at all, and often go unreported (Robertson, 2014). This is due to a variety of reasons including under-reporting and difficulties in trace-back to food items discussed above but also partly due to better national and international standards for drinking water (Chalmers, 2012; Painter et al., 2015), which are largely lacking for food produce.

Cryptosporidiosis outbreaks are generally identified through various surveillance systems, laboratory reporting, drug purchase or prescribing data, and media broadcasts (Chalmers, 2012). Surveillance systems such as FoodNet, National Notifiable Diseases Surveillance System (NNDSS), and National Outbreak Reporting System (NORS) by the US CDC, the Australian NNDSS, and similar systems by the European CDC (ECDC) and jurisdiction nations are in place in developed countries. Despite this, current outbreak detection methods lack sensitivity, specificity and timeliness i.e. there is a considerable lapse of time before an outbreak is recognised (Hellard et al., 2000; Dale et al., 2010; van de Venter et al., 2015). For example, in the Milwaukee outbreak in 1993, only a small number of cases were investigated prior to the identification of the outbreak and confirmation of its cause; similarly the 2011 outbreak in Skellefteå, Sweden, went unnoticed by authorities for months (Bjelkmar et al., 2017). This is due in part to the fact that only approximately one-tenth of individuals who develop gastroenteritis visit their local doctor, and of these, less than 10% have a faecal specimen collected and not all of these will have their faecal samples tested for microorganisms (Tam et al., 2012; McHardy et al., 2014; Ryan et al., 2017a). For those who do have a faecal sample tested for *Cryptosporidium*, microscopy is still the routine detection method, which lacks specificity and sensitivity (Checkley et al., 2015; Ryan et al., 2016). In fact, most cases of cryptosporidiosis are not identified and many apparently sporadic cases that may be part of small outbreaks do not get recognised as being part of an outbreak (Briggs et al., 2014). These problems are compounded in developing countries due to the remoteness of communities, lack of transport and communication infrastructures, and a shortage of skilled health care workers and laboratory facilities to ensure accurate and rapid diagnosis (WHO, 2015a,b). In addition, in foodborne outbreaks, even if a potential food source is identified in an outbreak, laboratory testing may not detect *Cryptosporidium,* as samples from the relevant food product may not be available for testing or oocysts may not be present in the samples taken due to heterogeneous distribution of the parasite in the product.

The timeliness of outbreak investigations is also vital for limiting foodborne outbreaks, and to minimise the impact of an outbreak on both patients and the health system. Yet despite this, no guidance or standards currently exist regarding what might be considered a timely investigation (van de Venter et al., 2015). A person is usually unable to remember all the types of foods consumed during the incubation period of the infection and this coupled with the long incubation period for *Cryptosporidium* (up to 12 days or more) means that it takes longer for cases to be detected, resulting in increased risk of recall bias and can lead to either not identifying the source of a foodborne outbreak or a delay in identifying the source of an outbreak resulting in increased difficulties in identifying additional linked cases, given the increased potential for the movement of cases (van de Venter et al., 2015). Additional factors include the level of resources available to local

public health systems, the quality of surveillance, lack of effective public communications and the fact that many foodborne outbreaks are not published. For example, a review of published and unpublished foodborne outbreaks of diarrhoea in the UK found that few outbreaks reported to Public Health England led to peer-reviewed publication (O'Brien et al., 2006). This means that the importance of *Cryptosporidium* in foodborne disease outbreak causation, policy development and regulation may be overlooked.

Cryptosporidium oocysts have been identified on a wide variety of foods including fruit, vegetables, dairy products, meat and various species of shellfish (Robertson, 2014; Dixon, 2015). Of these, dairy products, fresh produce (particularly salads) and apple cider are some of the main food items implicated in foodborne cryptosporidiosis outbreaks (FAO/WHO, 2014) (Table 2). Apple cider has been linked to three foodborne outbreaks and in one study, molecular typing identified *C. parvum* subtype IIaA17G2R1 in cases ($n = 4$) and in the remaining contents of a jug of cider consumed by one of the cases (Table 2). Leafy salads have been linked to at least six foodborne outbreaks (Table 2), and while raw vegetable consumption has been linked to an outbreak in Denmark (Ethelberg et al., 2009), several case control studies have reported that consumption of raw vegetables has a protective effect against cryptosporidiosis (Robertson et al., 2002; Hunter et al., 2004; Roy et al., 2004). This is thought to be due to development of protective immunity from repeated exposure to low numbers of *Cryptosporidium* oocysts on raw vegetables. Over a third (9/25) of the foodborne outbreaks occurred in Nordic countries (Table 2), however this does not appear to be due to an increased prevalence of cryptosporidiosis in these countries but rather due to better outbreak investigation and reporting (Robertson and Chalmers, 2013).

Of the 16 foodborne outbreaks for which genotyping was conducted, *C. parvum* was responsible for ~81% (13/16) of outbreaks, both *C. parvum* and *Cryptosporidium ubiquitum* were identified in one outbreak and the remaining two outbreaks were caused by *C. hominis* (Table 2). This is in contrast to waterborne outbreaks of cryptosporidiosis, where *C. hominis* predominates, particularly in large outbreaks including the Milwaukee outbreak (Zhou et al., 2003; Chalmers and Davies, 2010; Widerström et al., 2014; Efstratiou et al., 2017). All the *C. parvum* foodborne outbreaks for which subtyping was conducted were caused by the IIa and IId subtype families, which are common in livestock (Xiao, 2010). This suggests that zoonotic transmission predominates in foodborne outbreaks, however it is important to note that relatively few genotyping studies have been conducted on foodborne outbreaks and identification of *C. parvum* does not necessarily indicate an animal origin of contamination. Therefore, further molecular typing studies are required to better understand the sources of contamination and transmission dynamics of foodborne outbreaks of cryptosporidiosis.

Another global challenge is climate change, which is likely to have a number of serious consequences for foodborne diseases, and cryptosporidiosis in particular, in both developing and developed countries (Semenza et al., 2012). For example, northern Europe is expected to experience a 5–20% increase in precipitation, whereas southern Europe is projected to experience a decrease of between 5% and 30% (Semenza et al., 2012). Temperature is one of the most critical factors governing the viability of oocysts in the environment; while *Cryptosporidium* oocysts are rapidly inactivated at temperatures >40 °C, warmer temperatures may increase the survival of oocysts in areas prone to soil subsurface freezing or lake ice covers, resulting in substantial numbers remaining infective after the winter period, where previously they may have been inactivated (King and Monis, 2007). An increase in the frequency and intensity of extreme precipitation events could flush infectious oocysts into waterways used for crop irrigation. Conversely, if there is significantly less rain, then infection risks also increase because oocyst concentrations are less diluted. The Global Waterborne Pathogen model for Human *Cryptosporidium* emissions predicts that while *Cryptosporidium* emissions in developing countries will decrease by 24% in 2050, in Africa, emissions to surface water will increase by up to 70% (Hofstra and Vermeulen, 2016), and this will undoubtedly have an impact on foodborne transmission. A QMRA tool, called CC-QMRA (Climate Change Quantitative Microbial Risk Assessment), has been developed to quantify the anticipated impacts in terms of relative infection risks under climate change scenarios for *Cryptosporidium* (and other organisms), to guide intervention strategies to limit or reduce the risk of infection (Schijven et al., 2013).

The increasing rise of organically farmed produce (and the use of animal manure), due to a growing market demand for these products, is also likely to increase foodborne transmission of *Cryptosporidium*, yet no systematic studies have been conducted and foodborne outbreak surveillance generally does not systematically collect information on food production methods, rendering traceback more difficult. Standardised testing methods such as the recently developed ISO 18744:2016 (http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber = 63252), for the detection and enumeration of *Cryptosporidium* oocysts (and *Giardia* cysts) on or in fresh leafy green vegetables and berry fruits will improve the detection of *Cryptosporidium* on food.

To enhance cryptosporidiosis surveillance, CDC launched CryptoNet—a DNA sequence-based surveillance system for cryptosporidiosis (http://www.cdc.gov/parasites/crypto/cryptonet.html) in 2010 (Painter et al., 2015). Molecular characterisation methods are being used to establish a database of foodborne (and other) outbreaks, which will inform analysis of national foodborne transmission patterns and assist in the development of targeted prevention guidance, to prevent the transmission of *Cryptosporidium*. For example, CryptoNet was recently used to identify and confirm epidemiological links among individual aquatic facility outbreak-associated cases in Alabama, Arizona and Ohio, USA in 2016 (Hlavsa et al., 2017). The current ISO 18744:2016 does not include any viability or molecular analysis and because the method includes spiking of positive control oocysts into the samples, the result of molecular typing assays will be obfuscated. Therefore, future method development needs to accommodate the addition of molecular testing, as this is essential in tracking transmission of foodborne cryptosporidiosis.

5. Prevention of foodborne outbreaks

In 2007, in recognition of the growing public health problem of foodborne diseases worldwide, the Beijing Declaration on Food Safety (http://www.wpro.who.int/foodsafety/documents/beijing_declaration_fos/en/) established the necessary steps towards reducing foodborne diseases. These included (i) establishing independent, competent food safety authorities; (ii) development of transparent regulation; (iii) ensuring adequate and effective enforcement of food safety legislation; (iv) establishment of food monitoring programmes with linkages to human and food-animal disease surveillance systems, to obtain rapid and reliable information on prevalence and emergence of foodborne diseases; (v) development of tracing and recall systems in conjunction with industry, to rapidly identify, investigate and control food safety incidents; (vi) development of food safety policies and priorities, including education; and (vii) promoting effective cooperation between developing and developed countries as well as among developing countries.

To date, more than 50 developed and developing countries have adopted the Beijing Declaration on Food Safety (WHO, 2007) and

Table 2
Foodborne outbreaks of cryptosporidiosis.

Year	Country	No. of cases	Age group affected	Species and *gp60* subtype of *Cryptosporidium* (if known)	Food item contaminated	Other information	References
2015	UK	424	Children and adults	*C. parvum*, IIdA24G1	Salad items from coffee shop chain	Thought to be salad items but not possible to conclusively determine	Trienekens et al., unpublished data[a]
2014	USA	11	Children and adults (2 months-76 years)	*C. parvum*, IIaA16G3R1	Unpasteurised goat milk	Source not identified. Goat stool was unavailable for testing, water from the producer's well tested negative	Rosenthal et al. (2015)
2012	Finland	250	Children and adults	*C. parvum*, IIdA17G1	Frisée salad	Five outbreaks occurred between October and November, 2012. The cases were connected by lunch meals at restaurants in four different cities. In two outbreaks, the same *C. parvum* IIdA17G1 subtype was found	Åberg et al. (2015)
2012	Norway	40	Children	*C. parvum*, IIaA19G1R1	Unknown	Outbreak occurred at recreational holiday farm and same subtype identified in animals (lambs and goat kids)	Lange et al. (2014), Johansen et al. (2015)
2012	UK	648	Mostly adults	*C. parvum* IIaA15G2R1.	Ready-to-eat, precut mixed salad leaves	Difficulties in trace-back of salad leaves, hinders investigations into the ultimate sources of foodborne outbreaks	McKerr et al. (2015)
2010	Sweden (Örebro)	16	Adults	*C. parvum*, IIdA20G1e identified in two cases	Not identified, possibly a food handler	IIdA20G1e,was described in a Swedish calf, suggesting a possible zoonotic source for the Örebro outbreak (Silverlas et al., 2010)	Gherasim et al. (2012)
2010	Sweden (Umeå)	89	Adults	*C. parvum*, IIdA24G1 identified in six cases	Salad garnish on chanterelle sauce	The Örebro and Umeå outbreaks were geographically and epidemiologically isolated	Gherasim et al. (2012)
2009	USA	46	Adults and children	*C. parvum*, IIaA17G2R1 identified in seven cases	Unknown	Outbreak occurred at a summer camp in North Carolina and livestock on the farm were identified as the probable source	CDC (2011), Collier et al. (2011)
2009	Norway	74	Mostly children	*C. parvum*, IIaA19G1R1	Possibly peeled carrots	Same recreational holiday farm as the 2012 Norway outbreak. Contamination of food items by an infected food handler possible source	Rimšelienė et al. (2011), Johansen et al. (2015)
2008	Sweden	18		*C. parvum* IIaA16G1R1 (n = 10), IIaA15G2R1 (n = 1),IIdA22G1 (n = 3),and IId19G1 (n = 1)	Arugula salad	Source of contamination not identified. *gp60* typing conducted on 15/18 cases	Insulander et al. (2013)
2008	Sweden	21	Adults	*C. parvum*	Béarnaise sauce containing chopped fresh parsley (added to the sauce after heating)	Source of contamination not identified but the fresh parsley had been imported from Italy in plastic bags and was not rinsed before use	Insulander et al. (2008)
2008	Finland	72	Adults	*C. parvum*	Salad mixture (from several countries) suspected.	Source of contamination not identified.	Pönka et al. (2009)
2006	Japan	4	Adults	*C. parvum* IIaA16G2R1	Raw meat dish called "Yukke: Korean-style beef tartare" and/or raw liver	Source of contamination not identified	Yoshida et al. (2007)
2006	Germany		Information not provided,	Unknown	Milk and dairy products		EFSA (2007)
2005	Denmark	99	Adults	*C. hominis*	Canteen salad (carrots and red peppers)	Infected food handler possible source	Ethelberg et al. (2009)
2003	USA	144	Adults and children	*C. parvum* (n = 11), *C. ubiquitum* (n = 1) IIaA15G2R1 and IIaA17G2R1	Ozonated apple cider	IIaA17G2R1 identified in the remaining contents of a jug of cider that a laboratory-confirmed case-patient had partially drunk. Ozonation failed to inactivate *Cryptosporidium* oocysts. Cattle most likely source, as this is a common subtype in cattle	Blackburn et al. (2006)
2001	Australia	8	Children	Not determined	Unpasturised milk	Positive for *Cryptosporidium* by ELISA	Harper et al. (2002)
1997	USA	54	Adults	Not determined	Thought to be foods containing uncooked green onions eaten by a group attending a dinner banquet.	Infected food handler, also the green onions were not washed before delivery at the restaurant and were not washed at the restaurant	CDC (1998)

Table 2 (*continued*)

Year	Country	No. of cases	Age group affected	Species and *gp60* subtype of *Cryptosporidium* (if known)	Food item contaminated	Other information	References
1998	USA	152	Adults (University students and staff)	*C. hominis*	Most likely source were vegetables and fruits served at a university canteen, which were served raw.	*C. hominis* also detected in a food handler with diarrhoea, who had prepared the vegetables and fruits	Quiroz et al. (2000)
1996	USA	31	Mostly adults (median age was 27 years)	Not determined	Apple cider	Not determined but thought to be from a contaminated well on the property used for washing the apples. The cider mill was located across the road from a dairy farm	CDC (1997)
1995	UK	50	School children	Not determined	Cow's milk	Thought to be due to pasteurisation failure	Gelletlie et al. (1997)
1995	USA	15	Mostly adults	Not determined	Chicken salad	Possibly an infected food handler	CDC (1996)
1993	USA	160	Mostly school children		Fresh-pressed apple cider	*Cryptosporidium* oocysts were detected in the apple cider, on the cider press, and in the stool specimen of a calf on the farm that supplied the apples	Millard et al. (1994)
1990	Russia	13	Infants	Not determined	Milk kefir (probiotic fermented milk drink)	Oocysts were found in milk sediments on the filter taken from a tank at a local milk factory. Contamination assumed to have come from the local farm	Romanova et al. (1992)
1986	Mexico	1	Adult	Not determined	Salad	Purchased from a street vendor in Tepic, Mexico	Sterling et al. (1986)
1984	UK	24	Adults and children	Not determined	Possibly undercooked sausage or contaminated milk	Source not identified. Concurrent Campylobacter infection	Casemore et al. (1986)

[a] Trienekens, S., Kanagarajahy, S., Campos-Matos, I., Verlander, N., Charlett, A., Shaun, S., Inns, T., Elson, R., Chalmers, R., Gordon, R., Hawkins, G., Smith-Palmer, A., Alexander, C., Williams, C., Nichols, G. 2017. National *Cryptosporidium* outbreak linked to salad. 6th International *Giardia* and *Cryptosporidium* Conference. Havana, Cuba. P6.

the WHO Initiative to estimate the global burden of foodborne disease (WHO, 2015a,b) was an important part of the implementation of the Beijing Declaration. It aimed not just to estimate the global burden of foodborne diseases, but also to strengthen the capacity of countries to assess the impact of food safety measures and advise on the cost-effective use of resources. This is because accurate burden of illness estimates are essential for decision makers seeking to allocate resources to address the issues caused by foodborne pathogens. However, much work remains and foodborne diseases continue to be an important issue and have hampered the achievement of the United Nations (UN) Millennium Development Goals (MDGs) (Kuchenmüller et al., 2009; WHO, 2015a,b). The MDGs are eight specific development goals that aimed to combat extreme poverty around the world, to be met by 2015 (MDG, 2015). Foodborne diseases have specifically impacted the goal of reducing the children under-five years of age mortality rate by two-thirds by 2015, because children (and malnourished people) are particularly vulnerable to foodborne disease and more likely to die.

In developed countries, a risk-based approach to food production is used where several critical control points are monitored and is based on several strategies including the implementation of Good Agricultural Practices (GAP). A GAP framework considers the implementation of best practices regarding worker's health and hygiene, soil and water quality, sewage treatment, wildlife and livestock management, manure and biosolids management, field sanitation and hygiene, and harvest and transportation (Sant'Ana et al., 2014). The implementation of food safety management tools such as Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP), at postharvest steps, also helps to reduce, eliminate or prevent the spread of foodborne diseases. QMRA overlaps with HACCP, and is widely used in the Water Industry. QMRA is a four-tiered approach that interprets laboratory monitoring data in terms of public health impact and involves hazard identification, exposure assessment, dose–response modeling, and risk characterisation (Haas et al., 1999). Identifying the appropriate parameters, as well as defining the assumptions to be included in QMRA, is challenging, particularly for the food industry. However, QMRA has been used to identify produce most likely to become contaminated by *Cryptosporidium* (and other foodborne pathogens), identify stages in their harvesting, processing and handling that carry the most risk of foodborne transmission and identify the potential impact on public health. For example, QMRA was conducted to evaluate the public health impact of *Cryptosporidium* (and *Giardia*)-laden water irrigating produce in Mexico, to provide a foundation for the creation of food quality guidelines for irrigation of fresh produce (Mota et al., 2009). In the UK, QMRA was used to predict the number of humans infected with *Cryptosporidium* (and other pathogens), through consumption of root crops grown on agricultural land to which treated sewage sludge had been applied (Gale, 2005). The authors concluded that assuming a 12-month gap between application of sewage and harvest, that the risk was low with only one predicted resultant *Cryptosporidium* infection in the human population every 45 years (Gale, 2005). In the USA, the Food Safety Modernization Act (FSMA) has also adopted a risk-based approach and has shifting the focus from responding to foodborne contamination to preventing it (https://www.fda.gov/Food/GuidanceRegulation/FSMA/). As a result of this, the USFDA have established science-based minimal standards for the safe production and harvesting of produce including raw fruits and vegetables, and foreign suppliers are required to meet the same standards as domestic producers.

Due to the globalisation of food production, outbreaks of foodborne cryptosporidiosis that were once contained within a small community may now take place on larger scale. In order to facilitate *Cryptosporidium* foodborne outbreak investigations, methods for identifying the food products involved need to be improved. In outbreaks where the traditional epidemiological approaches fall short and the outbreak occurs over a longer period and/or in a larger geographical area, additional statistical tools including an adjusted likelihood ratio approach has been helpful in analysing the association between case and food product distribution through ranking different food products as the possible source of the outbreak (Norström et al., 2015).

In developing countries, in addition to the initiatives outlined above, access to improved WASH (Freeman et al., 2013) is crucial for limiting foodborne transmission and secondary spread. However, an estimated 663 million people still lack improved drinking water sources and 2.4 billion people have no access to improved sanitation, respectively (UNICEF, 2015). The UN MDGs successfully halved the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015 (UN, 2017), but has fallen short on its goal of halving the proportion of people without sustainable access to sanitation.

The application of molecular techniques is central to the prevention of future foodborne outbreaks, due to their ability to detect the occurrence, source and epidemiology of foodborne cryptosporidiosis. For example, in 2010 in Sweden, an increase in *Cryptosporidium* cases in the Stockholm/Uppsala area was investigated, which subsequently led to the discovery of a *C. parvum* foodborne outbreak in the geographically distant city of Umeå in northern Sweden (Gherasim et al., 2012). Typing at the *gp60* locus identified a rare *C. parvum* subtype (IIdA24G1) from both the Umeå outbreak and the Stockholm area cases, thus indicating a possible outbreak in the Stockholm area and establishing a link between these two events (Gherasim et al., 2012). At the same time, a foodborne outbreak occurred in the city of Örebro and typing was able to establish that this outbreak was not linked to the previous two, as a different *C. parvum* subtype (IIdA20G1e) was identified (Gherasim et al., 2012). There are limitations to the genotyping resolution of *gp60* and this has led to multiple studies looking into the potential use of multilocus sequence typing (MLST). A recent study based on whole-genome sequencing of 17 different *C. hominis* isolates developed a new sequence-based typing panel with higher resolution than *gp60* (Beser et al., 2017). The assay based on a one-step PCR of nine loci, which can be sequenced using a Next Generation Sequencing (NGS) platform, was used to show that the Östersund and Skellefteå outbreaks in Sweden in 2010 and 2011 were linked (Beser et al., 2017). This type of high level genotyping will be of great value in rapidly identifying and preventing the spread of foodborne outbreaks in the future.

Syndromic surveillance, defined as the real-time (or near real-time) collection, analysis, interpretation and dissemination of health-related data (Triple, 2011), also has the potential for early detection of foodborne outbreaks and limiting their spread. For example, an analysis of the waterborne outbreak of cryptosporidiosis in Skellefteå, Sweden reported that if monitoring of call patterns to the national Swedish telephone health advice line had been in place, that the outbreak, which began in early January 2011 and peaked in April, would have likely ended during January (Bjelkmar et al., 2017).

6. Conclusions

As most *Cryptosporidium* spp. are indistinguishable by traditional microscopy or immunodetection assays, only molecular diagnostic methods can effectively track foodborne outbreaks of cryptosporidiosis. Thus, rapid and efficient multidisciplinary collaboration combining conventional epidemiological analysis with molecular typing is essential for early detection of outbreaks, estimating their magnitude and limiting their spread by the identification of the contaminated food item. Molecular characterisation of *Cryptosporidium* specimens needs to shift from predominantly supporting outbreak investigations to becoming nationally (e.g. CryptoNet) and internationally (e.g. European Surveillance System (TESSy) systematic. Advancing molecular typing from single-gene to multilocus or whole-genome sequencing (which will increase discriminatory power), and increasing local and national capacity to collect and share epidemiological data is essential. This systematic analysis of foodborne outbreaks will also provide information for future prevention by identifying the main causes, understanding the risks and ensuring effective interventions are in place.

Uncited reference

Robinson et al. (2010b).

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