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Waterborne outbreaks of cryptosporidiosis

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Abstract. Water is the most commonly reported vehicle of transmission in *Cryptosporidium* outbreaks. While mains drinking water quality is highly regulated in industrialised countries, treated recreational water venues remain highly variable and these have emerged as important settings in the transmission of cryptosporidiosis. Epidemiological investigations of outbreaks benefit from supplementary microbiological evidence and, more recently, the application of molecular typing data to link isolates from cases to each other and to suspected sources. This article documents how waterborne *Cryptosporidium* outbreaks are identified and reported, how such outbreaks have acted as drivers of regulatory change, and some of the recent developments in the detection and investigation of these outbreaks and their spread, especially the application of molecular typing assays.

Key words: Cryptosporidium, genotyping, recreational, drinking, water.

Riassunto *(Epidemie di cryptosporidiosi trasmesse con le acque)*. L'acqua è il più comune veicolo di trasmissione di epidemie dovute a *Cryptosporidium*. Mentre la qualità delle acque potabili distribuite da acquedotti è fortemente regolamentata nei paesi industrializzati, la qualità delle acque trattate di ambienti ricreativi chiusi è fortemente variabile e questi ambienti si sono rivelati importanti nella trasmissione della criptosporidiosi. Le indagini epidemiologiche sulle epidemie traggono beneficio dalle evidenze microbiologiche e, più recentemente, dall'applicazione dei dati di tipizzazione molecolare per collegare i ceppi isolati con i casi d'infezione e con le fonti di contagio sospette. Quest'articolo documenta come vengono individuate e notificate le epidemie di *Cryptosporidium* trasmesse con le acque, come tali epidemie hanno agito da guida per migliorare la normativa, ed alcuni recenti sviluppi nella rilevazione e nelle indagini di queste epidemie e della loro diffusione, in particolare l'applicazione dei saggi di tipizzazione molecolare.

Parole chiave: Cryptosporidium, genotipizzazione, acqua ad uso ricreativo, acqua potabile.

INTRODUCTION

Human infection with the protozoan parasite *Cryptosporidium* causes the gastrointestinal disease cryptosporidiosis. Of the 25 or so species currently recognised, 15 have so far been reported in humans, of which some are established as human pathogens: *C. parvum*, *C. hominis* (which are the most commonly detected species in human cryptosporidiosis worldwide) and *C. meleagridis* are supported by human infectivity and clinical outcome data from feeding trials in adult volunteers [1-3], *C. cuniculus* (formerly the rabbit genotype) caused a drinking waterborne outbreak in the United Kingdom (UK) [4], and *C. felis* and *C. canis* were associated with diarrhoea in children in a shanty town in Peru [5]. Dose response studies have shown similar ranges for some *C. parvum* isolates compared with *C. hominis*, and small numbers (< 10) of parasites ingested can cause disease [1, 2]. Other *Cryptosporidium* species are rarely reported human infections, or have never been found in humans, and many are considered adapted to farmed animal or wildlife hosts [6] *(Table 1)*.

Transmission is by the faecal-oral route, from either humans or animals, depending on the *Cryptosporidium* species; for example, *C. hominis* has a human infection cycle while *C. parvum* also has susceptible animal hosts causing mainly gastrointestinal disease in young ruminants. The natural host for *C. cuniculus* is the European rabbit *(Oryctolagus cuniculus)* [13] and for *C. felis*, cats and *C. canis*, dogs. Although *C. meleagridis* was originally identified in farmed turkeys [14] current distribution and risk factors for human acquisition are not known; many cases report no contact with birds, the parasite species has a wide host range and other bird-restricted species are not considered a threat to human health. Although some *Cryptosporidium* spp. are highly infectious person-to-person, it is the parasite's ability to survive in the environment and its resistance to chlorine disinfection that support transmission via drinking and recreational waters, and other vehicles such as food. *Table 2* shows the human risk factors for acquisition of *Cryptosporidium* spp. and how these relate to settings where outbreaks have occurred.

Symptoms of cryptosporidiosis, which usually occur between 2 to 12 (usually 5 to 7) days after ingestion of oocysts (the transmissive stage of the life cycle), include watery diarrhoea, abdominal pain, nausea and/or vomiting, low grade fever and malaise, and may last for up to three weeks during which time apparent recovery may be followed by

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Table 1 | *Cryptosporidium species and selected genotypes and their association or not with human cryptosporidiosis*

Cryptosporidium **species**	**Mean oocyst dimensions (µm)**	**Major host(s)**	**Association with human cryptosporidiosis or infection**	**Selected references**
Most commonly associated with human cryptosporidiosis				
C. hominis	4.9 x 5.2	Humans	Common in sporadic cases and outbreaks; infectivity data from experimental infections in adults	[2]
C. parvum	5.0 x 4.5	Humans, ruminants	Common in sporadic cases and outbreaks; infectivity and dose response data from experimental infections in adults	[1]
C. meleagridis	5.2 x 4.6	Homoeothermic birds; mammals including humans	Sporadic cases reported, more frequent in some populations, for example as common as ***C. parvum*** in Peru and Thailand; infectivity data from experimental infections in adults	[3, 6]
Less commonly associated with human cryptosporidiosis				
C. canis	5.0 x 4.7	Dog	Epidemiologically linked to diarrhea in children in a shanty town in Lima, Peru; occasional sporadic cases in various countries, especially developing countries	[5, 6]
C. cuniculus	5.6 x 5.4	Rabbit, humans	Caused a waterborne outbreak in UK; occasional and seasonal sporadic cases in UK, individual reports from France, children in Nigeria	[4]
C. felis	4.6 x 4.0	Cat	Epidemiologically linked to diarrhea in chldren in a shanty town in Lima, Peru; occasional sporadic cases in various countries	[5, 6]
C. ubiquitum	5.0 x 4.7	Various mammals	Sporadic cases in various countries, especially developed countries	[6]
C. viatorum	5.4 x 4.7	Humans	Sporadic cases emerging in UK and Sweden, linked to visits to the Indian sub-continent, South America and Africa	[7]
Rarely associated with human cryptosporidiosis				
C. andersoni	7.4 x 5.5	Cattle	Individual reports from UK, Australia, Malawi	[6, 8]
C. bovis	4.9 x 4.6	Cattle	Individual reports from Australia and India	[8]
C. fayeri	4.9 x 4.3	Red kangaroo	Individual report from Australia	[8]
C. muris	7.0 x 5.0	Rodents	Individual reports from various developing countries	[6]
C. scrofarum	5.2 x 4.8	Pig	Individual report from Czech Republic	[9]
C. suis	4.6 x 4.2	Pig	Individual reports from UK and Peru	[6]
C. tyzzeri (syn. mouse genotype I)	4.6 x 4.2	Mice	Individual report from Czech republic	[10]
Chipmunk genotype I	4.8 x 4.2	Chipmunk; possibly other Sciuridae	Individual reports from USA, France, Sweden	[6]
Horse genotype	4.6 x 4.2	Horses	Individual reports from UK, USA	[11, 12]
Monkey genotype	not reported	Monkey, human	Individual reports from UK, Malawi	[6]
Skunk genotype	not reported	Skunk; possibly other mustelids	Individual report from UK	[6, 11]
Not associated with human cryptosporidiosis or infection				
C. macropodum	5.4 x 4.9	Eastern grey kangaroo	No association	
C. ryanae	3.7 x 3.2	Cattle	No association	
C. wrairi	5.4 x 4.6	Guinea pig	No association	
C. xiaoi	3.9 x 3.4	Sheep	No association	
C. baileyi	6.2 x 4.6	Chicken	No association	
C. galli	8.3 x 6.3	Chicken	No association	
C. fragile	6.2 x 5.5	Black spined toad	No association	
C. serpentis	6.2 x 5.3	Snakes	No association	
C. varanii	4.8 x 4.7	Mainly lizards; snakes	No association	
C. molnari	4.7 x 4.5	Sea bream	No association	
C. scopthalmi	4.4 x 3.9	Turbot	No association	
Various genotypes	Usually within the 4-6 range	Various, or not known if found in environmental samples and no host yet identified	No association	

Table 2 | *Exposure risk factors for human acquisition of cryptosporidiosis, and related outbreak settings*

Risk factor	*Cryptosporidium* species involved	Groups of people at risk	Outbreak settings
Drinking contaminated water	*C. parvum* *C. hominis* *C. cuniculus*	All consumers	Community with mains water supplies Small communities and settings with small or private water supplies
Eating contaminated food	*C. parvum* *C. hominis*	All consumers; people attending events or functions	Community Food establishments (*e.g.* catering establishments, institutions) Specific events (*e.g.* agriculutural fairs)
Traveling to less industrialised countries	*C. parvum* *C. hominis* *C. meleagridis* *C. viatorum*	Sporadic cases; groups of travellers	Various
Recreational activities involving water immersion	*C. parvum* *C. hominis*	Mainly children	Swimming pools Paddling or wading pools Water parks Fountains Natural waters
Contact with farmed animals, especially young ruminants; contact with animal dung	*C. parvum*	Those exposed through occupational and recreational activities (*e.g.* veterinary students, farmers, visitors to petting farms)	Veterinary schools, agricultural colleges, petting farms, farms opened to the public, residential outdoor activity centres
Changing nappies; toileting young children	*C. hominis*	Individual carers and those exposed through occupational activities (*e.g.* nursery/day-care centre employees)	Nurseries; day care centres
Contact with another person with diarrhoea	*C. hominis* *C. parvum* Others – some evidence that all species infecting humans can be transmitted person to person	Individual carers, family members, close contacts	Nurseries, day care centres, institutions such as schools, hospitals, prisons

temporary recurrence [15]. Oocysts may continue to be shed in faeces for many days after symptoms have ceased. About 10% cases may be hospitalised. Treatment is by supportive therapy to prevent dehydration; there is no licenced specific treatment in the EU, although nitazoxanide is licenced by the United States (US) Food and Drug Administration for immunocompetent patients over 1 year of age. Severely immunocompromised patients, especially those with T-cell immune deficiencies, risk chronic or intractable disease and infection of sites other than the gastrointestinal tract [16]. Immune reconstitution is important in these patient groups, as treatment modalities are otherwise undefined, and prevention of infection through risk reduction is paramount. For example, since the late 1990's, the Department of Health in England advised that those with compromised T-cell function should boil all drinking water (including bottled water) [17]. However, whether, given improved drinking water quality and reduction in *Cryptosporidium* risk since then [18], this permanent blanket advice remains necessary there is currently under review. Also of great concern are the longer term sequelae of infection in both the general population and in malnourished children in whom, even following asymptomatic infection, reduced cognitive function and failure to thrive have been reported (reviewed by Putignani and Menichella [19]). Even in non-immune compromised people, there is some evidence to suggest there may be different long term health effects depending on infecting species: for example, those cryptosporidiosis patients infected with *C. hominis* (but not *C. parvum*) were more likely to report joint pain, eye pains, headaches and fatigue in the two months following infection than controls

[20]. The relationship between clinical outcome in terms of severity of acute disease and long term sequelae requires further investigation.

Although people of any age can become infected, most cases of cryptosporidiosis are reported in children under 5 years largely due to intestinal tract immaturity and lack of mucosal immunity [20]. In non-industrialised countries, the peak incidence is in infants less than 1 year old with adult cases rarely identified, and in industrialised countries mainly in 2-4 year olds with a second, smaller peak in adults of child-rearing age. This is likely due to differences in general hygiene and feeding practices for infants, and age-related immunity generated by repeated exposure (for example, low-level exposure through drinking water) in older age groups. However, any immunising effect would come at a high risk to the health of the most vulnerable sectors of the population: young children and immunocompromised patients. The epidemiology of human cryptosporidiosis globally has been reviewed most recently by Putignani and Menichella [19].

The clinical problems of cryptosporidiosis described above contributed to the inclusion of the parasite in the World Health Organisation's (WHO) Neglected Diseases Initiative in 2004 [21]. Further, large waterborne outbreaks have highlighted the clinical and economic importance of *Cryptosporidium*. Waterborne cryptosporidiosis outbreaks are more commonly reported than outbreaks involving other vehicles; up to the end of 2010, a total of 185 outbreaks had been identified and reported globally [22, 23], contrasted with less than 20 foodborne outbreaks [24]. This is only partly because of the features of *Cryptosporidium* favouring waterborne transmission, as some of these also favour the foodborne route:

- multiple hosts for some human pathogenic species, for example, *C. parvum* (mainly humans and young ruminants);
- ubiquitous distribution (*Cryptosporidium* spp. occur worldwide);
- large numbers of oocysts (10^{10}), the transmissive stage, are shed by susceptible hosts during acute infection;
- oocysts are shed containing fully infective sporozoites – no secondary hosts or maturation conditions are required;
- transport vectors may provide further distribution of oocysts within or to the acquatic environment;
- small size of oocysts (4-6 μm for human-infective species) means they may pass between sand grains in filter beds, although other forces including sedimentation and adsorption also interplay during filtration, and application of a coagulant (flocculant) improves removal;
- oocysts can be discharged in sewage effluent in significant numbers;
- oocyst are robust and can survive for months in cool, moist environments; they also survive chlorination and;
- small numbers of ingested oocysts can cause disease [25].

Furthermore, the large scale of some drinking waterborne cryptosporidiosis outbreaks (the Milwaukee outbreak in 1993 involved an estimated 403 000 cases of cryptosporidiosis [26]) led to greater emphasis on monitoring and intervention of water supplies, and greater awareness and investigation of water as a transmission vehicle, including recreational waters such as swimming pools. There is a perception of cryptosporidiosis as a waterborne disease, perhaps at the expense of investigation in other possible routes and investment in their interventions. However, where chlorine disinfection is widely used to treat drinking water, *Cryptosporidium* is indeed one of the most common waterborne pathogens. For example, in a review of 89 waterborne outbreaks of infectious intestinal disease (IID) involving 4321 cases in England and Wales, *Cryptosporidium* was the causative agent in 69% [27]. In recreational waters, *Cryptosporidium* is the leading microbial cause of outbreaks in both the UK and USA [27, 28]. Despite global distribution of *Cryptosporidium*, reports of waterborne outbreaks are weighted heavily towards industrialised countries in the continents of Australia (especially New Zealand), Europe (especially UK and Ireland) and North America [19, 23]. This distribution may reflect national variations in surveillance, reporting, monitoring, and investigation of cases and outbreaks, as well as highlighting risk factors such as intensive stocking of farmed animals, environmental contamination, weather conditions and events, discharge of sewage effluent into drinking water sources, and the use of surface water sources. The pathways and host, parasite and environmental factors that determine the risk of infection, and thus public health outcomes, are shown in *Figure 1*, and how they contribute to waterborne *Cryptosporidium* outbreaks has been reviewed previously [for example, see 19, 22, 29]. Risebro and colleagues used a fault tree analysis to examine the contribution and interpretation of events in outbreaks in the EU occurring between 1990 and 2005 [29]. Of 31 protozoal drinking water outbreaks, 29 were *Cryptosporidium*, and most of these outbreaks were attributed to chronic filtration failures or livestock and rainfall in the catchment, contributing concurrently in 11 outbreaks. Interestingly, the most recent outbreaks in the UK have been attributed to human or wildlife sources *(Table 3)*. It is now possible, using molecular methods, to differentiate the *Cryptosporidium* species found in water samples and supplement catchment data to track the source of contamination to humans, farmed animals or wildlife and to assess the level of risk posed to public health beyond that previously possible from oocyst counts alone.

The aim of this article is to document how waterborne *Cryptosporidium* outbreaks are identified and reported, how such outbreaks have acted as drivers

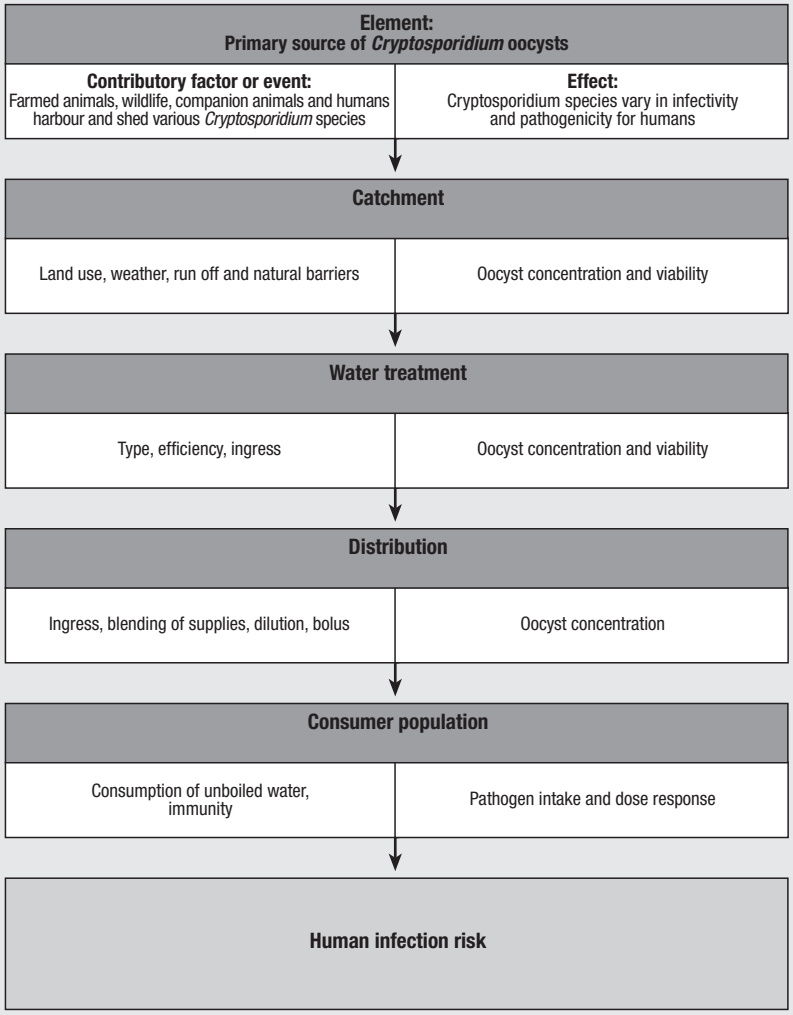

Fig. 1 | *Factors influencing drinking waterborne cryptosporidiosis: elements, contributory factors or events, and their effects.*

of regulatory change, and some of the recent developments in the detection and investigation of these outbreaks, especially the application of molecular typing assays.

IDENTIFICATION OF *CRYPTOSPORIDIUM* OUTBREAKS

Waterborne cryptosporidiosis outbreaks are identified through various surveillance systems, including passive and active morbidity reporting such as syndromic surveillance, laboratory reporting, sentinel surveillance systems, drug purchase or prescribing data, and media broadcasts. Poor water quality results, incidents or treatment failures will alert providers and authorities to a potential or existing outbreak. For example, following a water quality incident in the East Midlands, England in 2008 *(Table 3*; outbreak number 08/278), when oocysts were detected in treated water with a long history of non-detects in routine monitoring, syndromic surveillance data collected under the Qsurveillance scheme showed a significant increase in general practictioner consultations for diarrhoea and gastroenteritis in the week of the incident in the water distribution areas, compared with no increase in the unaffected areas [30]. Of the 33 cases of laboratory confirmed cryptosporidiosis identified during the outbreak investigation, 23 were *C. cuniculus*, the outbreak species (see below) [4] but QSurveillance data estimated an excess of 422 diarrhoea cases during the outbreak, an increase of about 25% over baseline weekly levels [30]. Thus, syndromic surveillance described the extent of cryptosporidiosis in the general population and provided reassurance that there was no further widespread impact.

The symptoms of cryptospordiosis are non-specific, so laboratory detection of the parasite, usually in stools, is required to confirm the infection. This is either by microscopy with prior staining using tinctorial, fluorescent or immunofluorescent stains, or immunoassay-based test kits, or more recently molecular assays. Routinely, detection in both clinical diagnostic and water testing laboratories, is of the genus: species identification can only

Table 3 | *Selected waterborne outbreaks of cryptosporidiosis in England and Wales 2001-2010 with molecular typing*

HPA Centre for Infections outbreak reference number	Government Office region	Year	Month	Setting or vehicle	Number of cases (laboratory confirmed)	Number of cases genotyped and result	Genotyping environmental isolates (where requested)	Strength of association (where indicated)
Drinking water								
02/1701	South East	2002-2003	November-January	Public water supply	➢ 31 (31)	28 *C. hominis*		Strong
05/552	South East	2005	September-November	Public water supply	140 (76)	76 *C. hominis*		Strong
05/790	Wales	2005-2006	October-January	Public water supply	231 (231)	225 *C. hominis* IbA10G2	Surface water and tap water also contained *C. hominis* IbA10G2 Other *Cryptosporidium* species and genotypes also detected in catchment samples	Strong
08/278	East Midlands	2008	June-July	Public water supply	➢ 400 (23)	23 *C. cuniculus* VaA18	Water in istribution, tap water and dead rabbit gut contents *C. cuniculus* VaA18	Strong
Recreational water								
01/347	South East	2001	June	School outdoor swimming pool	152* (10)	5 *C. hominis*		Possible
01/528	South West	2001	October-November	Club swimming pool	3 (3)	3 *C. hominis*		Possible
03/121	South East	2003	February	Swimming pool	20 (20)	4 *C. hominis* 11 *C. parvum*		Probable
03/220	Yorkshire and the Humber	2003	January-April	Swimming pool	66 (48)	21 *C. hominis*	Pool water and first backwash sample *C. hominis*, second backwash sample *C. parvum*	Strong
03/411	West Midlands	2003	August	Interactive water feature	122 (35)	31 *C. hominis* 1 C. *meleagridis*		Probable
03/409	South East	2003	August-September	Swimming pools	17 (17)	2 *C. hominis*		Strong
03/401	South West	2003	September	Interactive water feature at an open farm	63 (32)	29 *C. parvum* IIaA16G2 1 *C. parvum* IIaA19G2 2 *C. hominis*	Water feature *C. parvum* Goat and goat handler both *C. parvum* IIaA19G2;	Probable
~	North West	2004	March	Swimming pool	4 (4)	3 *C. hominis*		
04/186	Yorkshire and the Humber	2004	May-June	Swimming pool	7 (7)	3 *C. hominis*		
04/371	Yorkshire and the Humber	2004	October	Swimming pool	10 (9)	9 *C. hominis*		

Continues

Table 3 | *Continued*

HPA Centre for Infections outbreak reference number	Government Office region	Year	Month	Setting or vehicle	Number of cases (laboratory confirmed)	Number of cases genotyped and result	Genotyping environmental isolates (where requested)	Strength of association (where indicated)
05/554	South East	2005	September-October	Community swimming pools	➢ 88 (88)	76 *C. hominis* 7 *C. parvum*		Possible
05/623	London	2005	August-December	Swimming pools and community spread	➢ 129 (129)	13 *C. hominis*		Strong
06/36	North West	2006	January	Holiday park swimming pool	12 (11)	6 *C. hominis*		
~	West Midlands	2006	January	Club swimming pool	4 (4)	2 *C. hominis*		
06/481	North West	2006	June	Community swimming pool	6 (4)	4 *C. parvum*		
06/739	East Midlands	2006	June-July	Hotel swimming pool	13 (13)	7 *C. hominis*		
~	South East	2006	July	Comminity splash pool		2 *C. hominis*		
~	Wales	2006	September	Community swimming pool	9 (5)	4 C. hominis		
~	Wales	2006	October	Club swimming pool	13 (7)	7 *C. hominis*		
06/714	South West	2006	October	Hotel swimming pool	4 (4)	4 *C. hominis*		
06/607	Yorkshire and the Humber	2006	November	Club swimming pools	14 (14)	2 *C. hominis*		
06/668	East Midlands	2006	November	Holiday Park swimming pool	53 (27)	6 *C. hominis*		
06/670	North West	2006	November	Community swimming pool	4 (4)	2 *C. hominis* 2 *C. parvum*		
~	South East	2007	February	Community swimming pool	15 (5)	5 *C. hominis*		
~	West Midlands	2007	October	swimming pools	57 (39)	18 *C. hominis* 4 *C. parvum*		
08/375	Eastern	2008	November	School swimming pool	17 (17)	4 *C. hominis*		
09/64	Wales	2009	August	Community swimming leisure pool	106 (46)	44 *C. hominis*		
09/94	South West	2009	August	Caravan park swimming pool	7 (7)	7 *C. hominis*		
09/109	Yrokshire and the Humber	2009	September-October	Caravan park swimming pool	6 (5)	4 *C. hominis*		

Continues

Table 3 | *Continued*

HPA Centre for Infections outbreak reference number	Government Office region	Year	Month	Setting or vehicle	Number of cases (laboratory confirmed)	Number of cases genotyped and result	Genotyping environmental isolates (where requested)	Strength of association (where indicated)
~	Yorkshire and the Humber	2009	September-October	Swimming pool	6 (6)	1 *C. parvum*		
09/111	North West	2009	June-July	small warm swimming pool	8 (8)	3 *C. hominis*		
~	South East	2009	November	Community swimming pool	15 (11)	7 *C. hominis*		
~	Yorkshire and the Humber	2009	November	Community swimming pool	10 (?)	4 *C. hominis*		
~	North West	2010	September	Community swimming pool (swim club members)	48 (3)	3 *C. hominis*		
10/107	South East	2010	October	Community swimming pool	30 (19)	*C. hominis*		

**A concurrent norovirus outbreak accounts for some of the cases.*

be done by specific molecular "genotyping" assays, and is not usually performed by primary diagnostic laboratories. Not all diarrhoea patients will seek medical attention, have a stool sample taken or a *Cryptosporidium* test applied, and laboratory practice is varied. A request for "ova, cysts and parasites" will not include a test for *Cryptosporidium*: where not routinely sought, this must be specified on the request form. *Cryptosporidium* diagnosis is statutorily notifiable in only a few countries and cross-country comparisons are hampered by this [31]. For example, *Cryptosporidium* is included in Directive 2003/99/EC of the European Parliament and the Council of the European Union (EU), and cryptosporidiosis is therefore a notifiable disease within the EU with laboratory-confirmed case data collected through the European Surveillance System (TESSy). In 2009, reports were provided by 21 out of 31 EU and European Economic Area/European Free Trade Association countries; 8016 cases were reported across 13 countries and zero cases were reported by eight countries, an overall case rate of 2.7 per 100 000 population. The highest confirmed case rate was reported in Ireland (10.0 per 100 000 population) followed by the UK (9.3 per 100 000) and Belgium (4.1 per 100 000) [32]. Thus, *Cryptosporidium* is under diagnosed and underreported, but to varying extents. Even at a local level, testing and reporting practice is variable [33]. One study of IID in the UK has estimated the reporting ratio (*i.e.* the ratio of disease rates in the community and presenting to general practice relative to the rate of reported diagnoses to national surveillance) to be 8.2 (95% CI 2.1 to 31.7), estimating the annual number of cases in the community to be 43 834 (95% CI 11 393 to 168 655) [34]. This equates to an estimated annual incidence of 69.5 cases per 100 000 UK population, and the authors acknowledge the study may have underestimated *Cryptosporidium* rates as the case definition for acute gastroenteritis excluded cases of duration of illness over two weeks.

Surveillance data for England and Wales have shown that about 10% of reported cases of cryptosporidiosis are part of identified and reported outbreaks at a variety of settings *(Table 1)* [35], using the following definitions of an outbreak:

- an incident in which two or more people experiencing a similar illness are linked in time or place; or;
- a greater than expected rate of infection compared with the usual background rate for a place and time.

In investigations involving water, an incident may be defined as a suspected, anticipated or actual event involving microbial or chemical contamination of food or water. The Health Protection Agency (HPA) Centre for Infections (formerly the Communicable Disease Surveillance Centre) and local authorities in England and Wales have conducted structured surveillance of outbreaks of IID since 1992 [36]. Outbreaks are classified, according to the robustness of epidemiological and microbiological evidence, as definite, probable or possible defined from the following criteria:

a. the pathogen found in patients was also found in water samples;
b. documented water quality or treatment failure;
c. significant result from analytical epidemiologi-

cal study, demonstrating association between water and illness;

d. suggestive evidence that outbreak is water related from a descriptive epidemiological study, excluding obvious alternative explanations;

Which are combined to indicate the strength of association:

strong = a+c or a+d or b+c;

probable = b+d or a only or c only;

possible = b only or d only [37]. Outbreaks were summarised bi-annually until 2006 in the Communicable Disease Report, and are now reported in the Health Protection Report (www.hpa.org.uk).

A similar classification is in use in the USA where the Centers for Disease Control and Prevention (CDC), the Environmental Protection Agency (EPA), and the Council of State and Territorial Epidemiologists have collaborated on the Waterborne Disease and Outbreak Surveillance System (WBDOSS) for collecting and reporting data on waterborne disease outbreaks since 1971 for drinking water and 1978 for recreational water [28]. Two criteria must be met for a health event to be defined as a waterborne disease outbreak: 1) two or more persons must be linked epidemiologically by time, location of exposure to water, and illness characteristics, and 2) the epidemiologic evidence must implicate water or volatilization of water-associated compounds into the air surrounding the water as the probable source of illness. Outbreaks are classified according to the strength of both epidemiologic and clinical laboratory data, and environmental data implicating water as the vehicle of transmission:

Class 1 = epidemiologic and clinical laboratory data and environmental data are provided and are adequate;

Class 2 = epidemiologic and clinical laboratory data are provided and are adequate, but environmental data is not provided or are inadequate;

Class 3 = epidemiologic and clinical laboratory data are provided but are limited, and environmental data are provided and are adequate;

Class 4 = epidemiologic and clinical laboratory data are provided but are limited, and environmental data is not provided or are inadequate.

These classes, first delineated in the 1989-1990 surveillance report [38], have been updated to reflect the increasing use of molecular characterization of pathogens both in clinical specimens and environmental samples; molecular data that link people who had an identical water exposure are considered adequate to support a Class I or Class II assignment, and molecular data that link at least one person to the implicated water exposure now are considered adequate water quality data to support a Class I or Class III assignment [28]. The use of molecular data in the investigation of particular incidents and outbreaks is described below. However, as with many pathogens, the *Cryptosporidium* contamination event may have passed by the time of sampling and/or recognition of an outbreak. For *Cryptosporidium* the incubation period between ingestion and the onset of illness is long (up to two weeks) and the interpretation of microbial and water treatment process data requires careful interpretation. Differences in *Cryptosporidium* monitoring strategies for drinking water in the UK and USA are explored further below.

DRINKING WATERBORNE OUTBREAKS AS DRIVERS OF REGULATION

Although the first human cases of cryptosporidiosis were reported in 1976 [39, 40], the first outbreak linked to drinking water was identified in 1984, at Braun Station, Texas, USA [41]. Over 200 individuals were involved, when sewage contaminated a groundwater supply. The next outbreak was on an even larger scale: in 1987 an estimated 13 000 people were affected at Carrollton, Georgia, USA when mains water became contaminated from a surface supply during a period of operational irregularities at the conventional water treatment works [42]. In both cases the drinking water met existing water quality standards, based on WHO Guidelines for Drinking Water Quality, focussing on monitoring *E. Coli*. At the end of 1988 and into 1989, over 500 individuals were affected in Swindon and Oxford, England, when oocysts in contaminated surface source water broke through the conventional mains water treatment and in to supply [43]. These early outbreaks startled both public and water industry perception of drinking water in industrialised nations; filtration and chlorine disinfection were commonplace and assumed to control waterborne disease, and people no longer commonly became sick through the mains water supply. In 1989, *Cryptosporidium* was not even considered in the US Environmental Protection Agency (EPA) Surface Water Treatment Rule to control *Giardia* and viruses or in the companion Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems, published in 1990. The 1993 outbreak in Milwaukee focussed the work initiated to understand the sources, routes of transmission, detection and prevention of spread of the parasite.

In addition to work seeking improvements in methods to detect, and water treatment to control *Cryptosporidium*, the outbreaks in the 1980's and early 1990's prompted regulatory agencies to develop rules for public health protection through monitoring, removal or inactivation. This has been approached differently in USA and UK.

In the USA, *Cryptosporidium* monitoring was first required under the Information Collection Rule (ICR) of 1997, requiring surface water sources of supplies to more than 100 000 people to be monitored for *Cryptosporidium* oocysts, *Giardia* cysts and viruses for 18 months. Sources with ≥ 1000 of either protozoan per 100 L, or viruses ≥ 100 per 100 L,

required final water monitoring. A total of 93% of 5829 surface water samples were reported as non-detects for *Cryptosporidium*. However, results based on testing small sub-samples and extrapolation of counts to the original volume are now considered unreliable. During the ICR period, a standard method was published by the EPA, stipulating the acceptable sampling filter types, elution and oocyst recovery processes and microscopical detection procedures for enumeration of oocysts, and, critically, that the oocyst count be expressed per volume of water sampled. In 1999 the method was validated for simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts, currently published as EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA, 2005. Method 1623 now supports promulgation of EPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), which is the current regulation for all public water systems that use surface water or ground water that is under the direct influence of surface water, finalised in 2006. This rule requires monitoring of source waters to determine the level of treatment required for *Cryptosporidium* reduction by removal or disinfection. Mean oocyst counts, based on a two year, monthly sampling programme, are used to classify ("bin") supplies in one of four categories and determine the extent of treatment required, if any, above conventional full treatment. Systems classified in higher bins must provide additional water treatment to further reduce *Cryptosporidium* levels by 90 to 99.7 percent (1.0 to 2.5-log), depending on the bin, using treatment and management options in a "microbial toolbox". All unfiltered water systems must provide at least 99 or 99.9 percent (2 or 3-log) inactivation of *Cryptosporidium*, depending on the results of their monitoring. Suitable removal is through filtration provided by granular media, cartridge filters or membranes, and approved disinfectants effective against *Cryptosporidium* are chlorine dioxide, UV, and ozone. Systems must conduct a second round of monitoring six years after completing the initial round to determine if source water conditions have changed significantly. The EPA estimates that full compliance with the LT2ESWTR will reduce the incidence of cryptosporidiosis by 89 000 to 1 459 000 cases per year, with an associated reduction of 20 to 314 premature deaths. Additional expected benefits include reduced exposure to other pathogens, such as *Giardia*, that can co-occur with *Cryptosporidium*.

In the UK, the Oxford and Swindon outbreak in 1987-1988 led to the establishment of the group of experts, chaired initially by Sir John Badenoch and latterly by Professor Ian Bouchier. A series of reports, published in 1990, 1995 and 1998 set out what was known about *Cryptosporidium*, its occurrence in the environment, its importance as a waterborne infection for humans, the outbreaks and lessons learned from them, the treatment requirements for *Cryptosporidium* deficiencies in detection and enumeration methods, the need for methods to establish oocyst viability, characterise the species present and their infectivity for humans, the risks from groundwaters infiltrated by surface waters, and established good epidemiological practice in investigation of outbreaks and the need for clarification in the role and function of incident and outbreak control teams and their members. A correlation between outbreaks and inadequacy occurring in elements of the multi-barrier approach (source, treatment, distribution, monitoring and response) was identified in the third report (available online at www.dwi.defra.gov.uk/research/bouchier/index.htm). This was initiated following an outbreak in north west London and Hertfordshire in 1997 when 345 cases were reported following contamination of a groundwater source, a type previously considered to present a low risk from *Cryptosporidium*, by infiltration of surface water containing oocysts [44].

Although the legal standards for all EU member states are set out in the European Drinking Water Directive 1998, and are themselves informed by the WHO guidelines on drinking water quality, national standards are also set. The first, and only, regulatory requirements for *Cryptosporidium* in finished water anywhere in the world were introduced in England and Wales in 1999 as part of the Water Supply (Water Quality) (Amendment) Regulations 1999 and incorporated in to the Water Supply (Water Quality) Regulations 2000 in England and 2001 in Wales. The key driver for these regulations was the lack of admissible evidence to prosecute the water company associated with an outbreak in 1995 in Torbay, south west England, when 575 cases occurred associated with a lowland river with direct abstraction and bankside infiltration of unfiltered water [45, 46]. The regulatory requirement mandated water undertakers to conduct risk assessments with respect to *Cryptosporidium* on all water treatment works, considering the source water, catchment characteristics and treatment provided. Sites with a "significant risk" classification, had to treat the water to ensure an average of less than 1 oocyst in 10 L of treated water supplied, measured by continuous sampling of at least 40 litres of water per hour. Compliance was demonstrated by continual monitoring and reporting of results to the regulator, the Drinking Water Inspectorate (DWI), unless all particles > 1μm were continuously removed. The initial risk assessment reported in the DWI's annual report in 2001 (http://dwi.defra.gov.uk) identified 332 sites as being at significant risk, of which 158 were works treating surface waters and 174 were groundwater abstractions. Some sites were decomissioned and others subjected to improvement. In 2004, the DWI reported that none of the 146 307 samples taken between 2000 and 2003 exceeded the treatment standards, with most samples below 0·02 oocysts/10 L.

Improvements in drinking water quality driven in part by the England and Wales regulations,

and by long-term water company investment programmes, led to a substantial reduction in both *Cryptosporidium* cases, especially in the first half of the year, and in reported mains drinking water outbreaks [18, 47] at a time when swimming pool outbreaks appeared to increase in number (see below). Only four outbreaks linked to public water supplies have been recorded in England and Wales since 2000; one in 2002, two in 2005 and one in 2008, all with strong evidence for association with drinking water (*Table 3*). In November and December 2002 an outbreak involving 31 laboratory confirmed cases caused by *C. hominis* was reported in a population of 158 558 in South East England served by a mixture of water from a groundwater source and a surface water-treatment plant at significant risk, and where the continuous monitoring samples never exceeded treatment standards (*Table 3*, outbreak 02/1701) [48]. A second outbreak of 140 laboratory confirmed cases caused by *C. hominis* in the same area occurred between September and November, 2005 and once again, oocyst counts were below the treatment standard (*Table 3*, outbreak 05/552). The recognition that even small numbers of oocysts detected in drinking water can cause outbreaks contributed to the revocation of the treatment standard in the amended water quality regulations, The Water Supply (Water Quality) Regulations 2000 (Amendment) Regulations 2007. Furthermore, an outbreak in north west Wales in 2005, involving 218 cases of cryptosporidiosis (again, *C. hominis*) was linked to a surface water supply derived from a sparsely populated catchment. The treatment works, despite the absence of effective treatment or barriers in the catchment to remove *Cryptosporidium*, was not continually monitored as it had not been given a significant risk assessment [49]. The outbreak was controlled in the short term by a notice to boil drinking water, which was in place for 9 weeks until a UV treatment plant could be installed, although oocyst disinfection (inactivation) was not permitted by the regulations at the time, which focussed on oocyst removal. The elimination of the treatment standard in the 2007 regulations permitted application of disinfection such as UV for the control of *Cryptosporidium* in water supplies. Never-the-less, drinking water remains a risk factor for cryptosporidiosis in England and Wales [18].

As demonstrated above, outbreaks of cryptosporidiosis have been reported through drinking water that met WHO guideline microbiological standards and/or the *Cryptosporidium* treatment standard imposed in England and Wales. Subsequently, a preventive, risk based approach, derived from the food industry [50], in the form of the requirement for a water safety plan [51], now complements microbiological guidelines, and is therefore incorporated in further amendments to the regulations in 2010 in England and Wales as comprehensive risk assessments. A water safety plan is a systematic inventory of all hazards (including *Cryptosporidium*), an evaluation of the significance of these hazards and of the efficacy of control measures taken, and spans source water catchment, treatment and distribution of water supplies. The risk assessment is supported by testing and enforcement. However, one of the key differences between the USA and UK approaches remains: the former is historically based on source water monitoring for *Cryptosporidium* to inform subsequent levels of treatment required and the latter has the historical legacy of an emphasis on final water monitoring. Regulations supporting the Drinking Water Directive and water safety plan approach require raw water monitoring to identify risks to deterioration of raw water quality; there is no list of parameters for this purpose as it is up to the water company to assess the risks and monitor and treat accordingly.

SWIMMING POOL OUTBREAKS AND LACK OF REGULATION

Although the WHO *Guidelines for safe recreational waters* [52] provide a basis for standards, in swimming pool settings, in contrast with drinking water, there is a lack of legislation. The WHO guidelines, which are currently under revision, provide an authoritative referenced review and assessment of the health hazards associated with swimming pools, their monitoring and assessment, and activities available for their control through education of users, good design, construction, operation and management, and address a wide range of types of hazard, including water quality, physical hazards (leading to drowning and injury), contamination of associated facilities and air quality. In the USA, state and local governments establish and enforce regulations for protecting recreational water from contaminants but no federal agency has authority over treated recreational water and, apart from legislation to prevent entrapment, no minimum design, construction, operation, disinfection, or filtration standards exist. Swimming pool codes are enforced by individual state and local public health agencies but there is variation in regulation, compliance and enforcement. In the EU, the Bathing Water Directive 2006 sets out quality standards for natural waters designated for bathing but this does not cover treated waters. In the UK, the publication *Swimming pool water. Treatment and quality standards for pools and spas* [53] provides authoritative guidance and is viewed as best practice. Therefore, in a court of law, swimming pool operators would be, and indeed have been, prosecuted under the Health and Safety at Work etc. Act 1974, and the Management of Health and Safety at Work Regulations 1999, for causing an outbreak by failing to follow this guidance [54].

Globally, recreational waterborne outbreaks of cryptosporidiosis are reported slightly more commonly than drinking waterborne outbreaks: in the seven years between January 2004 and December 2010,

out of 120 reported waterborne *Cryptosporidium* outbreaks, 46% were associated with drinking water and 54% with recreational waters [23]. However, this analysis belies an apparent increase in swimming pool related *Cryptosporidium* outbreaks seen, for example, in the UK and USA. Between 1992, when surveillance for waterborne IID outbreaks began [36] and 2011, there were 56 outbreaks linked to swimming pools and 32 linked to public water supplies. However, just 29 (34%) of the swimming pool outbreaks were reported in the first ten years (1992 to 2001) compared with 56 (66%) in the following ten years (2012 to 2011) (Gordon Nichols, personal communication of provisional data). In the USA, *Cryptosporidium* outbreaks at recreational water venues also increased from 29 in 2005-6 to 60 in 2007-8 [28]. While the emergence of *Cryptosporidium* may be a continuing factor, other contributing factors may include changes in detection, investigation, and reporting of waterborne disease outbreaks driven by improved resources for the WBDOSS and strengthening of the outbreak response [28]. It is likely that recently improved understanding of risks associated with swimming pools and their investigation, has contributed to the apparent rise in outbreaks at these settings.

APPLICATION OF MOLECULAR ASSAYS FOR SPECIES DETERMINATION AND INVESTIGATION OF THE PROPAGATION OF OUTBREAKS

Molecular detection offers some advantages over standard methods such as EPA Method 1623, which are based on oocyst counts by immunofluorescence microscopy, such as detection of small numbers of organisms and potential for genotyping leading to species determination, this has not yet been adopted for operational or regulatory monitoring of drinking water. This is because only those oocysts containing sporozoites, and thus DNA, will be detected and so far, no reliably quantitative molecular method has been validated to replace oocyst counts. However, without assays determining the species/genotype, viability or infectivity, all oocysts detected by microscopy must be assumed to present a public health risk *(Figure 1)*. Viability and infectivity assays have been reviewed recently by Kothavade, who highlights the difficulties in applying these to the small numbers of oocysts often present in water samples, lack of interlaboratory trials and validation and difficulties in interpretation of the data [55]. In contrast, some genotyping assays have been standardised and applied to counted oocysts from microscope slides; thus both sets of data are collected: the oocyst count and the species, improving the data for assessment of risk to public health.

Cryptosporidium genotyping may be undertaken using material fixed and stained on microscope slides generated during *Cryptosporidium* monitoring by extracting *Cryptosporidium* DNA and applying conventional polymerase chain reaction (PCR) of the small subunit ribosomal RNA (ssu rRNA) gene prior to sequencing of amplicons (the benchmark method) or, more recently, using real-time PCR platforms *(Figure 2)*. Other methods, such as laser capture microscopy and reverse line hybridization are at developmental stages, and loop-mediated isothermal amplification have yet to be validated independently [55]. The benchmark assay is a specialist test requiring equipment and skill outwith the scope of most routine diagnostic or detection laboratories. The large genetic diversity in *Cryptosporidium* ssu rRNA sequences from source waters makes analysis complex. For example, an up to date reference database to compare accurate DNA sequences and subsequent phylogenetic analysis with proper interpretation are required, coupled with up to date knowledge of host-parasite relationships [56]. Rueker and colleagues have provided a detailed description and discussion of the sequence and phylogenetic analysis process, especially important in the identification of environmental *Cryptosporidium* isolates [56], providing a benchmark process. An ongoing Water Research Foundation (WaterRF) programme aims to further refine the benchmark assays and develop and validate simplified, rapid, assays for routine use in water testing laboratories for the differentiation of human pathogenic species (for this purpose defined as *C. hominis*, *C. parvum* and *C. meleagridis*) from animal-infective species (WaterRF 4284) (http://waterrf.org) *(Figure 2)*. Although the simplified assay, as proposed, is not likely to differentiate *C. cuniculus*, this species will be included in the *C. hominis* detections and may be differentiated by sequencing of PCR products, which is required for definitive results *(Figure 2)*.

Although there is no standard method for subtyping *Cryptosporidium* species, sequence analysis of the *gp60* gene is informative for *C. parvum* and *C. hominis* to a certain extent and sequence data can be compared readily [57]. Although the epidemiology of *gp60* subtypes was reviewed by Xiao in 2010 [57], new variants are frequently identified. The utility of the analysis has been demonstrated during the investigation of both zoonotic [58] and anthroponotic transmission, as illustrated in the investigation of waterborne outbreaks described below. However, single locus typing will under-estimate diversity, and a standardised, validated, internationally accepted, multi-locus scheme is required for epidemiological investigations of each species [59]. How subtype variation relates to virulence and pathogenicity is unclear; in fact, only putative virulence factors have been identified so far, although *gp60* subtypes have been associated with varying severity of illness [60]. Despite this, genotyping has proved to be beneficial to the epidemiological investigation of cryptosporidiosis, and extracted DNA can be stored long-term and re-tested retrospectively.

In non-outbreak situations, the data from typing sporadic cases of cryptosporidiosis provides a

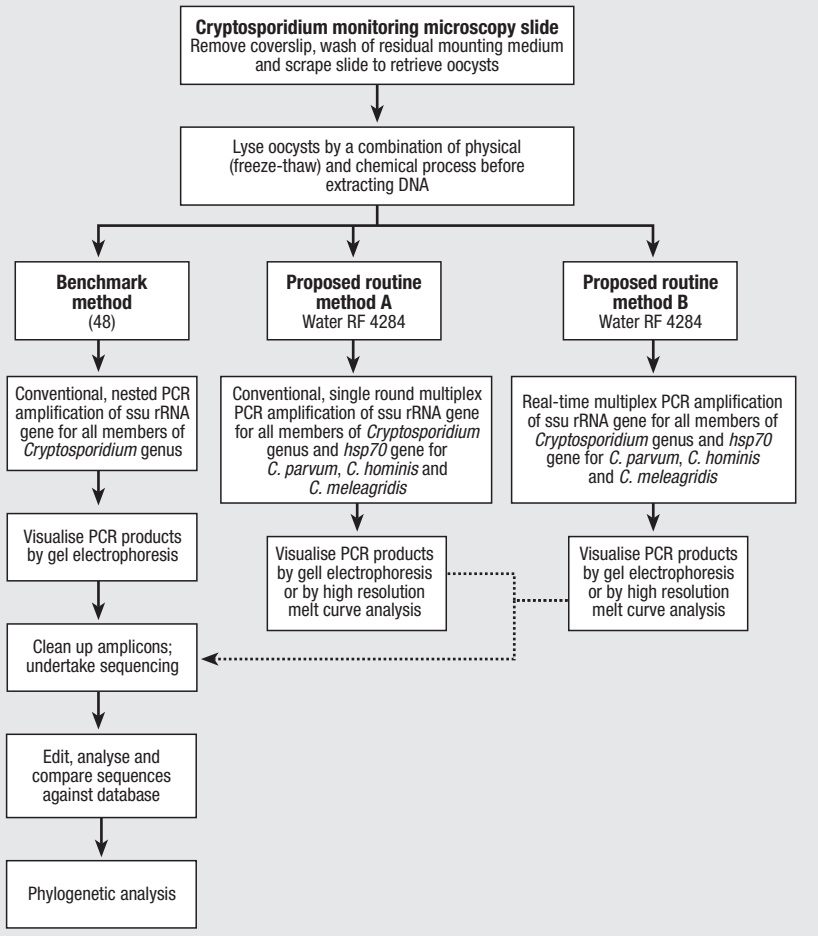

Fig. 2 | *Workflows for genotyping* Cryptosporidium *from regulatory monitoring microscopy slides.*

baseline against which trends can be identified and changes monitored. Since the improvements in water quality influenced by the 2000/1 regulations in England and Wales, the spring peak identified as being caused by *C. parvum* declined substantially [61]. Depite this, drinking water remains a risk for illness caused by *C. parvum*, as identified by Lake and colleagues in a case-control study design to investigate wider environmental and socioeconomic risk factors for human cryptosporidiosis in England and Wales [62]. In contrast to the decline in *C. parvum* cases in the spring, there has been no reduction in late summer/early autumn cases of *C. hominis* which predominates at this time of year. This is especially obvious in 2012, and recreational waters are implicated [63]. More swimming pool related outbreaks are caused by *C. hominis* than *C. parvum*, although both have occurred in the same outbreak *(Table 3)*, and may reflect multiple episodes of contamination.

When applied to *Cryptosporidium* isolates from catchment studies or routine drinking water monitoring, genotyping data can also further refine human health risk assessment by differentiating human pathogenic from solely animal-associated species and supports the other information gathered under drinking water regulatory frameworks. For example, a one year survey of *Cryptosporidium* oocysts detected in the Scottish Water Routine *Cryptosporidium* Monitoring Programme identified the species or genotypes present in 62.5% of 1042 oocyst-positive slides [64]. A high diversity of *Cryptosporidium* species and genotypes was present in source (no. = 456) and treated drinking (586) waters, with 2 or more in 16.9% samples; human-pathogenic species were present in fewer samples than non-pathogenic species. In source waters, *C. andersoni* (which is host-adapted to cattle) was most frequently identified (25.2% samples) followed by *C. parvum* (11.2%) and *C. ubiquitum* (7.2%). In drinking waters, *C. ubiquitum* was most frequent (12.6% samples), followed by *C. parvum* (4.3%) and *C. andersoni* (4.1%). In a long-term study of source waters in the agriculturally intensive South Nation River catchment in Ontario, Canada, *Cryptosporidium* species associated with livestock made up 39% of the total molecular detections, compared with wildlife associated species and genotypes accounted for 55% and *C. hominis* and *C. parvum* 1.6%, indicating a small risk to hu-

mans [56]. A study in Australia, where recreational activities within 2 km of drinking water sources is prohibited, compared *Cryptosporidium* species in recreational water and non-recreational water catchments [65]. This revealed a predominance of *C. hominis* in recreational water catchments which allowed swimming and camping, compared to non-recreational catchments which had a lower prevalence of *Cryptosporidium* and all the samples genotyped were *C. parvum*. Increasing population was strongly correlated with an increase in the prevalence of *Cryptosporidium* recreational catchments, and the data support government policy limiting activities to the outer catchment [65]. The predominance of host-derived species in some catchments, contributes to effective evaluation and selection of management practices to reduce *Cryptosporidium* contamination in source waters.

Retrospective analysis of clinical isolates can be enlightening, identifying, for example, that the Milwaukee outbreak was caused by *C. hominis* [66], where previous uncertainty over the origin of the oocysts identified cattle, slaughterhouses, and human sewage as potential candidates [26]. Further analysis of isolates collected in the Torbay outbreak [46] and north west London and Hertfordshire outbreak [44] established that these were also caused by *C. hominis* [67]. Even where species identification has been undertaken, further analysis of isolates from cases may be required to investigate outbreaks. Retrospective analysis of *C. parvum* isolates by multilocus fragment typing enabled more accurate mapping of outbreak-related cases to water supply zones [68]. Some nine years after the Milwaukee outbreak, *C. hominis* isolates, further analysed for variation in the *gp60* gene, were identified as indistinguishable from the outbreak isolate, year round in Milwaukee wastewater indicating continuing, stable transmission of human cryptosporidiosis in the city [69]. Analysis of *C. parvum* isolates from patients linked to a swimming pool outbreak in Stockholm, Sweden in 2002, at three loci (*gp60*, TP14 and *hsp70*) identified that two separate outbreaks had occurred simultaneously, as the genotypes segregated people using an outdoor pool from those that swam only in the indoor pool [70].

Latterly, genotyping methods have been applied in "real time" during incident and outbreak investigations and have been demonstrated to assist in attributing sources and establishing correct interventions. In the UK, isolates from clinical cases are genotyped routinely to the species-level, and in some outbreaks additionally from suspected sources *(Table 3)*. For example, during investigation of a drinking waterborne outbreak caused by *C. hominis* in north west Wales in 2005 (outbreak number 05/790), isolates found in clinical cases were indistinguishable by sequence analysis of the ssu rRNA gene and the *gp60* gene from isolates in the surface water source and in the treated water supply to the affected area, adding strength to the epidemiological evidence for the association with drinking water [49,71]. Although a diversity of *Cryptosporidium* species was identified in the catchment, the outbreak *C. hominis* subtype, IbA10G2, was only found in source waters under the influence of sewage contamination, and in the tap water, highlighting the need for water companies to throughly document wastewater inputs in surface waters [72]. During a water quality incident in the East Midlands, England in 2008 (*Table 3*, outbreak number 08/278), isolates in treated water were identified as *C. cuniculus* before any human illness was identified, and were matched subsequently by sequencing the ssu rRNA, *hsp70* and *gp60* genes to those from a dead rabbit found in a chlorine contact tank and 23 ensuing human cases [4]. This outbreak established *C. cuniculus* as a human pathogen. In two out of three drinking waterborne outbreaks in Northern Ireland in a single year, the confirmation of *C. hominis* re-directed the investigations away from farmed animals and towards raw sewage contamination in one outbreak and wastewater in the other, illustrating how genotyping isolates from cases can also eliminate alternative suspected sources [73].

Secondary spread from initial outbreaks at swimming pools, for example, through other swimming pools and community settings such as nurseries can lead to community wide outbreaks, as has been shown in Japan [74] and in 2007 in Utah, USA [75]. The ability of *Cryptosporidium* to survive for over 10 days even in properly chlorinated water, the protracted incubation period (commonly 5 to 7 days) and possible diagnostic delay which prolongs the time between infection and epidemiologic link with the source of the outbreak contribute to this, as does behaviour of cases who continue to swim while ill. They can introduce the protozoan to other recreational water venues, as was shown in the Utah outbreak where an estimated 20% of cases swam while ill with diarrhoea and identified approximately 450 potentially contaminated recreational water venues [75]. Subsequent to the Utah outbreak, increases in cases in 2007 were seen in neighbouring states of Colorado, Idaho, New Mexico, and Iowa. Subtyping 57 *C. hominis* isolates at the *gp60* gene identified the same, previously rare subtype (IaA28R4) in 40 (70%) samples [76]. Unfortunately, none of the Utah samples were available for typing, and it is impossible to know whether this increase was related. This would have been a powerful investigation in to the spread of cryptosporidiosis and highlights the importance of the availability of isolates for further investigation.

Thus, the benefits of genotyping in outbreaks are:

- characterising the outbreak in terms of the cause of the cryptosporidiosis;
- linking cases with each other and monitoring their spread, or excluding cases with non-related isolates;

- linking cases with isolates in suspected sources, or excluding other sources;
- providing supporting data for further investigation of the source of contamination;
- providing additional evidence for the strength of evidence for the association with water;
- providing new data for prevention and control of further outbreaks.

However, there are additional challenges to genotyping most especially from environmental samples, requiring mitigation strategies:

- sensitivity, as small numbers of oocysts may be present on slides and in the pesence of potential PCR inhibitors;
- specificity, as there is difficulty in selecting target gene sequences that are both conserved within all *Cryptosporidium* species but different or absent in other genera;
- not all isolates from environmental samples are typable, even when present in large numbers, for unknown reasons;
- in concurrent *Cryptosporidium* populations, it may be difficult to identify multiple sequences present, especially of minor genotypes which may be underdetected;
- genotypes have been found in source waters which have no known host; there is a need for more information on *Cryptosporidium* shedding by most host species;
- a limited amount of *Cryptosporidium* DNA may be available for subtyping from environmental samples;
- assays are time consuming, specialised and costly.

CONCLUSIONS

Despite the knowledge gained since the first drinking waterborne outbreaks, there are still gaps that need to be filled. Waterborne outbreaks have occurred where small numbers of oocysts have been detected, and conversely, high numbers of oocysts do not necessarily lead to increased disease [76]. This reflects the multifactorial dynamics of waterborne disease, including human behaviour (water consumption) and immunity of the exposed population, as well as the potential infectivity of the oocysts for humans, determined by their species and viability *(Figure 1)*. These present analytical and interpretive challenges to public health and quantitative microbial risk assessment. Nevertheless, early detection, investigation and appropriate control of outbreaks can reduce their impact, and are facilitated by molecular methods to establish the relationship between isolates from cases and suspected sources.

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Conflict of interest statement

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