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Review: Epidemiological evidence of groundwater contribution to global enteric disease, 1948–2015

Heather M. Murphy¹ · Morgan D. Prioleau¹ · Mark A. Borchardt² · Paul D. Hynds³

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Abstract Globally, approximately 2.2 billion people rely on groundwater for daily consumption. It is widely accepted that groundwater is more pristine than surface water but while this assumption is frequently the case, groundwater is not ubiquitously free of contaminants; accordingly, this presumption can result in an unfounded and potentially hazardous sense of security among owners, operators and users. The current paper presents a review of published literature providing epidemiological evidence of the contribution of groundwater to global human enteric infection. An emphasis is placed on enteric pathogens transmitted via the faecal-oral route, and specifically those associated with acute gastrointestinal illness (AGI). The review identified 649 published groundwater outbreaks globally between 1948 and 2013 and several epidemiological studies that show there is an increased risk of AGI associated with the consumption of untreated groundwater. The review identified that the following five pathogens were responsible for most outbreaks: norovirus, *Campylobacter*, *Shigella*, Hepatitis A and *Giardia*. Crudely, the authors estimate that between 35.2 and 59.4 million cases of AGI per year globally could be attributable to the consumption of groundwater. Although groundwater is frequently presumed to be a microbiologically safe source of water for consumption, this

review demonstrates that consumers served by an untreated groundwater supply remain at risk to enteric disease. The authors conclude that collaboration between microbiologists, hydrogeologists and epidemiologists is needed to better understand pathogen occurrence, persistence, detection and transport in groundwater as well as build stronger epidemiological evidence documenting the true magnitude of disease associated with groundwater globally.

Keywords Health · Enteric disease · Global · Epidemiology · Pathogens

Introduction

Groundwater is the world's most extracted raw material, both in terms of frequency and volume, with global withdrawals currently estimated to be 982 km³/year (Margat and van der Gun 2013). Globally, the authors estimate that approximately 2.2 billion people (31.5% of the global population) rely on groundwater for daily consumption. In the United States, 90.5 million people are served by community groundwater systems, while 48 million people have a private well (Colford et al. 2006a, b; US EPA 2015). In Canada, 4.1 million (11.7%), 2.6 million (7.4%) and >1 million people (3.1%) are reliant on private wells, large municipal groundwater systems (>1,000 people), and small municipal groundwater systems (<1,000 people), respectively (Murphy et al. 2016a, b). Analogous figures pertaining to Asia, Europe, Latin America and Australia are 1–1.2 billion, 200–500 million, 150 million, and 3 million people, respectively (Job 2009). A further 80% of the 900 million people in Africa (720 million people) employ a groundwater source (Macdonald et al. 2009).

It is widely accepted that groundwater typically represents a more pristine source of water for human consumption than

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surface-water resources, due to the inherently protective effects afforded by overlying soil and subsoil layers—i.e. increased residence times, physical, chemical and microbial attenuation, etc. (Medema et al. 2003). While this assumption is frequently the case, groundwater is not ubiquitously free of chemical and/or microbial contaminants; accordingly, this presumption can result in an unfounded and potentially hazardous sense of security among owners, operators and users—for example, in an effort to reduce waterborne gastrointestinal illness in developing regions of southern Asia, the international community heavily promoted a shift from using surface water to presumably cleaner groundwater sources during the 1970s (Flanagan et al. 2012). However, at the time, high rates of naturally occurring arsenic via concurrent biogeochemical and hydrologic processes in many of these regions had not been recognized, resulting in a widespread failure to assess the chemical quality of newly developed groundwater sources (Mukherjee et al. 2006).

Similarly, in developed regions, the presumption that groundwater is a universally safe resource has resulted in undesirable practices; recent studies of private well users in Ireland, Canada and the US have found a concurrence between the belief that groundwater represents a safe domestic water supply, and a lack of “protective action” by homeowners (i.e. source maintenance, periodic testing, water treatment; Strauss et al. 2001; Charrois 2010; Kreuzwiser et al. 2011; Hynds et al. 2013). In a survey of 245 private well owners in the Republic of Ireland, Hynds et al. (2013) found that water treatment, source maintenance and regular water quality testing were absent in 64, 72 and 40% of cases, respectively. Moreover, globally, at the governmental level, it is important to note that private groundwater sources remain unregulated in many provinces, states and countries.

Groundwater represents a significant source of potentially hazardous microbial pathogens, with subsurface pathogen occurrence, distribution and movement still poorly understood in many areas and environments. The body of evidence in the academic literature leaves little doubt that contaminated groundwater supplies are a significant source of endemic, sporadic, and epidemic gastrointestinal infections in both developed and developing regions (Gallay et al. 2006; Fong et al. 2007; Craun et al. 2010; Kvitsand and Fiksdal 2010; Giammanco et al. 2014; Guzman-Herrador et al. 2015; Lapworth et al. 2015). Between 1971 and 2008, approximately 30% of waterborne outbreaks in the US were associated with consumption of untreated groundwater, with these confirmed events responsible for at least 23,478 infections, 390 hospitalizations, and 13 deaths (Wallender et al. 2014). Just under 18% of all waterborne outbreaks occurring in Canada between 1974–2001 were associated with contaminated private water supplies, the majority of which rely on groundwater (Schuster et al. 2005; Murphy et al. 2016a). During the 22-year period 1989–2011, 76% of reported outbreaks with a

known water source in the Nordic region were related to a public or private groundwater supply (Guzman-Herrador et al. 2015).

The current paper presents results arising from a scoping global review of the available published literature comprising epidemiological evidence of the contribution of groundwater consumption to human infection, with an emphasis placed on enteric pathogens transmitted via the faecal-oral route, and specifically those associated with acute gastrointestinal illness (AGI). Within the published literature, the clinical definition of AGI has been characterized by a relatively wide range of symptoms and severity; for the purposes of the current study, it has been defined as the occurrence of three or more loose stools and/or vomiting over the course of one 24-h period (Murphy et al. 2014). In the interests of clarity, the review has been delineated into three distinct sections, as follows:

1. Global outbreaks with a confirmed groundwater etiology
2. Previous epidemiological studies linking enteric (epidemic and endemic) disease to public or private groundwater sources
3. The occurrence of enteric pathogens in groundwater (including wells and aquifers) and associated microbial risk assessment

It should be noted that while every effort was made to identify and include all relevant global studies, due to myriad limitations including potential publication bias and infrastructural or economic deficiencies per issue, a number of regions are likely over-represented, namely North America and Europe.

Groundwater supply types: public groundwater systems and private wells

At present, groundwater used for drinking is typically provided via municipal supplies overseen by a local authority, local committee-managed community water systems or privately owned and managed wells. All of these groundwater supply types may or may not be treated depending on the context and local, regional or national regulations. In developing countries, wells may be shared by communities or individually owned and, typically, undergo no treatment at the source (WHO/UNICEF JMP 2015). The primary distinction between municipal, community and private supplies are the regulatory frameworks within which they are managed. In developed countries such as in Canada, the United States and many European countries, private groundwater supplies remain unregulated and it is therefore the homeowner’s responsibility to maintain and manage their water supply (Atherholt et al. 2013; Murphy et al. 2016a; Irish Environmental Protection Agency 2016). In

the Republic of Ireland, a private borehole (or dug well) remains the only significant feature of a domestic residence, which is not regulated by any legislative instrument or standard (Ball 2000). Regulatory frameworks that define a private well, versus a public or community based groundwater system varies greatly from country to country; therefore, it is difficult to make comparisons. Globally, limited data are available on the proportion of private well owners that test their water annually, the proportion that treat their water prior to consumption, and the types of treatment systems used in these households. In Canada, 34% of private well owners reported that they treated their water for bacteria; however, it is unclear what type of treatment system these homeowners had in place (Statistics Canada 2013). In New Jersey, USA between 2002 and 2007, 50,800 domestic wells (out of an estimated 400,000) were tested for total coliforms and faecal coliforms/*E. coli* (organisms used to suggest contamination of faecal origin). Based on “one-off” sample collection, 13.1 and 2.1% of wells were positive for total coliforms and faecal coliforms/*E. coli*, respectively, leading the authors to suggest that up to 13.1% of private wells in New Jersey were contaminated with faecal pollution at least once during the study period (Atherholt et al. 2012). In a second study in New Jersey, Atherholt et al. (2015) reported that in bedrock, 21, 33 and 43% of wells tested once, twice or three times were positive for total coliforms. Sampling and analyses carried out in Ireland as part of the EPA Groundwater Monitoring Program during the 10-year period 1998–2008 suggests annual mean bacterial contamination (total coliforms, *E. coli*, faecal coliforms) rates of 25–35% across the national monitoring network which includes municipal, community, and monitoring wells (US EPA 2015). Subsequent works by Bacci and Chapman (2011) and Hynds et al. (2012) have reported similar rates in private boreholes in the Republic of Ireland.

The regulatory framework of municipal and community managed groundwater varies by country and often by state or province within that country. For instance, in the United States all systems serving >25 households or connections are considered community water supplies and are subject to some regulation. The US EPA Groundwater Rule has no explicit disinfection requirements for community groundwater systems unless system deficiencies or source contamination issues have been identified (US EPA 2008). Each state adopts their own version of the Groundwater Rule, which can be more stringent. In some states, community water supplies utilizing a groundwater source need to be disinfected (e.g. Pennsylvania, New York, West Virginia), whereas in other states, no treatment of these supplies is employed unless the water system has significant deficiencies in terms of water quality (e.g. Washington State, Wisconsin, Florida).

Groundwater-related outbreaks of enteric infections

In an effort to capture a representative sample of confirmed groundwater-related outbreaks of enteric infection from around the world, over the past three decades a scoping review was undertaken. The NCBI PubMed archive of biomedical and life sciences was searched using the keywords “groundwater” and “outbreak”. Overall, 98 articles dating back to 1985 were recovered, of which 94 were in English. Forty-four articles discussed either multiple or single outbreaks related to groundwater and have been included here. The remaining articles were either excluded as they did not pertain to groundwater specifically, or they were retained because they discussed pathogens found in groundwater under non-outbreak conditions and are further discussed in section ‘Pathogens of concern in groundwater’. Table 1 summarizes the available literature pertaining to groundwater-related outbreaks of enteric infection stratified by country/region. As shown, the highest number of reported outbreaks were reported in articles from the United States ($n=19$). The majority of available articles focused on outbreaks in middle and high-income countries, with very little coverage of outbreaks in low-income regions. The breakdown of articles by region was North America ($n=19$), Europe ($n=13$), Asia ($n=8$), Africa ($n=1$), and the Middle East ($n=1$).

Pathogens associated with groundwater outbreaks

Table 2 presents a breakdown of the most frequently reported pathogens with respect to the groundwater-related outbreaks included in the current scoping review. The review identified the following 17 pathogens responsible for groundwater related outbreaks, listed from most to least outbreaks: norovirus (39), *Campylobacter* (31), *Shigella* (24), Hepatitis A (21), *Giardia* (16), *Salmonella* (12), *E. coli* O157:H7 (9), *Cryptosporidium parvum* (7), rotavirus (2), SRSVs (2), Adenovirus (1), Enterovirus (1), *Arcobacter* (1), *Entamoeba* (1), *Vibrio cholera* (1), and *Yersinia* (1). These outbreaks are further examined in section ‘Pathogens of concern in groundwater’ where the principal pathogens of concern in groundwater based upon confirmed outbreak etiologies (Table 2) and their occurrence during groundwater surveillance activities are discussed (Table 4). Of those articles included in the current scoping review, which reported on groundwater-related outbreaks with a confirmed or suspected etiology, 46% were associated with bacterial pathogens, 40% were related to viruses and 14% were related to protozoa. The likely factors contributing to the higher occurrence of reported outbreaks with a viral or bacterial etiology are their comparatively longer survival outside a host, and their

Table 1 Published groundwater-related enteric infection outbreaks from 1948 to 2013 stratified by country/region (ordered from most to least outbreaks)

Country	Number of groundwater outbreaks	Period or year	References
United States	~386	1948–2011	Adam 2016; Balbus and Embrey 2002; Barwick et al. 2000; Beer 2015; Blackburn 2004; Borchardt et al. 2011a; Casemore 2006; Craun 1992; Craun et al. 2010; Curriero et al. 2001; Fong et al. 2007; Herwaldt et al. 1991; Moore 1993; Parshionikar et al. 2003; Reynolds et al. 2008; Rose 1997; Straub et al. 1993; Wallender et al. 2014
Scandinavia ^a	139	1984–2012	Guzman-Herrador et al. 2015; Hänninen et al. 2003; Jakopanec et al. 2008; Kukkula et al. 1997; Kvitsand and Fiksdal 2010; Miettinen et al. 2001
Israel	~52 ^b	1976–1997	Tulchinsky et al. 2000
South Korea	35	1999–2012	Cho et al. 2014; Jung et al. 2015; Kim et al. 2005; Koh et al. 2011; Lee et al. 2011
European Union ^c	24	1990–2005	Risebro et al. 2007
Taiwan	1	1998	Jean et al. 2006
India	2	2004–2010	Datta et al. 2012
UK	2	1992–1993	Bridgman et al. 1995; Willocks et al. 1998
Italy	2	2003–2011	Giammanco et al. 2014; Migliorati et al. 2008
France	1	2000	Gallay et al. 2006
Malaysia	1	2013	Muhamad Harish et al. 2015
South Africa	1	2006	Bessong et al. 2009
Greece	1	1996	Alamanos et al. 2000
Switzerland	1	1998	Maurer and Stürchler 2000
Canada	1	2000	Auld et al. 2004; Balbus and Embrey 2002

^a Included Finland, Norway, Denmark and Sweden

^b From community water systems, unclear if all related to groundwater

^c Included the United Kingdom (England, Scotland, N. Ireland, Wales), Finland, France, Spain, Sweden, Greece, Germany, Republic of Ireland, Italy, Netherlands

smaller size resulting in lower rates of physical attenuation and higher rates of movement within the subsurface environment, as compared with protozoa (Macler and Merkle 2000).

Reported causes of pathogen occurrence in groundwater during outbreak and non-outbreak conditions

The confirmed or likely causes attributed to the groundwater-related outbreaks included in the current scoping review are summarized in Table 3. In articles pertaining to the detection of enteric pathogens in groundwater irrespective of public health events (Table 4), the suspected sources/causes of pathogen contamination were similar as those presented in Table 3, including septic systems/sewage, agricultural activities, precipitation events, incorrect well construction and/or maintenance, inadequate treatment, and surface-water intrusion (Brooks et al. 2004; Cheong et al. 2009; Giwa et al. 2015; Lee et al. 2011; Ritter and Sibley 2002; van der Hoek et al. 2011). Additionally, Borchardt et al. (2011a) and

Atherholt et al. (2012) have reported that local geological setting, and particularly the presence of fractured bedrocks, likely contributes to the transport of microbial contaminants and faecal indicators to groundwater supplies. A previous study by Hynds et al. (2012) sought to model microbial ingress to private wells situated in diverse hydrogeological settings in the Republic of Ireland; study findings indicate that the modeling clusters (i.e. explanatory variables) of greatest significance were well design parameters (i.e. source protection; 47% of calibrated variance), location (setback distance and gradient) of the closest septic tank (25.9%), local geological setting including subsoil permeability and thickness (23.5%), and short-term antecedent rainfall (4.7%). Accordingly, evidence suggests and the authors would tend to agree that the majority of groundwater contamination events, and subsequent human infections, are caused by >1 temporal and/or spatial factor. Thus, source contamination may be geographically and/or temporally specific, which should be appropriately reflected in source protection and management strategies.

Table 2 Groundwater-related outbreaks of enteric infection (1948–2013) stratified by associated (confirmed/suspected) causative pathogen, including articles reporting on multiple outbreaks

Causative organism	Number of groundwater outbreaks	References
Bacteria		
<i>Arcobacter</i>	1	Fong et al. 2007
<i>Campylobacter</i> (spp. and jejuni)	31	Auld et al. 2004; Hänninen et al. 2003; Jakopanec et al. 2008; Kvitsand and Fiksdal 2010; Lee et al. 2011; Maurer and Stürchler 2000; Miettinen et al. 2001; Wallender et al. 2014
<i>E. coli</i> O157: H7	8	Balbus and Embrey 2002; Barwick et al. 2000; Beer 2015; Fong et al. 2007; Herwaldt et al. 1991; Lee 2002; Wallender et al. 2014
<i>Salmonella</i> spp., <i>Salmonella typhimurium</i>	12	Kvitsand and Fiksdal 2010; Muhamad Harish et al. 2015; Lee 2002; Tulchinsky et al. 2000; Wallender et al. 2014
<i>Shigella</i> spp., <i>Shigella sonnei</i>	24	Alamanos et al. 2000; Barwick et al. 2000; Kvitsand and Fiksdal 2010; Maurer and Stürchler 2000; Moore 1993; Tulchinsky et al. 2000; Wallender et al. 2014
<i>V. cholerae</i>	1	Datta et al. 2012
<i>Yersinia</i>	1	Wallender et al. 2014
Protozoa		
<i>Cryptosporidium parvum</i>	7	Barwick et al. 2000; Bridgman et al. 1995; Rose 1997; Sherline H. Lee 2002; Willocks et al. 1998
<i>Entamoeba</i> spp., <i>Entamoeba histolytica</i>	1	Wallender et al. 2014
<i>Giardia intestinalis</i> , <i>Giardia</i> spp., <i>G. lamblia</i>	16	Barwick et al. 2000; Moore 1993; Sherline, Lee 2002; Wallender et al. 2014
Viruses		
Adenovirus	2	Kukkula et al. 1997; Divizia et al. 2004
Enterovirus	1	Jean et al. 2006
Hepatitis A	21	Herwaldt et al. 1991; Moore 1993; Wallender et al. 2014
Norovirus (Norwalk-like virus)	39	Beer 2015; Blackburn 2004; Borchardt et al. 2011a; Galloway et al. 2006; Giannanco et al. 2014; Herwaldt et al. 1991; Kim et al. 2005; Koh et al. 2011; Kukkula et al. 1997; Kvitsand and Fiksdal 2010; Miettinen et al. 2001; Migliorati et al. 2008; Park et al. 2015; Parshionikar et al. 2003; Wallender et al. 2014
Rotavirus	2	Galloway et al. 2006; Kukkula et al. 1997
Small round structured viruses (SRSV(s))	2	Kukkula et al. 1997; Maurer and Stürchler 2000
Other		
Not specific	Not specified	Adam et al. 2016; Beer 2015; Curriero et al. 2001; Kukkula et al. 1997; Maurer and Stürchler 2000; Migliorati et al. 2008; Risebro et al. 2007

Table 3 Reported causes of pathogen occurrence in groundwater during outbreak and non-outbreak conditions

Cause	Number of outbreaks	Reference
Agricultural activities	1	Auld et al. 2004
Geology (fractured bedrock)	1	Borchardt et al. 2011a
Heavy rainfall	6	Auld et al. 2004; Bridgman et al. 1995; Fong et al. 2007; Giammanco et al. 2014; Jean et al. 2006; Willocks et al. 1998
Inadequate treatment	14	Alamanos et al. 2000; Auld et al. 2004; Bessong et al. 2009; Borchardt et al. 2011a; Bridgman et al. 1995; Datta et al. 2012; Fong et al. 2007; Gallay et al. 2006; Giammanco et al. 2014; Jakopanec et al. 2008; Kim et al. 2005; Maurer and Stürchler 2000; Migliorati et al. 2008; Parshionikar et al. 2003
Septic system/municipal sewage	9	Bessong et al. 2009; Borchardt et al. 2011a; Bridgman et al. 1995; Fong et al. 2007; Gallay et al. 2006; Giammanco et al. 2014; Jakopanec et al. 2008; Maurer and Stürchler 2000; Parshionikar et al. 2003
Surface-water intrusion	9	Bessong et al. 2009; Borchardt et al. 2011a; Fong et al. 2007; Gallay et al. 2006; Giammanco et al. 2014; Jean et al. 2006; Kukukula et al. 1997; Migliorati et al. 2008; Willocks et al. 1998
Well construction	2	Borchardt et al. 2011a; Willocks et al. 1998

The epidemiology of groundwater-related infections

In epidemiology, disease occurrence is defined at distinct levels; illnesses within the community may be defined as endemic or epidemic (CDC 2012). Endemic disease refers to the constant presence, or prevalence of a disease within a specific population and/or in a particular region. An example of endemic illness is the relatively constant and predictable annual rates of chickenpox (varicella zoster virus) among children in Canada, the UK, and the US (Seward et al. 2008). “Endemic” and “sporadic” are often interchangeably used to describe levels of illness; however, this is not necessarily accurate as sporadic typically refers to low rates of infection which are neither constant nor predictable. Epidemic disease refers to sudden spikes in disease above local endemic levels. The terms epidemic and outbreak are often used interchangeably (CDC 2012).

Upon examination of the published literature, it is clear that endemic, sporadic, and epidemic levels of illness are associated with groundwater sources globally. Outbreak-associated infections are more frequently tracked, and reported within healthcare systems, and are thus more often linked with the causative organism(s) and source(s). Endemic and sporadic disease associated with groundwater supplies are typically much more difficult to track; cases are by definition isolated and more likely associated with healthy adults (i.e. mild gastrointestinal symptoms with low levels or absence of associated sequelae) resulting in frequent self-medication, high levels of under-reporting and/or under-diagnosis (Schuster et al. 2005, Thomas et al. 2013); Guzman-Herrador et al. 2015; Thomas et al. 2013). Furthermore, previous studies have shown evidence of significant disease source and exposure misclassification, particularly with respect to populations served by private water supplies (Jones et al. 2006). Accordingly, the most effective way of quantifying levels of endemic (and sporadic) waterborne infection within a particular region and/or population is by designing and performing appropriate epidemiological studies. The following sections provide a summary of epidemiological study types and the epidemiological and hydrogeological evidence that has been acquired from several published studies (see section ‘Epidemiological studies of endemic or sporadic groundwater-related infections’).

Epidemiological studies of endemic or sporadic groundwater-related infections

The global burden of disease associated with contaminated groundwater supplies remains unknown. As mentioned in the previous section, endemic and sporadic cases of AGI not associated with outbreaks are difficult to track for numerous reasons (Hunter et al. 2011; Murphy et al. 2016a; Thomas et al. 2013). Epidemiological studies such as randomized

Table 4 Pathogens positively identified in groundwater supplies globally (under non-outbreak and outbreak conditions) 1985–2015

Name of organism	Countries	Number of studies/articles	Reference
Bacteria			
<i>Arcobacter spp./Campylobacter spp.</i>	France, US/Canada, New Zealand	5	Close et al. 2008; Hynds et al. 2014b; Tissier et al. 2012
<i>Clostridium perfringens</i>	South Korea	1	Joung et al. 2013
<i>E. coli O157 (STEC)</i>	South Korea	4	Hynds et al. 2014b; Joung et al. 2013
<i>Enteropathogenic E. coli (EPEC)</i>	US/Canada ^a	4	Hynds et al. 2014b
<i>Salmonella spp.</i>	Nigeria, South Korea, US/Canada	7	Giwa et al. 2015; Haznedaroglu et al. 2012; Hynds et al. 2014b; Joung et al. 2013
<i>Shigella</i>	Nigeria, South Korea	2	Giwa et al. 2015; Haznedaroglu et al. 2012
<i>V. cholerae</i>	Nigeria, Cameroon, England	3	Akoachere et al. 2013; Giwa et al. 2015; Koch 2013
Protozoa			
<i>Cryptosporidium spp.</i>	US/Canada ^a	7	Darnault et al. 2003; Hynds et al. 2014b
<i>G. lamblia</i>	US/Canada ^a	3	Hynds et al. 2014b
Viruses			
Adenoviruses	South Korea, US/Canada ^a	6	Cheong et al. 2009; Hynds et al. 2014b
Astroviruses	Slovenia	1	Steyer et al. 2011
Echovirus 1	United States		Yates et al. 1985
Enteroviruses	South Korea, US/Canada ^a	16	Hynds et al. 2014b; Joung et al. 2013; Lee et al. 2011
Hepatitis A	US/Canada ^a	7	Hynds et al. 2014b
Human enteric viruses	South Korea, US/Canada ^a	5	Cheong et al. 2009; Fout et al. 2003; Hynds et al. 2014b
Noroviruses	South Korea, Slovenia, US/Canada ^a	13	Cheong et al. 2009; Hynds et al. 2014b; Joung et al. 2013; Lee et al. 2011; Steyer et al. 2011
Poliovirus 1	United States		Yates et al. 1985
Reovirus	US/Canada ^a	2	Hynds et al. 2014b
Rotaviruses	Slovenia, South Korea, US/Canada ^a	6	Cheong et al. 2009; Hynds et al. 2014b; Steyer et al. 2011

^a Result for the US/Canada extracted directly from Hynds et al. 2014b, some of these results may have been linked to outbreaks

controlled trials (RCTs), community intervention trials, observational, case-control, and ecological investigations are methods that may be used to understand and quantify the health risks associated with the consumption of untreated groundwater. The majority of the epidemiological studies linking groundwater to enteric disease have been conducted in North America, with the most studies conducted in Wisconsin, USA.

Randomized controlled trials

Randomized controlled trials (RCTs) are considered the “gold standard” of epidemiological studies for determination of causal links; other study designs are limited in their ability to establish clear causality (as opposed to statistical association) between exposure and disease (Rothman 2012). RCTs are extremely time and resource intensive. In these trials, one group of households is randomly assigned to use an in-home intervention such as a household water filter installed under the tap. The other group of households serves as a control group. The two groups of households are, in theory, representative of one another with the only difference being the household water intervention. Under this assumption, the

researchers may attribute differences in illness rates between the two groups to the presence or absence of the household intervention being examined (Rothman 2012). To date, no RCT has been undertaken to specifically investigate the burden of illness associated with consumption of untreated groundwater (Murphy et al. 2014), thus representing an important data and knowledge gap to be filled.

Community intervention trials

Community intervention trials (CITs) are similar in design to RCTs; however, the intervention is assigned at the community level as opposed to the household level. To date, one community intervention trial has been published globally on untreated groundwater supplies (Borchardt et al. 2012). In this trial, 14 untreated public groundwater supplies were selected in Wisconsin, USA, half of which were randomly assigned to be treated with a UV treatment system and half of which remained untreated (Borchardt et al. 2012). At the mid-point (24 weeks) of the study, the intervention was reversed (i.e. untreated wells had UV systems installed, while treated wells had their systems disconnected). Household served by these municipal wells were blinded to the intervention. Borchardt

et al. (2012) found that a 6–22% increase in AGI was associated with viral presence (primarily norovirus and enterovirus) in untreated community groundwater supplies in Wisconsin. The fraction of AGI caused by the water supply approached 63% in children under 5 years old when norovirus was abundant in the water (Borchardt et al. 2012). Community intervention trials of this nature have several advantages over a household intervention trial. Firstly, the entire water supply for the community is altered; therefore, the intervention is more controlled because people who consume water from outside the home (i.e. at work or at school) are still subject to the intervention provided that they stay within the community. In a household intervention trial, it is more difficult to control for the consumption of water outside of the home. Another advantage of a CIT is that blinding of participants at the community level is easier and possibly more reliable as households cannot tamper with the intervention. One disadvantage of the CIT is that private water supplies cannot be evaluated using this approach. In addition, if the intervention occurs at the water treatment plant, it is difficult to control the quality of water reaching the home (i.e. no control for contamination of distribution system or household plumbing). Since only one trial has been published, it is difficult to extrapolate the results to other countries and contexts. More trials are needed to better understand the burden of waterborne disease associated with untreated community water supplies.

Case-control studies

Case-control studies are designed to determine if a particular exposure is associated with a specific outcome (Lewallen and Courtright 1998). Case control studies can be either retrospective (use existing data) or prospective (collect current or future data) in nature (Rothman 2012). The study design involves the identification of cases (a group known to have the outcome of interest (e.g. AGI) and controls (a group known to be free of the outcome). Subsequently, available pertinent information with respect to individual demographics and exposures are collated and comparatively analyzed to detect (1) which subjects in each group had the exposure(s) of interest (i.e. consumption of untreated groundwater), and (2) whether this exposure is significantly associated with the outcome of interest (i.e. AGI; Lewallen and Courtright 1998).

The authors have identified four prospective case-control studies, which, sought to examine cases of AGI, with consumption of untreated groundwater included as an exposure of interest. In a study of children aged ≤ 18 years in Wisconsin, found that 11% of cases characterized by diarrhoea were directly attributable to consumption of private well water containing faecal enterococci (a faecal indicator organism; Adjusted OR, 6.18; 95% CI, 1.22–31.46). Similarly, (Denno et al. 2009) found that infection with *Salmonella* spp. was associated with consumption of private well water in

Washington State [OR 6.5 (1.4–29.7)]. Fullerton et al. (2007) found that infants aged 0–6 months were at risk to *Campylobacter* infection if they were drinking well water in US case control study (OR 4.4; CI 1.4–14). In another nested case control study conducted in a metropolitan area in the US it was found that there was an increased risk of getting AGI when a household was served by well water compared to surface water (aOR 1.38; CI 1.01–1.87; Gorelick et al. 2011).

Ecological studies

Ecological studies represent an observational (i.e. no intervention) approach characterized by ≥ 1 variable being measured at the group (not individual) level—for example, incidence rates of likely or confirmed waterborne infection. Studies may be undertaken either retrospectively or prospectively, with groups/populations typically defined either geographically or temporally. Potential exposures (e.g. drinking water source, rainfall, livestock density, etc.) and outcomes (e.g. AGI incidence) are averaged within each examined population and comparatively analyzed using statistical methods such as—logistic regression, spatial autocorrelation, clustering, principal components analysis, and factor analysis (Craun and Calderon 2006). Disease/infection occurrence is thus compared between populations characterized by differing levels of spatial and/or temporal exposure. Ecological studies are frequently and most appropriately employed for initial investigation of causal hypotheses, particularly where existing relevant data are available (Craun and Calderon 2006). One weakness of ecological studies is that one cannot draw conclusions for individuals, as the data is analyzed at a group level (ecological fallacy). Perhaps the most famous, and almost certainly the first example of an ecological study in modern epidemiology is the “Ghost Map” developed by Dr John Snow in 1854 in response to a major cholera outbreak in London. The “Ghost map” was a geographical grid that showed the cases of cholera and their proximity to a public well believed to be the source of cholera (Hempel 2013).

In Ontario, Canada, Strauss et al. (2001) conducted a prospective ecological study of 235 rural households served by private wells. Data was collected on self-reported AGI and well water samples were measured for faecal indicator organisms. No relationship was established between self-reported AGI and wells containing faecal indicator organisms. Conversely, in a similar study from Ontario, Canada, *E. coli* present in well water was significantly associated with gastrointestinal illness, with this relationship modified by the distance of the septic tank to the well (Raina et al. 1999). Wells located >20 m from a septic tank were associated with greater odds of contributing to gastrointestinal illness than those <20 m from the septic tank (OR 2.16; 95% CI 1.04, 4.42).

Uhlmann et al. (2009) employed geographic information systems (GIS) as part of an observational study which

demonstrated a 5.2-fold increased risk of AGI among those served by private wells in British Columbia than those served by municipal groundwater supplies. Similarly, Dangendorf et al. (2002) have presented evidence of a significant association between disease incidence and the number of people served by groundwater in Germany. ÓhAiseadha et al. (2017) investigated the potential risk factors for 989 confirmed primary verotoxigenic *E. coli* (VTEC) infections in the Republic of Ireland during the period 2008–2013. Spatial geo-referencing and derivation were used to positively identify three predictors of VTEC notification, namely private well usage (OR 6.896, $p < 0.001$), cattle density (OR 1.002, $p < 0.001$) and septic tank density (OR 0.978, $p = 0.002$).

Pathogens of concern in groundwater

The following section provides an outline of the most significant enteric pathogens with respect to groundwater contamination and human illness, including what is known about their survival and movement in the subsurface. A summary of previous studies comprising data on enteric pathogens isolated from global groundwater supplies under both outbreak and non-outbreak conditions are presented in Table 4. While this summary is not exhaustive, it will assist in providing a broad context on what is currently known about enteric pathogen presence in groundwater supplies, in addition to highlighting any significant knowledge gaps. Knowledge around the types and concentrations of pathogens along with their persistence in groundwater supplies is needed to inform risk assessment models. In the absence of epidemiological data, quantitative microbial risk assessment (QMRA) can be used to estimate and to quantify risks of AGI attributable to specific pathogens found in groundwater supplies. Examples of QMRA models employed for groundwater are presented in see section ‘Quantitative microbial risk assessment’.

Bacteria

Bacteria are the microbial group of most importance in terms of both frequency of isolation in drinking water and the number of reported waterborne disease outbreaks (Gray 1997). Although numerous bacterial species may and have been found to contaminate aquifers, some are more prevalent than others. As previously outlined, the most widespread waterborne disease throughout history has been cholera, with the first major epidemic in Europe killing over one million people from 1830–1832 (Wyn-Jones 2000). However, many of these traditional waterborne diseases (i.e. cholera and typhoid) have largely disappeared from Europe and North America, due to advances in microbiology, epidemiology and medicine, as evidenced by Craun et al. (2006), who report that during the period 1920–1940, 70% of waterborne disease outbreaks in

the United States presented as typhoid fever, compared to 22 and 11% of outbreaks during the periods 1941–1960 and 1961–1970, respectively. Although typhoid fever has not been a major causative agent of waterborne infection since then, many of the “classical” waterborne infections are still of major importance in poorer developing countries. The primary bacterial pathogens associated with groundwater supplies are now outlined.

Salmonella spp.

Salmonella are bacterial pathogens present in both human and animal faeces and are capable of causing a range of enteric infections depending upon the serotype involved, including gastroenteritis, salmonellosis, typhoid fever and paratyphoid fever (Gill et al. 2003). *Salmonella* is primarily transmitted through improper handling and cooking of food; however, it can also be found and transmitted via water. The most common symptoms of *Salmonella* gastroenteritis include diarrhoea, fever, and abdominal cramps, with illness typically lasting 4–7 days (Bemrah et al. 2003; CDC 2016a). Boehm et al. (2012) report that *Salmonella* can persist in dark freshwater microcosms for at least 53 days with little to no decay. One documented waterborne outbreak of *Salmonella* associated with a groundwater supply occurred in Gideon, Missouri in 1993, with the outbreak affecting approximately 650 people, 15 of whom were hospitalized, with 7 associated deaths. In this case, the outbreak was associated with community wells, all of which tested positive for *Salmonella typhimurium* (Angulo et al. 1997).

Pathogenic *E. coli*

E. coli are bacteria, ubiquitously found in the lower intestine of warm blooded animals including humans with a number of strains capable of causing infection (Haas et al. 1999). These pathogenic strains are characterized into five main virotypes (Todar 2007), of which the enterohaemorrhagic (EHEC) serotype O157 is perhaps the best known.

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neo-natal meningitis, with gastroenteritis being the most prevalent form of illness associated with waterborne serotypes. Survival of *E. coli* is similar throughout the serotypes, and they are capable of multiplying in warm nutrient-rich conditions (e.g. septic tank effluents; Gray 2004). Approximately 100 organisms are required to cause infection, with gastroenteric symptoms comprising profuse watery diarrhoea, nausea and dehydration. The majority of waterborne *E. coli* outbreaks detailed in the literature focus on the verotoxin-producing, EHEC strain O157.

Verotoxigenic (or verotoxin-producing) *E. coli* (VTEC) including serotype O157:H7 is associated with a wide range of severity of illnesses from mild diarrhoea to haemorrhagic

colitis and haemolytic uraemic syndrome (HUS), which causes the intravascular lysis of red blood cells (Garvey et al. 2010). In severe cases, HUS can result in irreversible renal failure, requiring haemodialysis, and in a minority of cases, may prove fatal (Coffey et al. 2007). HUS associated with VTEC is the most common cause of renal failure in children (Garvey et al. 2010). Animals, and in particular cattle and other ruminants, are a primary environmental reservoir (Szewzyk et al. 2000; Rosen 2000; Garvey and McKeown 2008), resulting in this virulent *E. coli* strain's association with rural areas' private groundwater sources. Drinking troughs and moist mixed cattle rations have been cited as sources of VTEC on farms (Hancock et al. 2001).

Perhaps the most notable outbreak of groundwater-related *E. coli* O157:H7 infection was that of Walkerton, Ontario, Canada in 2000 where seven people died and over 2,000 became ill as a result of exposure to *E. coli* O157:H7 and *Campylobacter jejuni* found in the community groundwater supply (Holme 2003). In 1999, a serious outbreak of *E. coli* affected the state of New York with more than 1,000 people affected and 2 deaths. It was reported that a total of 122 cases of *E. coli* O157:H7 were confirmed, 65 people were admitted to hospital and 11 children developed HUS. Two people (a 3-year old girl and a 79-year old man) died (Charatan 1999; Hruday and Hruday 2004). The source of the infection was a contaminated well at the Washington County Fair, with runoff from cattle manure after torrential rain thought to be responsible (Charatan 1999). A study by Schets et al. (2005) reported *E. coli* O157:H7 presence in 2.7% of private groundwater supplies in the Netherlands, with pulsed field gel electrophoresis (PFGE) analysis suggesting that cattle were the most probable source.

Shigella spp.

Shigella are a genus of bacteria closely related to *E. coli* and *Salmonella* (CDC 2016a), although they have a lower environmental survival rate than both of these organisms (Bitton 2005). The disease is characterized by a short period of watery diarrhoea with intestinal cramps and general malaise, followed by permanent emission of bloody stools, known as haematochezia (DuPont 2010; CDC 2016a). *Shigella* is characterized by an extremely low threshold dose and therefore has a high potential for person-to-person (secondary) propagation (Wikswa and Hall 2012). Alamanos et al. (2000) reported a waterborne outbreak of shigellosis which occurred in a community of 2,213 persons in north-western Greece during October 1999. In all, 288 inhabitants of the village of Eleoussa, suffered from gastroenteritis, with the peak of the epidemic occurring during the first 3 days. The highest risk of developing gastroenteritis was observed in the 0–14-year age group (41.4%) and decreased significantly with age ($p < 0.01$). Patients over 65 years were more frequently hospitalized than those in other age groups ($p <$

0.05). *Shigella sonnei* was isolated from both water and human faecal samples (Alamanos et al. 2000). Environmental conditions suggested that infection was caused via contaminated groundwater sources. *Shigella* has been linked to 20 groundwater outbreaks in the United States between 1971–2008 (Wallender et al. 2014). Additionally, it has been isolated from groundwater supplies in Nigeria and South Korea (Giwa et al. 2015; Joung et al. 2013).

Campylobacter spp.

Campylobacter are a bacterial genus with at least 17 known species, of which *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter fetus* and *Campylobacter rectus* species are the most important from a human health perspective (Altekruse et al. 1999). *Campylobacter jejuni* and *Campylobacter coli* are two of the most common causes of bacterial gastroenteritis worldwide, with illness characterized by severe diarrhoea, cramping, fever, and abdominal pain (St-Pierre et al. 2009; Pagaya et al. 2015). Symptoms may subside after a number of days or persist for weeks and can become more severe in immunocompromised persons. Rarely, more severe symptoms may develop such as reactive arthritis, Reiter's syndrome, or HUS, with approximately one in every 1,000 cases leading to a severe neurological disorder called Guillain-Barré Syndrome (CDC 2016b).

Campylobacter species are found in the faeces of humans, livestock and a number of wildlife species, and are therefore common in the environment (Szewzyk et al. 2000; Whiley et al. 2013). A number of *Campylobacter* outbreaks have been positively associated with waterborne transmission routes, particularly in Scandinavian countries including Denmark (Engberg et al. 1998), Finland (Hänninen et al. 2003), Norway (Jakopanec et al. 2008), and Sweden (Andersson et al. 1997). Waterborne exposures are often associated with faecal contamination of the water source from agricultural waste run-off, bird droppings or sewage outflow (Hänninen et al. 2003). Survival in water can be from several weeks up to 3 months provided the temperature is low (Altekruse et al. 1999; Nicholson et al. 2005). Chlorination is a highly effective barrier against *Campylobacter* species in water and therefore, where waterborne illness occurs, it is largely associated with untreated private water supplies (Coffey et al. 2007). One waterborne *Campylobacter jejuni* outbreak in Denmark in 1995 resulted in an estimated 2,400 symptomatic infections, with contamination of the water supply positively traced to contamination of groundwater due to a break in a sewage pipe (Engberg et al. 1998). A recent QMRA from New Zealand predicted that consumers receiving their drinking water from shallow groundwater in an intensive dairy region (Waikakahi catchment, Canterbury) had an estimated 60–75% chance of contracting campylobacteriosis during the irrigation season (Hynds et al. 2014b; Murphy et al. 2016a).

Vibrio cholerae

V. cholera is a bacterial pathogen, and is the causative organism for cholera. Cholera is now rare in developed countries; however, it remains endemic in developing regions including Africa and Southeast Asia (Lutz et al. 2013; CDC 2016c). Of the approximately 221,000 cases reported globally in 2009, 98% were associated with African countries (WHO 2010). According to Rebaudet et al. (2013), cholera represents a “prototypical waterborne and environmental disease”. Cholera is a diarrhoeal disease with severity ranging from asymptomatic infection to severe diarrhea and vomiting resulting in death. As part of the current scoping review, the presence of cholera in groundwater supplies under outbreak and non-outbreak conditions were retrieved from three articles (Akoachere et al. 2013; Giwa et al. 2015; Koch 2013). In the city of Douala, Cameroon, cholera outbreaks are common, particularly in New Bell District where the water table is high and inhabitants rely on shallow wells for drinking and household uses (Akoachere et al. 2013). These shallow supplies are extremely vulnerable to microbial contamination. Densely populated urban environments such as those found in major cities in Nigeria experience similar conditions, whereby people rely on groundwater that can be heavily contaminated with faecal waste due to a lack of adequate sanitation (Giwa et al. 2015). These are ideal conditions for cholera and other pathogens to enter the water supply.

Interestingly, while three-quarters of all cholera cases in Africa between 2009–2011 occurred in inland areas, *V. cholera* has rarely been isolated from water supplies in these regions (Rebaudet et al. 2013). Moreover, the natural habitat of *V. cholera* is typically brackish or saline water (Lutz et al. 2013). Sorensen et al. (2015) recently found that *V. cholera* was persistent in 41% of groundwater samples analyzed from Kabwe, Zambia, with the bacteria exhibiting an ability to enter a viable but non-culturable (VBNC) state in order to survive in unfavorable environments.

Viruses

Viruses are pathogens of particular concern with respect to groundwater as they are shed in large quantities in the environment, while their size results in lower attenuation and relatively rapid transport to and within aquifers (Macler and Merkle 2000; Bradbury et al. 2013). Culturable enteric viruses have been found in numerous groundwater supplies across the US (Abbaszadegan et al. 2003; Borchardt et al. 2003, 2004, 2007, 2012; Bradbury et al. 2013; Lambertini et al. 2012). A recent systematic review of groundwater systems in North America found that 88.5% of US studies included for meta-analysis reported the presence of enteric viruses in analyzed groundwater samples, with viruses more frequently identified than either bacterial or protozoan pathogens (Hynds et al.

2014b). There are over 140 distinct known types of human pathogenic virus, with those of most concern being the enteric viruses which cause gastrointestinal illnesses including enteroviruses, rotaviruses, astroviruses, calciviruses, hepatitis A virus and norovirus (Gray 2004).

Norovirus

Norovirus (previously called Norwalk-like viruses and more commonly known as the “winter vomiting bug”) is an RNA virus (taxonomic family Caliciviridae), identified as the leading cause (~90%) of epidemic non-bacterial gastroenteric outbreaks around the world (Hall et al. 2013). In the United States alone, noroviruses have been estimated to cause between 19–21 million illnesses per year, including 57–800 deaths, 56,000–71,000 hospitalizations, 400,000 emergency department visits and 1.7–1.9 million out-patient visits (Hall et al. 2013). The primary transmission routes are faecally contaminated food or water, and person-to-person contact (Lee 2011). Infected individuals are associated with shedding rates of 10^5 – 10^9 particles per gram in feces; therefore, leaking septic and sewage systems are significant sources of groundwater contamination (Lopman et al. 2012; Bradbury et al. 2013; Hynds et al. 2014b). Additionally, norovirus is excreted by adults for up to 3 weeks, and up to 47 days in young children (Murata et al. 2007). It is also capable of surviving and remaining infectious in water for up to 2 months (Lopman et al. 2012). Norovirus outbreaks are primarily associated with closed-communities such as hospitals, retirement homes, cruise ships, etc. (Gallimore et al. 2005; Coffey et al. 2007). Typically, acute gastroenteritis develops 24–48 h after exposure, lasting for 24–60 h. The disease is usually self-limiting, and characterized by nausea, vomiting, diarrhoea, and abdominal pain. General lethargy, weakness, muscle aches, headache, and low-grade fever may also occur (Zheng et al. 2010).

Consumers drinking water from a contaminated well in central Wyoming, which was suspected of having been contaminated by sewage, were found to be 4.5 times more likely to present with gastroenteric symptoms than those who had not consumed from the well, with norovirus confirmed as the causative infectious organism (Parshionikar et al. 2003). Manuala et al. (2005) previously found that a high proportion of norovirus contamination events occurring in Finland are attributable to groundwater systems, with almost 50% of groundwater outbreaks associated with private water wells. Reporting on a recent norovirus outbreak associated with a private well in a dolomite aquifer in northeast Wisconsin, (Borchardt et al. 2011b) found that 229 patrons of a new restaurant were affected during the incident, which was positively confirmed as being due to ingress of septic tank effluent into a nearby private well—both the septic tank and well conformed to state regulations in this case. Both Borchardt et al. (2012) and Bradbury et al. (2013) have found enteric

viruses including norovirus in deep confined aquifers assumed to be free of pathogens; bedrock fractures and improperly sealed well casings both represent pathways for viral intrusion to and within these aquifers. In the current review, norovirus was the pathogen most frequently responsible for groundwater outbreaks reported within the academic literature (Table 2).

Adenovirus and enterovirus

Adenoviruses and enteroviruses are non-enveloped DNA viruses, approximately one third of which are capable of causing human infection. Adenoviruses in particular are unusually stable in the presence of chemical and physical agents and extreme pH conditions, thus permitting prolonged survival outside a host (Mena and Gerba 2009). A recent study has found that adenovirus remained relatively stable in seeded groundwater at 10 °C for at least 160 days and up to 301 days (Rigotto et al. 2011). This is a concern, as 10 °C is comparable with annual mean groundwater temperatures in many temperate regions. Studies have shown that adenoviruses survive longer in water than enteroviruses and hepatitis A virus, likely due to their double-stranded DNA (Ogorzaly et al. 2010; Carratalà et al. 2013). Both adenovirus and enterovirus may be transmitted via the faecal-oral route, diarrhea with oral transmission typically causing relatively mild infection; the typical incubation period for gastroenteritis is 3–10 days (Mena and Gerba 2009).

Bradbury et al. (2013) found that 47% (67/147) of samples collected over a 2-year period from six deep (220–300 m) community groundwater wells contained enteroviruses and/or adenoviruses. Based on results of the present review, both enteroviruses ($n=16$) and adenoviruses ($n=6$) have been identified in groundwater supplies during non-outbreak conditions (Table 4). Mena and Gerba (2009) report that adenovirus is responsible for more recreational waterborne outbreaks than any other enteric virus. To date, just two outbreaks in Finland and Albania, and one outbreak in Taiwan have been linked to groundwater for adenovirus and enterovirus, respectively (Table 2); nevertheless, given that both organisms manifest via respiratory symptoms, the authors suspect that their link to waterborne exposures may be significantly under-reported. The waterborne adenovirus outbreak reported by Kukkula et al. (1997) is of particular interest, as ingress of contaminated river water at a wellhead was found to be the source of the outbreak. In all, some 1,500–3,000 people, accounting for 25–50% of the affected Finnish community, were affected. Laboratory findings confirmed adenovirus, norovirus, and group A and C rotaviruses as causative agents.

Rotavirus

Rotavirus is a genus of double-stranded RNA virus and the most common cause of severe diarrhoea among infants and

young children worldwide (Glass 2006). Globally, it is estimated to be responsible for an estimated 610,000 child mortalities per year, accounting for approximately 5% of all deaths in those <5 years (Glass 2006). Rotavirus may be found in water sources, including private wells, which have been contaminated with faeces from infected humans, with sewage overflows or inoperative sewage systems primarily responsible for contamination (Parashar et al. 2006). The virus is highly resistant in the natural environment and can be present in large amounts in wastewater (Macler and Merkle 2000). Previous studies (Abbaszadegan et al. 2003; Azadpour-Keeley and Ward 2005) have found that enteric viruses may survive for many weeks in groundwater environments, with Sobsey et al. (1995) reporting that there was little, if any, viral inactivation in samples from a deep well in North Carolina over an 8-week period. More recently, Espinosa et al. (2008) demonstrated that rotavirus can survive and remain virulent for >210 days in groundwater. In addition, they demonstrated that rotaviral infectivity is tolerant to residual chlorine concentrations of between 0.1–1.5 mg/L, higher than those recommended by the World Health Organization (WHO) for water used for human consumption. Symptoms of rotaviral infection in humans include vomiting and watery diarrhoea (Parashar et al. 2006). Infections often occur as winter epidemics with a high level of secondary (person-to-person) transmission, which is mainly of faecal–oral origin. As few as 10 viral particles are sufficient to cause symptomatic illness (CDC 2016c).

Rotavirus has been isolated from groundwater supplies in North America, South Korea and Slovenia (Table 4) and has been responsible for groundwater related outbreaks in France and Finland (Table 2). Both groundwater-related outbreaks were characterized by the presence of multiple causative pathogens—for example, a large waterborne outbreak occurred in France during August 2000. Local residents that reported having drunk tap water were characterized by a three-fold increased risk for illness, with the risk increasing in concurrence with consumed volume. *Campylobacter coli*, group A rotavirus and norovirus were identified in patient stool samples, with a community groundwater source probably contaminated by agricultural run-off, and a chlorination failure identified as the likely causes of the outbreak (Gallay et al. 2006).

Hepatitis A

Hepatitis A virus (HAV) is a waterborne virus responsible for 50% of global hepatitis cases (Bosch et al. 2008). Hepatitis A virus (HAV) is a viral pathogen capable of causing both acute and chronic illness, and most frequently associated with consumption of contaminated food or water; while rarely responsible for human mortality, infection is capable of significantly incapacitating patients for up to several months (Bosch et al. 2008).

The disease is frequently asymptomatic, with symptomatic infection usually appearing 2–6 weeks subsequent to exposure and presenting as fever, fatigue, loss of appetite, vomiting, and nausea (CDC 2015). The virus has been responsible for a significant number of virus-related groundwater outbreaks globally (Table 2) and has been recovered from groundwater supplies in North America (Table 4). Between 1971 and 2008, 21 outbreaks (8.5%) in the US with known etiology were attributed to hepatitis A in untreated groundwater supplies (Wallender et al. 2014). It was the most common pathogen linked to outbreaks in untreated groundwater supplies during this time period in the US, followed by *Shigella* (20, 8.1%) and *G. intestinalis* (14, 5.7%; Wallender et al. 2014).

Protozoa

Protozoa (from the Greek words proton, meaning “first”, and zoa, meaning “animals”) are a subkingdom of microorganisms. Most are free-living, some of which can cause disease, whereas others are obligate parasites (i.e. cannot reproduce outside a host; Haas et al. 1999). They form structures called cysts, oocysts or spores (depending on the life cycle of the organism), which are resistant to adverse conditions such as high temperatures and disinfection (Karanis et al. 2007). Pathogenic (i.e. infectious) protozoa are primarily excreted in the faeces or urine of infected human or animal host, and transmitted during the infectious (oo) cyst stage via the faecal-oral route (Haas et al. 1999). This section focuses on the two most important protozoa found in groundwater supplies that can cause AGI: *G. lamblia* and *Cryptosporidium spp.*

Giardia spp.

Giardia spp. is a protozoan parasite, with *G. lamblia* being the most commonly encountered species, particularly in water supplies (Hunter and Thompson 2005). , et al (sis is the most common protozoan infection of the human intestine worldwide (Haas et al. 1999), with prevalence ranging from 2–5% in the developed world to 20–30% in the developing world (Marshall et al. 1997). Symptomatic illness is characterized by acute diarrhoea, foul-smelling stools, upper or mid-epigastric cramps, bloating and flatulence, with patients usually expressing feelings of malaise, nausea and sulfuric belching. The prolonged duration of diarrhoea, frequently continuing for 2–4 weeks, is a typical feature of *giardiasis* (Haas et al. 1999; Ashbolt 2004).

G. lamblia is found in a wide range of animals (Gray 2004), with cysts capable of surviving for long periods in groundwater (and surface water) environments, particularly at cold temperatures during the winter months (2–3 months at <10 °C; up to 1 month at 21 °C; LeChevallier et al. 1991; Coffey et al. 2007). Furthermore, when in its cyst form, it comprises a protective wall facilitating chlorine resistance.

During the period 1980–1985, there were 502 outbreaks of waterborne disease in the USA, with *Giardia* positively confirmed as the causative organism in 52% of these cases. One large outbreak occurred at a Colorado ski resort, when sewage contamination of a groundwater supply resulted in 123 people contracting symptomatic infection. During the period 1995–2004, *Giardia* has been identified as the primary causative agent in 13% of 108 reported gastroenteric outbreaks associated with drinking water in the US (Yoder and Beach 2007). It should be noted that the proportion of these outbreaks directly attributable to contaminated groundwater could not be established. In the present review, *Giardia* was found to have been responsible for 16 groundwater related outbreaks worldwide (Table 2), and has been isolated from groundwater supplies in North America (independently of a confirmed human health impacts; Table 4).

Cryptosporidium spp.

Cryptosporidium spp. are protozoan parasites, with 21 currently recognized species of which two are particularly important in the context of human infection: *Cryptosporidium parvum* and *Cryptosporidium hominus*. *Cryptosporidium parvum* is the species primarily responsible for clinical illness in humans and animals (Fayer et al. 2000; Hrudney and Hrudney 2004). Cryptosporidiosis is now acknowledged as being a significant gastrointestinal disease in infants, young children and immunocompromised individuals (Chen et al. 2002; Cohen et al. 2006). The incubation period after ingestion of viable oocysts is 5–28 days with a mean of 7.2 days (Marshall et al. 1997; Haas et al. 1999) with symptoms lasting for up to 2 weeks. Clinical symptoms include an influenza-like illness, diarrhoea (characteristically cholera like), malaise, abdominal pain, anorexia, nausea, flatulence, mal-absorption, vomiting, mild fever and weight loss (Smith and Rose 1990; Gray 2004), with infection typically non-fatal and self-limiting in healthy adult hosts. However, the severity and duration of the disease is significantly higher among immunocompromised individuals (Gatei et al. 2006). Low-level exposure to oocysts (1–10) can initiate infection (Gray 2004), with studies indicating that a single oocyst may be adequate to cause infection (Boak and Packman 2001).

Cryptosporidium oocysts are capable of survival outside the intestine for considerable periods of time (Marshall et al. 1997; Fayer et al. 2000). A study by Robertson and Gjerde (2006) found that *Cryptosporidium* oocysts with viable morphology were detected after approximately 20 weeks during winter in an aquatic environment in Norway. However, as stated by Reynolds et al. (2008), the interaction between pH, temperature and exposure time may have adverse effects on the survival of *C. parvum* oocysts in water and soils, and therefore an exact environmental survival rate is difficult to ascertain.

Cryptosporidium are smaller in size than *Giardia*, and as a result have been more frequently isolated in groundwater supplies due to their ability to be more readily transported in the subsurface environment. Additionally, *Cryptosporidium* oocysts are more resistant to low temperatures and are therefore expected to survive longer than *Giardia* in groundwater environments (Carmena 2010). While *Cryptosporidium* has been more frequently found in groundwater supplies than *Giardia*, it has been linked to fewer groundwater-related outbreaks (Table 3). In a study by LeChevallier et al. (1991), *Cryptosporidium* was found more frequently than *Giardia* in surface waters, thus suggesting that the pathogen may be more prevalent in the environment. Although, *Cryptosporidium* was found more frequently in groundwaters cited in the present review, Zmirou-Navier et al. (2006) report that the probability of disease when infected by *Cryptosporidium* is around 70%, whereas for *Giardia* it can be as high as 91%, thus perhaps explaining why *Cryptosporidium* has not caused as many outbreaks in groundwater. Rose (1997), provides ranges for the occurrence of *Cryptosporidium parvum* oocysts in a range of media including raw sewage, filtered treated wastewater, surface waters and groundwaters, with a range of 0.004–0.922 oocysts per liter provided for groundwater samples, suggesting a background level of oocysts existing in groundwater bodies have the potential for human infection.

The low associated human threshold dose, coupled with high environmental resistance to environmental factors including disinfection by chlorination, has led to a number of large cryptosporidiosis outbreaks associated with contaminated drinking water throughout the world. Smith and Rose (1998) report that during the 10-year period 1988–1998, *Cryptosporidium* was the causative organisms in at least 19 waterborne outbreaks of cryptosporidiosis in the US (surface water and groundwater), affecting over 425,000 individuals. Three of these outbreaks were associated with contaminated wells and one with a suspected spring (Smith and Grimason 2003). Willocks et al. (1998) report on 345 confirmed cases of cryptosporidiosis in North Thames during spring 1997. A case control study, in addition to oocyst detection rates and descriptive epidemiology associated with the outbreak, indicated that the outbreak was associated with drinking unboiled tap water that originated from a deep chalk borehole. Garvey and McKeown (2005) report that a significant decrease in cryptosporidiosis cases in northwest England during the period 1997–2000, was likely attributable to developments in public water supplies, which would suggest that some burden of illness was associated with private water supplies. Hunter et al. (2011) have shown that the predicted risk of cryptosporidiosis (and giardiasis) among users of very small private supplies in England and France are substantially greater than for public water supplies and well above the tolerable risk threshold.

Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) represents a methodology that can be used to understand and estimate the risk of infection or illness associated with (individual or population) exposure to a specific pathogen (Haas et al. 1999). QMRA findings may be used to develop and undertake improved risk management and communication strategies (Vose 2000). As set out by Lammerding and Fazil (2000), the QMRA process comprises four steps, namely hazard identification, hazard characterization, exposure assessment, and risk characterization. The hazard (waterborne pathogen) is characterized by the likelihood of exposure (amount of water consumed), the infectivity and attack rate associated with the pathogen (the amount of infectious pathogen required to cause infection or illness). The methodology is pathogen-specific, and therefore in order to assess the risk of multiple pathogens, QMRAs need to be performed for each individual pathogen of concern (Teunis et al. 1997; Macler and Merkle 2000; Howard et al. 2003). Previous QMRAs of enteric infection attributable to consumption from groundwater sources have been undertaken for *Cryptosporidium* spp. (Cummins et al. 2010; Razzolini et al. 2016), *E. coli* O157 (Haas et al. 2000; Hynds et al. 2014a), *Giardia* spp. (Razzolini et al. 2016), and rotavirus (Rutjes et al. 2009), in addition to a recently published national-scale QMRA of multiple groundwater pathogens (Murphy et al. 2016b).

Waterborne disease burden estimates

The authors have identified six studies within the published literature attempting to quantify the burden of waterborne enteric disease attributed to groundwater supplies at a national level (Colford et al. 2006b; Macler and Merkle 2000; Messner et al. 2006; Reynolds et al. 2008; Murphy et al. 2016a, b). Three of these studies endeavored to provide estimates of disease burden among the population served by community water systems (CWS) in the United States (Colford et al. 2006b; Messner et al. 2006; Reynolds et al. 2008). Two of these studies included public and community systems served by groundwater but did not include private wells. Colford et al. (2006b) approximated the occurrence of 1.33–3.88 million illnesses per year attributable to community groundwater supplies, while Messner et al. (2006) estimated approximately 5.4 million cases of AGI annually. The third study considered both community and non-community groundwater supplies in the US and used QMRA to estimate the risk of viral infections from groundwater supplies (Reynolds et al. 2008). In this study, they estimated that 5.4 million and 1.1 million cases of symptomatic viral infections may be attributable to community and non-community groundwater supplies annually in the US, respectively. Prior to these estimates, Macler and

Merkle (2000) provided a range of 890,000 to 5.9 million cases per year as a result of contaminated groundwater in the US.

More recently, Murphy et al. (2016a) have provided estimates of the AGI burden attributable to consumption of contaminated groundwater from private wells and small systems (serving less than 1,000 people) in Canada via a QMRA approach. Five key waterborne pathogens were selected, namely *Cryptosporidium*, *Giardia*, norovirus, *Campylobacter*, and *E. coli* O157. Findings indicate the incidence of AGI associated with consumption of contaminated groundwater from untreated private wells and chlorinated small systems (most common form of groundwater treatment in North America) to be 0.027 cases per person-year and 0.016 cases per person-year, respectively. Resulting estimates are markedly lower than those produced in the United States; a crude application of the generated Canadian incidence rates to the current global groundwater reliant population (2.2 billion), produces a global range of 35.2–59.4 million cases of illness per year.

Discussion

Overall findings

In this review, it was shown that untreated groundwater is a major source of enteric disease globally. The review identified 649 published groundwater outbreaks globally between 1948–2013 (Table 1). These outbreaks only represent the tip of the iceberg in terms of the number of illnesses attributable to groundwater, since AGI can be difficult to track due to its sporadic nature and due to under-reporting and under-diagnosis. Several epidemiological studies showed that there was an increased risk of AGI associated with the consumption of untreated private or municipal well water. Using results from a multi-pathogen QMRA conducted in Canada, the authors crudely estimate that between 35.2–59.4 million cases of AGI per year globally could be attributable to the consumption of untreated groundwater. The review identified 17 pathogens as being responsible for groundwater related outbreaks of which the top five pathogens were: norovirus, *Campylobacter*, *Shigella*, Hepatitis A and *Giardia* (Table 2). The main causes for pathogen intrusion into groundwater during outbreak and non-outbreak conditions included: septic system/ municipal sewage, heavy rainfall, well construction, geology, inadequate treatment, surface-water intrusion and agricultural activities (Table 3).

Study limitations

It is important to note that the present review did not utilize a formalized “systematic” approach, and is instead considered a scoping review. Typically, scoping reviews focus on

comprehensive coverage, as opposed to realizing a specified standard of evidence (McCull et al. 2009), and are thus typified by a broad focus, rather than an exhaustive review (Gentles et al. 2010; Pham et al. 2014). Accordingly, the current study is associated with a number of inherent limitations, as follows: (1) due to database selection, some relevant studies may not have been identified, (2) only published peer-reviewed academic journal papers were considered for inclusion, thus evidence from white papers, national databases, academic theses, and gray literature were not sought or employed, (3) no quality assessment and/or critical appraisal of included/excluded literature was undertaken, thus gaps in the published literature associated with low research quality were not identified, and (4) some level of publication bias will be present, i.e. study outcome(s) may have influenced the decision to publish (e.g. unconfirmed pathogen etiology, unconfirmed pathogen source, unconfirmed illness, etc.). In the current review, the authors consider it likely that publication bias due to insufficient or absent infrastructure are likely to create an imbalance between the availability of literature from developed and developing regions. Due to these stated limitations, study findings, while indicative, should be employed with caution.

Future research needs

Over the past 50 years, significant progress has been made in improving our understanding of the extent and potential consequences of groundwater contamination by enteric pathogens. Research has advanced on several fronts including: groundwater sampling methods, pathogen detection methods such as qPCR and other molecular approaches, pathogen transport in the subsurface as related to hydrogeological concepts and colloid filtration theory, pathogen survival in groundwater, the relationship between faecal indicator organisms and pathogens, the role of environmental factors such as temperature and precipitation, and finally, early estimates on the burden of illness from consumption of pathogen contaminated groundwater. Nevertheless, both data and knowledge gaps remain, thus necessitating further research and collaboration between health professionals, microbiologist and hydrogeologists in this area. Key areas of research for more research include: (1) pathogen transport and persistence in groundwater supplies, (2) presence, detection and microbiological sampling methods, (3) epidemiological studies.

Pathogen transport and persistence in groundwater supplies

An advanced science should seek to develop the necessary tools and subsequent capability to predict an outcome of interest; however, unfortunately, accurate prediction of the susceptibility of an individual groundwater source to pathogenic contamination remains a challenge. Some of this difficulty

likely stems from the natural variability associated with sources of enteric pathogens in the environment, stemming from the rise and fall of infections within the host population releasing these pathogens to the environment via faecal wastes. The remaining difficulty lies with the unique subsurface transport characteristics of pathogens and the conceptual leap needed to transfer our nanoscale particle-level understanding of pathogen attachment/detachment to transport at the macro-scale (Hunt and Johnson 2017, this issue). Empirical models to predict well susceptibility to pathogens have been developed (Schijven et al. 2010). An alternative approach is the use of advanced statistical models to identify the set of independent variables (i.e., risk factors) most highly associated with well vulnerability, for example, proximity to faecal source, well casing depth, depth to water, etc. (Howard et al. 2003; Hynds et al. 2012). Allen et al. (2017, this issue) explore this statistical approach in a univariate fashion with a small set of wells. While these models might be specific to hydrogeological setting, (e.g., fractured bedrock, alluvial), any tool with reasonable predictive value for “at-risk” groundwater supplies should be welcomed by regulators and others responsible for protecting public health. The paper by Bradford and Harvey (2017) in this issue provides suggestions for future research specific to pathogen transport in groundwater.

Understanding pathogen survival in groundwater is important for establishing safe setback distances between faecal sources (e.g., septic systems) and drinking water wells, and for evaluating groundwater exposures to pathogens and the consequent health risks. Many studies on pathogen survival in groundwater are conducted via laboratory microcosms or groundwater mesocosms. Such study designs likely do not adequately represent the direct and indirect effects on pathogen survival from attachment to aquifer sediments, predation from invertebrates and protozoa, bacterial pathogen growth from nutrient inputs into the aquifer, and the complex interactions among the aquifer’s native microbial community. Thus, further studies are needed which seek to adequately account for the abiotic and biotic conditions found in natural groundwaters, as these will likely yield the most accurate measurements of microbial subsurface survival.

Presence, detection and microbiological sampling methods

Additionally, and perhaps more fundamentally, an improved understanding of the effects of sample volume and sampling frequency on the likelihood of false-negative results for pathogens, or for that matter, faecal indicator organisms in groundwater, is required. At present, logistics, costs, and precedent are the typical determinants of many sampling design programs, and rarely is the false-negative rate considered. Optimal sample volumes

and frequency of sampling for pathogens in groundwater are unknown. Avoiding false-positives may be of little consequence with regard to health risk, but this too requires additional research before the importance of standardized sampling can be dismissed.

While there are a number of studies on the occurrence of pathogens in groundwater, the data tend to be biased towards particular geographic regions and pathogen types—for example, in the USA, groundwater pathogen studies are over-represented in the upper Midwest, whereas few studies have been conducted in the southeastern region. Studies have tended to focus on viruses in groundwater, but there is a dearth of data on groundwater contamination by bacterial pathogens such as *Salmonella spp.*, *Campylobacter spp.*, *Listeria*, and pathogenic *E. coli*. Interestingly, colloid filtration theory predicts bacterial pathogens are the optimal size for transport through the subsurface to groundwater (Hunt and Johnson 2017, this issue), and with additional studies more may be learned regarding the existence of predominate pathogens in groundwater bacteria.

Epidemiological studies

As reported in this review, there are too few human health studies to accurately evaluate the burden of illness from pathogen-contaminated groundwater, the kind of data needed to develop effective public health policies. Groundwater-borne disease outbreaks in the USA and Europe are frequently underreported, and measurement of sporadic illness from groundwater exposures, potentially representing a significant fraction of total illness, requires expensive and labor-intensive epidemiological studies. The burden of illness attributable to household private wells and non-disinfected groundwater-supplied public water systems, the relationship between groundwater pathogen levels and disease risk, and whether measurements of faecal indicator organisms in groundwater truly protect public health are all highly uncertain.

Lastly, and most importantly, there is an immediate need for hydrogeologists to contribute their expertise in efforts to minimize pathogen-contaminated groundwater and minimize the risk of groundwater-borne disease. Hydrogeologists possess an inherent understanding of the complex and unpredictable nature of groundwater contamination. In collaboration with microbiologists and epidemiologists, hydrogeologists have an opportunity to help achieve these global public health goals.

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