GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

GIARDIA DUODENALIS

Érica Boarato-David
São Paulo State University
São Paulo, Brazil

Semiramis Guimarães
São Paulo State University
São Paulo, Brazil

Simone Cacciò
Istituto Superiore di Sanità
Rome, Italy

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Summary

Giardia duodenalis (synonyms, *G. lamblia* and *G. intestinalis*) is a flagellated protozoan and the etiological agent of giardiasis, a very common gastrointestinal disease of humans and animals. The simple life cycle of this parasite comprises a vegetative stage, the trophozoite, and a transmittable stage, the cyst. Transmission occurs directly, by contact between hosts, and indirectly, through ingestion of contaminated water and food.

Humans are infected by two of the eight currently recognized genotypes of *G. duodenalis*, namely assemblages A and B, which are likely to represent different species. Of the many factors influencing the epidemiology of giardiasis, three are of utmost importance. First, very large numbers of cysts are shed in the environment with the feces of infected hosts. Second, the infectious dose is low, theoretically, a single cyst may cause infection, and cysts are immediately infectious upon excretion. Third, cysts are remarkably resistant to environmental stress and can persist for months while retaining their infectivity. Consequently, there is a large potential for environmental contamination with *Giardia* cysts. This has been confirmed by surveys of water from all over the world, which have included samples from wastewater, surface water, drinking water, and swimming pools. Perhaps not surprisingly, waterborne outbreaks of giardiasis have occurred in many countries, and *Giardia* is second only to *Cryptosporidium* as a cause of waterborne gastroenteritis caused by protozoan parasites.

This Chapter will first describe the essentials of *Giardia* biology, then will present data on the occurrence and survival of cysts in the environment and in water and wastewater, and finally focus on the different treatment processes and their efficacy in removal and/or inactivation of cysts from water.

Giardia

*Giardia*is an important protozoan enteric pathogen spread by excreta and sewage associated with waterborne disease worldwide.

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

Giardiasis is a very common gastro-intestinal infection of humans; the World Health Organization has estimated that about 200 million people in Asia, Africa, and Latin America have symptomatic infections (WHO, 1996; Yason and Rivera, 2007). The parasite has a global distribution, but the prevalence of infection is higher in developing regions of the world, where *Giardia* is common in both children and adults (Cacciò and Sprong, 2014). In recognition of the burden of disease caused by the parasite, and to underline its link to poverty, the WHO has included giardiasis in the list of neglected diseases since 2004 (Savioli et al., 2006).

Infection rates have been reported in both developing countries (range 8-30%) and industrialized countries (range 1-8%) (Cacciò, 2015). Rates are probably higher in individuals with diarrhea, but our current understanding is limited by the lack of reporting systems and monitoring programs in many countries, and by the high rate of asymptomatic carriage of *Giardia* in humans (Cacciò and Sprong, 2011). This suggests that giardiasis is strongly underdiagnosed and underreported.

1.1.1 Symptomology

Giardiasis infections are associated with watery diarrhea that is often described as foul-smelling. Diarrheal stools may be greasy and intermittent. Individuals may experience abdominal cramps, bloating, gas, nausea, and fatigue. These symptoms last for weeks and could be chronic and recurring. Failure to thrive can occur particularly in young children with weight loss in many.

1.2 Taxonomic Classification of the Agent(s)

Organisms in the genus *Giardia* are classified, based on morphology of the cyst and the trophozite, in the phylum Metamonada, subphylum Trichozoa, superclass Eopharyngia, class Trepomonadea, subclass Diplozoa and order Giardiida (Thompson and Monis, 2012). The taxonomy of *Giardia* species is still controversial and confusing, with different names being used for the same species. Historically, and under the assumption of strict host specificity (i.e., a different species for each host), as many as 51 species of *Giardia* have been described, including 30 from mammals (of which 2 were from humans), 14 from birds, 4 from amphibians, 2 from reptiles and one from fish. The first important taxonomic revision was introduced by Filice, who, after a critical revision of the data, proposed to consider only 3 morphologically distinct groups, namely *Giardia muris*, *G. agilis* and *G. duodenalis*. More recently, advances in microscopic techniques allowed for ultrastructural description of trophozoites, and the recognition of two new species from birds, *Giardia ardeae* (Erlandsen et al., 1990) and *Giardia psittaci*(Erlandsen and Bemrick, 1987). Another species, *Giardia microti*(Feely, 1988), that infects various rodents, was recognized as a separate species due to the unique presence of fully differentiated trophozoites in the cysts. Therefore, six species are currently recognized in the genus, of which *Giardia duodenalis* (also referred to as *Giardia intestinalis* or *Giardia lamblia*) has the largest host range and infects many mammals, including humans (Table 1).

Table 1. Giardia species currently recognized as valid, their host distribution and distinctive morphological characteristics

Species	Host	Distinctive morphologic features	Length / width of the trophozoite (mm)
Giardia agilis	Amphibians	Long and narrow trophozoites with club-shaped median bodies.	20-30 / 4-5
Giardia ardeae	Birds	Rounded trophozoites, prominent notch in ventral disc and rudimentary caudal flagellum. Median bodies round-oval to claw-shaped.	~10 / ~6
Giardia duodenalis	Humans, domestic and wild mammals	Pear-shaped trophozoites with claw-shaped median bodies.	12-15 / 6-8
Giardia microti	Rodents	Trophozoites similar to *G. duodenalis*. Cysts contain fully differentiated trophozoites.	12-15 / 6-8
Giardia muris	Rodents	Rounded trophozoites with small, round median bodies.	9-12 / 5-7
Giardia psittaci	Birds	Pear-shaped trophozoites, with no ventro-lateral flange. Claw-shaped median bodies.	~14 / ~6

With the development of procedures for the propagation of trophozoites in axenic conditions, molecular comparison of *Giardia* strains from different hosts became possible. Investigation of protein (isoenzyme) polymorphism was first used to reveal large amounts of genetic variability among *Giardia duodenalis* strains. Importantly, clustering analysis of the isoenzyme data identified strongly supported groups of genetically related strains that, in most cases, were derived from specific hosts (Monis et al., 1999). These groups are nowadays referred to as Assemblages, to underline the fact that they share genetic similarities but are not identical to each other. The validity of Assemblages has been fully corroborated by DNA sequence data that, after the introduction of amplification techniques (e.g., PCR), were mainly generated from field isolates (i.e., not expanded by *in vitro* growth). On the basis of molecular studies of parasites isolated from many hosts, eight Assemblages (A-H) have been described, among which Assemblages A and B infect humans and other animals, Assemblages C and D are restricted to carnivores, Assemblage E to hoofed animals, Assemblage F to cats, Assemblage G to rats, and Assemblage H to marine mammals (Feng and Xiao, 2011) (Table 2).

Table 2. The currently recognized G. duodenalis Assemblages, their host distribution and proposed taxonomy

Assemblage	Host distribution	Proposed species name
A	Humans and other primates, livestock, dogs, cats, and some species of wild mammals	Giardia duodenalis
B	Humans and other primates, dogs, cats, and some species of wild mammals	Giardia enterica
C	Dogs and other canids	Giardia canis
D	Dogs and other canids	Giardia canis
E	Hoofed livestock	Giardia bovis
F	Cats	Giardia cati
G	Rats	Giardia simondi
H	Marine mammals (pinnipeds)	NR

NR - Not Reported

The genetic differences separating the eight Assemblages are very large, paralleled by phenotypic differences in terms of adaptability to in vitro culture, metabolism, and susceptibility to drugs, and to infection with a double stranded RNA virus (Thompson and Monis, 2012). Finally, comparisons at the whole genome level have reinforced the concept that Assemblages represent distinct species (Adam et al., 2013).

Therefore, it has been proposed (Monis et al., 2009) that the different Assemblages deserve the status of species, and that previously assigned names are available for most Assemblages (Table 2). However, controversial points remain, mostly concerning the names to be given to the human-associated Assemblages A and B (the proposed names are *G. duodenalis* and *G. enterica*, respectively).

1.3 Transmission

Humans acquire infection by ingestion of the cysts via direct contact with feces from other infected persons (anthroponotic transmission), animals (zoonotic transmission), or by ingestion of contaminated food or water (foodborne or waterborne transmission). Dose-response has been well established and used for estimating risk with the ID50 (dose causing 50% infection in those exposed) at 35 cysts. (http://qmrawiki.canr.msu.edu/index.php?title=Table_of_Recommended_Best-Fit_Parameters#tab=Protozoa) (Rendtorff, 1954)

1.3.1 Transmission routes

Human to human transmission via feces is an essential element in the epidemiology of giardiasis. Direct transmission occurs most commonly in environments where hygiene levels are compromised, such as child day-care centres or in disadvantaged community settings. The rising incidence in such settings has led to the designation of giardiasis as a re-emerging infectious disease in the developed world (Savioli et al., 2006).

The issue of zoonotic transmission of giardiasis has dominated the debate for about 30 years, since the WHO first recognized giardiasis as a zoonosis. Pets and livestock, along with some wild animals, have long been considered as the likely reservoirs (Feng and Xiao, 2011). Molecular epidemiologic surveys have demonstrated that host-specific *Giardia duodenalis* Assemblages are more common in their respective hosts than zoonotic Assemblages. Nevertheless, Assemblage A and, more occasionally, Assemblage B have been identified in many animal species (Ryan and Cacciò, 2013). It has been argued that studies in defined endemic areas, where transmission dynamics and host range are known, should provide a better understanding of the significance of animals as reservoirs and conditions where zoonotic transmission can occur (Thompson and Monis, 2012). However, studies in Australia, Peru, India, and Thailand generated controversial results, with some supporting zoonotic transmission (from dogs to humans) while others found no support (Ryan and Cacciò, 2013). Nevertheless, there is a tendency to consider that the zoonotic risk posed by *Giardia* isolates from pets, livestock and wild animals is low, but it cannot be excluded from current data (Ryan and Cacciò, 2013).

Waterborne transmission also plays a very important role in the epidemiology of human giardiasis, worldwide. A recent review reported that, of the 199 global outbreaks caused by protozoa during the period 2004-2010, 70 (35%) were caused by *Giardia*(Baldursson and Karanis, 2011). The majority of these outbreaks were reported from North America, UK, and Asia (Robertson and Lim, 2014); this is likely the result of better detection and monitoring systems rather than epidemiological differences in various parts of the world. Outbreaks can be large, as was the case of the Bergen (Norway) outbreak of 2004, with a total of 1300 laboratory confirmed cases (Nygård et al., 2006). The water treatment plant involved in this outbreak was one of the oldest in Norway and the treatment in place during the outbreak was just chlorination. Leaking sewage pipes contaminating the drinking water supply combined with insufficient water treatment was the likely cause of the outbreak.

In the US, in the period from 1971 to 2011, 242 outbreaks of giardiasis were reported to the Centers for Disease Control and Prevention (Adam et al, 2016). These outbreaks affected ~41,000 persons, and resulted from waterborne (74.8%), foodborne (15.7%), person-to-person (2.5%), and animal contact (1.2%) transmission. Most waterborne outbreaks were associated with drinking water (74.6%), followed by recreational water (18.2%).

Foodborne transmission has been more difficult to prove. Natural contamination with *Giardia* cysts has been demonstrated for fruits (strawberries), vegetables (dill, lettuce, basil, mung bean sprouts and radish sprouts) and shellfish (species of oysters, mussels and clams) (Escobedo et al., 2010; Adell et al. 2014). This finding has public health implications because these products are frequently consumed raw without thermal processing. Voluntary consumption of mud or sand (geophagia), which is particularly common in children and individuals with mental problems, may also contribute to the transmission of *Giardia*(Giacometti et al., 1997). Factors contributing to under-reporting of foodborne outbreaks include the lack of appropriate diagnostic tools and the fact that foodborne outbreaks may be more widespread than waterborne outbreaks, thus appearing sporadic. Most foodborne outbreaks of giardiasis have been ascribed to direct contamination of food by a food handler.

1.3.2 Reservoirs

Giardia duodenalis is able to infect a wide range of hosts (Table 2), mostly mammals. The prevalence of infection is particularly high among pets, with estimates of 10% in well-cared-for dogs, 36–50% in puppies and kittens, and up to 100% in breeding establishments and kennels (Ballweber et al., 2010). In food production animals, prevalence at the farm level varies between 45% and 100%. The cumulative incidence on a farm is 100% in cattle and goats and close to 100% in sheep, implying that every animal on that farm will get infected (Geurden et al., 2010). Some wild animals, such as beaver and nutria, are often

infected with *Giardia* and have been considered important reservoirs of cysts infectious to humans.

Considering the high number of cysts that can be excreted by infected animals (ranging from 10^1 to 10^6cysts/gram), particularly by young animals, they are considered as an important source of environmental contamination. However, the actual role of animals in the transmission of giardiasis to humans remains uncertain (Feng and Xiao, 2011).

1.3.3 Incubation period

The incubation, or pre-patent period, before cysts appear in the faeces, may be short in both humans and other animals, commencing as early as 3 days post-infection, but can range up to 3 weeks depending upon host species Assemblage, infectious dose, host immune responses, and other host factors.

1.3.4 Period of communicability

The duration of infection may vary from a few days to several months. The first appearance of any symptoms usually coincides with the onset of cyst excretion. Cyst excretion is characteristically intermittent in both humans and other animal species, and cysts are resistant to various environmental stressors and thus can survive for at least 2 months in suitable temperature and moisture conditions.

1.3.5 Population susceptibility

In humans, giardiasis is mainly a pediatric infection, with the highest prevalence observed in children aged 1-4 years. This is true for both industrialized and developing countries, and is thought to be due to lower hygiene and higher susceptibility of children (with immature immune system) at the first exposure to the parasite (Cacciò and Sprong, 2014). A secondary peak is observed in adults aged 30-40; in this case, women represent the risk category, likely because of direct transmission of the parasite from children to their mothers. Other risk groups include institutionalized children in developing and developed countries, returning travelers from developing countries, immigrant/refugees and adopted children from endemic areas (Cacciò and Sprong, 2011; Escobedo et al, 2010).

Giardiasis is not considered an opportunistic parasitic infection, because no prolonged, or more severe, symptoms are observed in HIV-infected patients, and therapy is independent of immune status. A prevalence between 1.5% and 17.7% is reported in a few published studies of HIV patients (Stark et al., 2009).

1.4 Population and Individual Control Measures

1.4.1 Hygiene measures

Prevention at the individual level is mainly based on good hygienic practices, such as frequent hand washing with soap and avoiding contact with the feces from infected persons or animals. A recent meta-analysis evaluated the effect of various interventions (hygiene, sanitation, water supply, water quality, and multiple interventions) on the reduction of diarrhoeal diseases in developing countries (Fewtrell et al., 2005). Some of the studies considered focused on *Giardia*. All of the above-mentioned interventions significantly reduced the risks of diarrhea, with a similar degree of impact. Interventions on water quality (e.g., point-of-use water treatment) were found to be more effective. The importance of personal hygiene is further stressed by the role played by infected food handlers in foodborne outbreaks.

A recent study that investigated factors involved in episodes of gastroenteritis in child day-care centres found that disinfecting fomites with chlorine, cleaning vomit with paper towels (and cleaner), daily cleaning of bed linen/toys, formal policies for cohorting and exclusion for ill children and staff, were all protective factors (Enserink et al., 2015). Travelers in countries where giardiasis is endemic should drink bottled water, avoid consuming raw fruits and vegetables, and minimize exposure to untreated water (lakes, rivers and streams). Public health measures are also necessary to protect water supplies against contamination by human or animal feces.

1.4.2 Drug therapy

Although giardiasis is usually a self-limiting disease, treatment of confirmed cases is necessary to cure symptoms and shorten the duration of acute infection, therefore reducing the risk of post-infectious complications and prolonged excretion of infectious cysts. The value of treatment in hyperendemic communities is questionable, due to the high rate of re-infection (Gilman et al., 1988).

Six classes of drugs are approved for treatment of giardiasis, including 5-nitroimidazole and benzimidazole derivatives, quinacrine, furazolidone, nitazoxanide and paromomycin (Lalle, 2010). Treatment is usually the same for both immunocompetent and immunosuppressed patients. Among nitroimidazoles, metronidazole (MTZ; Flagyl) is the most commonly used, with a cure rate of 80-95%. The recommended dose of MTZ is 500 mg every 8 hours for 7-10 days, or 2 g for 3-5 days. MTZ is effective on trophozoites but has no effect on the viability of cysts. Side effects include gastrointestinal upset, headache, nausea, leukopenia, and a metallic taste in the mouth. In about 10-20% of the clinical cases, resistance to MTZ has been reported, with recurrence rates of up to 90%.

Other 5-nitroimidazole drugs are available with less side effects compared to MTZ and are optimized for single daily-dose therapies. Tinidazole (Fasigyn) proved to be 100% successful against *Giardia* in a single oral dosage of 2 g or 50 mg/kg. Secnidazole (Flagentyl) administrated in a single oral dose of 2 mg achieves clinical/parasistological cure rates of 80-100%, whereas ornidazole (Tiberal) is generally administrated as a single dose but for several days and can achieve 100% effectiveness.

In the case of treatment failure of first line drugs, other nitro-drugs can be used. The synthetic nitrofuran furazolidone (Furoxone) is administered as four doses per

day in adults (100 mg per dose) and children (1.5 mg/kg), and a cure rate of 80-96% has been reported for 7-10-day treatment courses. In about 10% of cases, gastrointestinal disturbance, nausea, vomiting, diarrhoea, malaise, itch, urticaria, hypersensitivity, and haemolysis in glucose-6-phosphate dehydrogenase-deficient patients, have been reported.

Nitazoxamide (Alinia or Annita) is a synthetic nitrothiazolyl-salicylamide derivative discovered in the 1980s as an anti-helminthic and later found to be active against *Giardia* and other intestinal protozoa. The drug is approved in the US for the treatment of both cryptosporidiosis and giardiasis in immunocompetent individuals. The recommended dose is 500 mg twice a day for 3 days, with an 85% clinical cure and 71–80% parasite eradication observed in patients. This drug is also well tolerated with few gastrointestinal side effects.

Benzimidazoles such as the benzimidazole carbamate compound albendazole, fenbendazole and mebendazole, exert a good anti-giardial efficacy along with anti-helmintic activity. Treatment of patients with albendazole is less efficacious than treatment with MTZ, with a 62 to 95% average efficacy of albendazole compared to 97% for MTZ. Side effects for benzimidazoles are similar, but less intense, to those reported for MTZ.

In cases of seriously refractory giardiasis, acridine yellow quinacrine can be used. The drug was first used as an antimalarial but have been found to also be effective against *Giardia*, with a reported cure rate of 92-95%. However, quinacrine can cause serious side effects including dizziness, headache, vomiting, psychotic response, reproductive tract cancers, development of abnormal uterine lesions, ectopic pregnancy, prolonged amenorrhea, and fetal exposure.

During pregnancy, paromomycin is considered the safest drug to treat giardiasis, especially during the first trimester. Paromomycin sulfate is a broad-spectrum aminoglycoside antibiotic with a 60-70% efficacy against giardiasis. Since paromomycin is poorly absorbed after oral administration, the drug does not reach the fetus. Nausea, increased gastrointestinal motility, abdominal pain, and diarrhea are the most common side effects, and like other aminoglycosides, systemic absorption of this antibiotic may cause ototoxicity and nephrotoxicity.

2.0 Environmental Occurrence and Persistence

Technological advances for detection and surveillance of waterborne pathogens have allowed for a better understanding of their global occurrence. *Giardia* cysts have been recovered from several environmental matrices, including watersheds used as a source of drinking water or for recreational activities, in both high and low income areas. In addition to water, cysts have been detected in other samples such as sewage, sludge, and soil. All types of ambient water bodies are vulnerable to contamination by *Giardia* through either land runoff or discharge of wastewater and sewage. The high numbers of infective cysts excreted in feces of a wide range of hosts and their ability to survive in the environment clearly increase dispersion and transmission. Many countries have legislation or follow guidelines for source water quality and environmental protection, yet *Giardia* continues to be one of the major causes of waterborne disease outbreaks. Thus, a better knowledge on environmental contamination by cysts including occurrence, persistence, and survival data would enhance our understanding of the epidemiology and help to establish public health priorities and preventive measures.

2.1 Detection Methods

Detection of *Giardia* cysts in the environment, particularly in aquatic systems, requires sensitive and specific methods. Efficient detection is complicated by many interfering factors, including the small size and dispersion of cysts in the environment and the presence of high amounts of interfering extraneous particulate materials (Carmena, 2010). A universally adopted method is not available, but the United States Environmental Protection Agency (USEPA 1623) (USEPA, 2005) has been widely used in different studies and became a standard for the simultaneous detection, identification, and enumeration of *Giardia* cysts and *Cryptosporidium* oocysts in environmental samples.

USEPA 1623 method is based on the following key steps: (1) filtration, usually of volumes between 10 and 1000 L; (2) concentration; (3) immunomagnetic separation (IMS) using magnetizable beads coated with specific antibodies; and (4) detection/enumeration by immunofluorescence assay (IFA) by epi-fluorescence microscopy (Skotarczak et al., 2009; Ramirez-Castillo et al., 2015). Since its introduction in 1996, a number of revisions have been proposed (USEPA Method 1623.1 EPA 816-R-12-001; Rhodes et al., 2012), particularly with regard to sample filtration and immunomagnetic separation, and different kinds of filters and IMS kits have been tested and validated for use (Skotarczak et al., 2009).

Despite various limitations, such as high cost, low recovery efficiency and the necessity of well-trained personnel, the IMS/IFA approach is widely used to detect *Giardia* cysts in water and other environmental samples. A number of factors, including the filtration system and the physicochemical and organic properties of the sample, such as turbidity, pH, inorganic compounds and suspended algae, are known to influence the recovery efficiency (Carmena, 2010). Alternative techniques have been proposed, including fluorescently activated cell sorting (FACS), which has high sensitivity and specificity, being also amenable to high throughput. However, high cost, difficult-to-operate cell sorters and challenges in processing turbid samples and have prevented its routine application (Health Canada, 2012).

Protocols for routine detection of *Giardia* in the environmental samples, including the USEPA 1623 method, are microscopy-based. Therefore, their use do not consent to determine the species and genotypes of the recovered cysts, which is necessary to infer their likely origin (human-derived versus animal-derived) and generate data useful to

track the source of pollution/contamination in environmental samples (Skotarczak et al., 2009; Ramirez-Castillo et al., 2015). The accurate identification and characterization of *Giardia* cysts can be obtained using molecular methods, of which those based on the Polymerase Chain Reaction (PCR) are the most widely used. A variety of PCR assays have been described, including conventional PCR, reverse transcriptase PCR (RT-PCR), real-time PCR (qPCR) and multiplex-PCR (mPCR). Depending on the PCR assays used above, they can amenable for indirect detection of viable cysts (RT-PCR), for quantitative evaluation of cyst number (qPCR), and for simultaneous detection of *Giardia* and other organisms in sample (qPCR and mPCR). These methods usually have high sensitivity and specificity, but their performance on environmental samples is influenced by the presence of inhibitors that can suppress or reduce the amplification efficiencies; thus, strategies to remove or minimize PCR inhibitors during nucleic acid preparation steps are essential to ensure optimal method performance (Skotarczak et al., 2009). Other molecular methods used for cyst detection are loop-mediated isothermal amplification (LAMP) and fluorescence *in situ* hybridization (FISH). LAMP is less affected by the presence of inhibitors, does not require thermal cyclers and has a low cost, whereas FISH allows enumeration and an indirect assessment of cyst viability.

2.2 Data on Occurrence

2.2.1 Raw sewage and sludge

Investigations of raw sewage (influent) and outgoing wastewater (treated/final effluent) have provided information on the presence of pathogens in a given population and on pathogen reduction resulting from treatment plant operation, respectively. A number of surveys have shown the frequent and ubiquitous presence of *Giardia* cysts in raw sewage influents and effluents samples from wastewater treatment plants (Table 3).

Table 3. Occurrence of Giardia cysts in raw sewage influent and treated effluent samples from wastewater treatment plants

Area	Sample Type	Percent Positive (# of samples) See footnotes a, b, c for method used	Concentration Average (range), Cysts/L	Reference
Raw Sewage Influents				
Brazil	Influent	90.5%[c] (48/53)	1.0E+05 (Mean)	Neto et al., 2006
Brazil	Influent	100.0%[a] (24/24)	30 to 1.9E+04	Hachich et al., 2013
Canada	Influent	95.0%[a] (18/19)	9.0E+03 (max)	Lalancette et al., 2012
China	Influent	100.0%[a] (33/33)	1.3E+02 to 3.6E+03	Fu et al., 2010
Germany	Influent	88.0%[a] (120/155)	50 to 7.5E+03	Ajonina et al., 2013
Greece	Influent	18.9%[d] (7/37)	NR	Spanakos et al., 2015
Italy	Influent	100.0%[c,e] (16/16)	2.1E+03 to 4.2E+04	Cacciò et al., 2003
Ivory Coast	Influent	83.0% (20/24)	1.5E+01 (Mean)	Yapo et al., 2014
Japan	Influent	100.0%[a] (24/24)	3.7E+02 to 3.9E+03	Oda et al., 2005
Malaysia	Influent	100.0%[b] (24/24)	4 to 8.5E+03	Lim et al., 2007
Norway	Influent	93.0%[a,e] (37/40)	1.0E+02 to 1.4E+04	Robertson et al., 2006
South Africa	Influent	82.0%[a,e] (46/56)	1 to 4.5E+03	Dungeni & Momba, 2010
South Africa	Influent	31.6%[a,e] (25/79)	NR	Samie et al., 2014
Spain	Influent	91.0%[b] (10/11)	9.8 to 1.8e+03	Gomez-Couso et al., 2005

Area	Sample Type	Percent Positive (# of samples) See footnotes a, b, c for method used	Concentration Average (range), Cysts/L	Reference
Spain	Influent	98.0%[a] (49/50)	2 to 1.4E+04	Castro-Hermida et al., 2010
Spain	Influent	100%[d] (26/26)	67	Reinoso et al., 2011
Spain	Influent	100.0%[b] (13/13)	NR	Rodriguez-Manzano et al., 2012
Tunisia	Influent	80.0%[a,g] (6/7)	6.6E+01 to 3.2E+02 .	Khouja et al., 2010
USA	Influent	55.3%[a,e] (131/237)	NR	Sulaiman et al., 2004
USA	Influent	100.0%[a] (30/30)	5.0E+02 (Max)	Harwood et al., 2005
USA	Influent	100.0%[a] (23/24)	1.4E+03 to 1.5E+04	Kitajima et al., 2014
Treated Sewage Effluents				
Brazil	Activated Sludge Effluent	95.6%[c] (87/91)	1.1E+03 (Mean)	Neto et al., 2006
Brazil	Activated Sludge Effluent	79.2%[a] (19/24)	5.0E-02 to 1.1E+02	Hachich et al., 2013
Bulgaria	Effluent	42.9%[b] (3/7)	11.4E+02 to 6.0E+2	Karanis et al., 2006
Canada	Activated Sludge Effluent	61.0%[a] (11/18)	4.7E+02 (Max)	Lalancette et al., 2012
China	Activated Sludge Effluent	100.0%[a] (33/33)	0 to 2.0E+03	Fu et al., 2010
China	anaerobic-anoxic-oxic (AAO) process	96.0%[a,f] (48/50)	0 to 49	Ma et al., 2016
China	Membrane bioreactor	100.0%[a,e] (12/12)	3 to 95	Zhang et al., 2015
Germany	Activated Sludge Effluent	88.0%[a] (120/155)	30 to 3.2E+03	Ajonina et al., 2013
Greece	Activated Sludge Effluent	5.6%[d] (2/36)	NR	Spanakos et al., 2015
Malaysia	Extended Aeration Aerated Lagoon Effluent	62.5%[b] (15/24)	1 to 1.5E+03	Lim et al., 2007
Norway	Activated Sludge Effluent	74.0%[a] (53/72)	1.0E+02 to 5.1E+04	Robertson et al., 2006
Poland	Activated Sludge Effluent	84.6% (11/13)	0.7 to 66	Sroka et al., 2013
South Africa	Activated Sludge Effluent	67.9%[a,e] (38/56)	1 to 1.7E+02	Dungeni & Momba, 2010
South Africa	Activated Sludge Effluent	31.6%[a,e] (25/79)	NR	Samie et al., 2014
Spain	Activated Sludge Effluent	87.5%[b] (14/16)	7 to 2.5E+03	Gomez-Couso et al., 2005
Spain	Undescribed	96.0%[a] (48/50)	2 to 6.0E+03	Castro-Hermida et al., 2010
Spain	Waste Stabilization ponds Anaerobic, facultative & maturations	73.0%[d] (19/26)	8.1 2.4 & 0.7 (means)	Reinoso et al., 2011
Spain	Activated Sludge Effluent	83.3%[b] (10/12)	0.4 to 13.2	Rodriguez-Manzano et al., 2012

Area	Sample Type	Percent Positive (# of samples) See footnotes a, b, c for method used	Concentration Average (range), Cysts/L	Reference
USA	Activated Sludge Effluent	80.0%	NR	Harwood et al., 2005
USA	Undescribed	96.0%[a] (23/24)	<0.4 to 6.2E+02	Kitajima et al., 2014

NR - Not Reported

Methods used [a]IMS-IFA, [b] IFA, [c] filtration and IFA, [d] flocculation and IFA

G. duodenalis [e] Assemblages A and B, [f]Assemblage AII, [g]Assemblages AI, AII and B

High prevalence rates of *Giardia* in raw wastewater were reported in both developed and developing countries. Surveys undertaken in North America (Harwood et al., 2005; Kitajima et al., 2014), Europe (Cacciò et al., 2003; Reinoso et al., 2011; Rodriguez-Manzano et al., 2012), Asia (Oda et al., 2005; Lim et al., 2007; Fu et al., 2010; Ma et al., 2016) and South America (Hachich et al., 2013) reported the presence of cysts in up to 100% of the raw sewage influent samples tested. The number of cysts /L of raw sewage was found to vary from hundreds to thousands. For example, very high numbers were reported in Italy (Cacciò et al., 2003) and in the United States (Kitajima et al., 2014) where cysts were detected at concentrations ranging, from $2.1x10^3$ to $4.2 x 10^4$ cysts/L and from $1.4x10^3$ to $1.5x10^4$ cysts/L, respectively. Similarly, in Norway (Robertson et al., 2006) and Spain (Castro-Hermida et al., 2010), high numbers of cysts were found in raw sewage influent samples that contained from 10^2 to $1.36x10^4$cysts/L and 2 to $1.44x10^4$cysts/L. In Brazil, cysts were detected at concentrations as high as 10^5cyst/L (Neto et al., 2006; Hachich et al., 2013). In contrast, relatively few studies have reported cysts at concentrations not exceeding 100 cysts/L (Reinoso et al., 2011; Yapo et al., 2014; Zhang et al., 2015). These data underscore the variability in cyst concentration in influent samples, which is likely influenced by the source of wastewater (e.g. domestic/agricultural), prevalence, intensity and duration of infection in the contributing population, population size, per capita water consumption, and cyst survival (Stott, 2013). In addition, cysts can be found across the whole year in influent samples, but, occasionally, seasonal variations have also been reported (Cacciò et al., 2003; Oda et al., 2005; Ajonina et al., 2013). Analysis of raw sewage for the presence of cysts has been suggested as a way for surveillance of *Giardia* infection in a community and to detect possible outbreaks (Jakubowski et al., 1991).

In many countries, the final product of a wastewater treatment plant, i.e. treated effluent, is discharged into receiving streams, rivers, or even ocean. Furthermore, the effluent may be used as reclaimed water for irrigation or potable reuse, as is the case in the USA and Africa (Dahl, 2014; Lahnsteiner and Lempert, 2007). Thus, a major goal of wastewater effluent management is to reduce pathogen load and avoid, or diminish, health risk (Harwood et al., 2005). Effluents from treatment plants often contain *Giardia* cysts, with prevalence between <10 and 100% (Table 3). In general, cyst concentration does not exceed $7.0x10^2$cysts/L, but can be as high as $6.0x10^3$cysts/L. In contrast, in a Norwegian survey, some sewage effluent samples showed cyst numbers greater than in the influent samples collected on the same day (Robertson et al., 2006). The authors suggested this was an artifact that may depend on (i) higher recovery of cyst from (cleaner) effluent samples, (ii) temporal fluctuations in parasite concentrations, (iii) lack of pairing the samples, (iv) uneven distribution of cysts in the samples, or a combination of these factors. In general, *Giardia* cysts have been typically detected in lower numbers in treated effluent than in influent samples. Nevertheless, detection of very low numbers, or no detection, of cysts in wastewaters does not guarantee that effluents are free of parasites, since enumeration and detection methods never allow full recovery or provide infective status of the cysts (Stott, 2013).

In sewage treatment plants, sewage sludge, also referred to as biosolids, is a by-product generated at various stages within the treatment process, in order to remove pollutants before discharging the treated effluent into the environment. This product is a mixture of organic and inorganic compounds, which makes it a valuable, cost effective and sustainable source of nutrients and organic matter that is widely used in agriculture and pasture as fertilizer/soil conditioner (Usman et al., 2012). However, a particular disadvantage and concern of land application of sewage sludge is related to its pollutant load, including a wide range of microbial pathogens (Usman et al., 2012). Thus, pathogen loads in sludge must also be reduced before application to land by appropriate stabilization and hygienization procedures. Unfortunately, sludge treatment is not available at all treatment works, especially smaller plants in developing regions, and thus can be an important issue when considering the risks to human health arising from its use.

Studies conducted in wastewater treatment plants for pathogen detection have demonstrated the presence of *Giardia* cysts in sewage sludge and its derivatives after various treatment methods (Table 4).

Table 4. Occurrence of Giardia cysts in sludge.

Area	Sample Type	Percent Positive (# of samples)	Concentration Average (range)	Reference
Brazil	Activated sludge	100.0%[a] (8/8)	4E+04 to 1.2 E+06 Cysts/L	Santos et al., 2004
Brazil	Activated sludge	100.0%[a] (22/22)	4E+04 to 4.4E+05 Cysts/L	Rondello Bonatti et al., 2007
Finland	Raw and activated sludge after lime stabilization	20.0%[b] (9/44)	NR	Rimhanen-Finne et al., 2001
Finland	Compost samples (10-week-old), and end products	44.0% and 35.0%[a]	0.6 to 5 Cysts/g and 0.5 to 2.8 Cysts/g	Rimhanen-Finne et al., 2004
Ireland	4 urban facilities Activated sludge and end products sewage sludge cake	NR[a,c]	5.4E+02 to 1.54E+03 Cysts/L and 3.0E-03 to 0.114 Cysts/g	Graczyk et al., 2007
Poland	Sewage sludge	100.0%[a,c,d] (6/6)	22 to 32 Cysts/g	Graczyk et al., 2008
Spain	Sludge from stabilisation ponds anaerobic and facultative ponds	NR[a,d]	6.8 E+02 and 12.6 Cysts/g dry weight	Reinoso et al., 2011
Tunisia	Sewage sludge	80.0%[a,b,e,f] (4/5)	2.4 Cysts/g	Khouja et al., 2010
Tunisia	Dry, dehydrated sludge	25.0%[b,g] (3/12)	NR	Ben Ayed et al., 2012
USA	Sewage Sludge Class B biosolids from 9 facilities five used anaerobic digestion and three used aerobic digestion	97.0% (33/34)	1.2 E+04 (± 1. E+04 SD) Avg Cysts/g dry weight	Rhodes et al., 2015

NR - Not Reported

[a]IFA, [b]PCR, [c]Fish, [d]Sucrose flotation, [e]IMS

As shown in some surveys, a widely used conventional wastewater treatment practice, such as the activated sludge digestion procedure, can itself be a source of environmental cysts (Santos et al., 2004; Rondello Bonatti

et al., 2007; Graczyk et al., 2007a; Graczyk et al., 2008). High concentrations of *Giardia* cysts were reported in two studies carried out in Brazil (Santos et al., 2004; Rondello Bonatti et al., 2007) in which cysts were detected in 100% of the activated sludge samples with concentrations ranging from $4.0x10^4$ to $1.2x10^6$cysts/L. These high concentrations reinforce the necessity of contaminant evaluation before its final use, especially in developing countries where improvements toward a safer use of sludge for land applications are hampered by poor or lack of appropriate legislation. A survey in Ireland showed cysts in activated sludge at levels ranging from $5.4x10^2$ to $1.1x10^3$cysts/L, while in its corresponding end-products (sewage sludge cake) lower concentrations between 3 and $1.14x10^2$cysts/g were detected (Graczyk et al., 2007a). A significant reduction of cyst load associated with the treated activated sludge was demonstrated however viable cysts were still detected. It is noteworthy that the observed cyst concentration is of public health concern, considering the low infectious dose. Therefore, even after treatment, sludge might still present a risk. Besides activated sludge, *Giardia* cysts have also been detected in sludge from stabilization ponds and from treatment based on composting procedures (Rimhanen-Finne et al., 2004; Reinoso et al., 2011). In Spain, cysts were found both in aerobic and facultative ponds with concentrations of $6.8x10^2$ and 12.6 cysts/g, respectively (Reinoso et al., 2010), with anaerobic ponds showing better treatment process for removing cysts. In Finland, low numbers of cysts were detected in 44 and 35% of the sludge samples obtained from sludge that have been composting for 10 weeks and in end-products (30 weeks of composting or after stabilization by quicklime and peat addition), respectively (Rimhanen-Finne et al., 2004).

Very few studies have been carried out to genetically characterize the cysts found in wastewater and sludge samples. In treatment plant influents and effluents that have been surveyed, Assemblages A and B, both of which are known to infect humans, were found (Cacciò et al., 2003; Sulaiman et al., 2004; Robertson et al., 2006; Dungeni and Momba, 2010; Khouja et al., 2010; Samie and Ntekele, 2014; Zhang et al., 2015). Taken together, the predominance of Assemblage A was noted. Not surprisingly, recent data on molecular characterization of *Giardia* isolates recovered from sewage sludge have revealed the presence of Assemblages and subtypes that are more frequently associated with human infections (Khouja et al., 2010; Ben Ayed et al., 2012).

In both developed and developing countries, municipal wastewater and its by-products are frequently applied to land. However, in developed countries where regulatory standards are applied, much of the wastewater is properly treated prior to use. In contrast, in developing countries, untreated raw wastewater is often used, mainly for crop irrigation (Pachepsky et al., 2011). In these countries, higher levels of pathogens in irrigation waters have been reported (Thurston-Enriquez et al., 2002). In this study, the occurrence of human pathogenic parasites was investigated in irrigation waters used for food crops in the United States and in some Central American countries, and the average concentrations of *Giardia* in samples were 5.59 cysts/L and 0.25 cyst/L, respectively.

Despite these observations, to date, there is still no clear evidence that infection with *Giardia* can be associated with wastewater use in agriculture. However, it has been suggested that exposure to wastewater with high *Giardia*concentrations carries an increased risk for infection among exposed farm workers and their families and consumers of crops that are normally eaten uncooked (Ensink et al., 2006). The study found a significantly increased risk of (asymptomatic) *Giardia*infection in wastewater farming households in Pakistan when compared with farming households using regular (non-wastewater) irrigation water. Even though irrigation with reclaimed water decreases food safety and poses a potential source of pathogens in foodborne outbreaks, there is still no clear association with illnesses to the use of wastewater for crop irrigation. Nowadays, considering that the concept of a sustainable world is becoming more and more common, the reuse of wastewater fulfills the aim to reduce environmental pollution and to recycle the organic matter of wastes in soil. Irrigation with raw wastewater is increasing in many areas, particularly in developing countries, where there is parallel increase in urban growth and urban food demands. Thus the systematic treatment of wastewater in communities with growing populations for safe reuse is often problematic. The increasing availability and use of wastewater will generate additional challenges for regulatory agencies charged with minimizing potential impacts on public health and the environment (Qadir et al., 2010).

2.2.2 Surface water - including recreational water

Among natural water resources, the risk of *Giardia* contamination is greater from surface waters. Surveys have demonstrated the presence of cysts in rivers, lakes, streams, reservoirs, canals, and others matrices used as a source of drinking water or for recreational activities (Table 5). Although most surveys have been conducted in developed countries, recent studies reported cysts detection in surface water samples in South America and Asia. During the last two decades, widespread occurrences of cysts in surface water samples have been demonstrated, with prevalence ranging from 12% (Robertson & Gjerde, 2001) to almost 100% (Anceno et al., 2007; Lim et al., 2008; Mahmoudi et al., 2013), and, in most cases, values greater than 50%.

Table 5. Occurrence of Giardia cysts in surface water, including recreational water

Area	Sample Type	Percent Positive (# of samples)	Concentration Average (range), Cysts/L	Reference
Belgium	Raw water in catchment sites	35.0%[a,d] (53/151)	35 (Max)	Ehsan et al., 2015a
Belgium	Swimming pools, lakes and splash parks	15.2%[a,d] (15/99)	3.0E-02 to 0.7	Ehsan et al., 2015[b]
Brazil	Raw water treatment plant	20.0%[a] (3/15)	0.2 (Mean)	Nishi et al., 2009
Brazil	Raw water Watershed catchments	46.1%[a] (6/13)	3.4 (Max)	Razzolini et al., 2010
Brazil	Source water intake of treatment plant	49.5%[a] (102/206)	0.1 to 97	Sato et al., 2013
Bulgaria	Rivers and lakes	8.5%[b] (4/47)	72 to 1.7E+02	Karanis et al., 2006
Canada	Streams	46.0%[b] (6/13)	5.0 E-02 to 0.6	Budu-Amoako et al., 2012
Canada	Rivers and source water intake of treatment plant	44.0%[a] (107/244)	0.1 to 97	Edge et al., 2013
Canada	Rivers	86.0%[a,e] (98/114)	38 (Max)	Prystajecky et al., 2014
China	Source water intake of treatment plant	18.0%[a] (9/50)	0.1 to 8	Feng et al., 2011
China	Lake	65.0%[a,c,e] (37/61)	3.23 (Max)	Xiao et al., 2013
France	River	67.0%[a] (38/57)	0.1 to 16.5	Coupe et al., 2006
France	Rivers	93.8%[a] (152/162)	5.0 E-02 to 51.2	Mons et al., 2009
France	River	88.0%[a] (21/24)	<2 E-02 to2.8	Jacob et al., 2015
Germany	Stream	4.0% (1/23)	0.67 (Max)	Gallas-Lindemann et al., 2013
Germany	River and tributaries	90.0% (27/30)	1.0E-02 to 1.32	Kistemann et al., 2012
Hungary	Raw water from treatment plants	48.4%[a] (15/34)	5.0 E-02 to 10.3	Plutzer et al., 2007
Hungary	Rivers and lakes	62.5%[a,f] (10/16)	0.1 to 8.5	Plutzer et al., 2008

Area	Sample Type	Percent Positive (# of samples)	Concentration Average (range), Cysts/L	Reference
Italy	River and water coúrses in treatment facilities	71.0% (15/21)	1E-02 TO 80	Briancesco and Bonadonna, 2005
Italy	River	57.0% (4/7)	1E-02 to 0.08	Vernile et al., 2009
Italy	Swimming pools	38.0% (8/21)	NR	Oliveri et al., 2006
Iran	River	75.0% (15/20)	3.2E+04 (Max)	Mahmoudi et al., 2013
Japan	River	92.0% (12/13)	4.0 E-02 to 0.58	Haramoto et al., 2012
Japan	Rivers	36.0%[a] (23/6423)	0.5 to 3.8	Haramoto et al., 2012
Malaysia	Rivers	39.0% (68/74)	0.7 to 1.3E+04	Lim et al., 2008
Malaysia	Rivers	30.0%[a] (18/60)	0.1 to 12	Azman et al., 2009
Malaysia	Lake	77.8%[a,h] (7/9)	0.17 to 1.1	Lim et al., 2009
Mexico	River	48.0%[a] (25/58)	0.17 to 16.33	Chaidez et al., 2005
Netherlands	Canals and rivers	97.0%[a] (31/32)	0.1 to 16.7	Schets et al., 2008
Netherlands	Lakes	36.4%[a] (20/55)	0.2 to 1.1	Schets et al., 2008
Norway	Rivers and lakes	11.8%[a] (48/408)	0.1 to 0.3	Robertson and Gjerde, 2001
Philippines	Rivers, ponds and natural lakes	75.0%[a] (15/20)	0.1 to 74.4	Onichandran et al., 2014
Poland	Rivers, lakes	60.6%[a] (20/33)	1.0E-03 to 0.28	Bajer et al., 2012
Portugal	Raw water treatment plants	58.0%[a,g] (40/69)	NR	Lobo et al., 2009
Portugal	River beaches	85.0%[a] (63/74)	3.27E+02	Júlio et al., 2012
Russia	Source water intake of treatment plant	26.0%[a] (26/87)	2.0 E-02 (Mean)	Egorov et al., 2002
Russia	Rivers and lakes	20.0%[b] (3/15)	7 to 3.7E+02	Karanis et al., 2006
Spain	River	85.7%[b] (6/7)	0.4 to 29.3	Gomez-Couso et al., 2005
Spain	Rivers and reservoirs	77.3%[a] (68/88)	2.5E+02 (Mean)	Carmena et al., 2007
Spain	River	67.0%[a,d] (78/116)	2 to 7.22E+02	Castro-Hermida et al., 2009
Switzerland	Streams	97.5%[a] (39/40)	5.0 E-02 to 10.8	Wicki et al., 2009

Area	Sample Type	Percent Positive (# of	Concentration Average (range), Cysts/L	Reference
Taiwan	Small water systems	46.2%[a] (12/26)	2.9E-03 to 10.3	Hsu et al., 2001
Thailand	Canals	98.3%[c,f] (118/120)	3.2E+04 (Max)	Anceno et al., 2007
USA	River	24.5%[a] (47/192)	1.0E-03 to 5.8E-02	Ryu et al., 2008
USA	Swimming pools	6.9%[c] (11/160)	NR	Shields et al., 2008

NR - Not Reported

[a]IMS-IFA, [b]IFA, [c]PCR; *G. duodenalis* [d]Assemblages A (AI and AII), B and E, [e] Assemblages A, B and E, [f]Assemblages A and B,

[g]Assemblage A(AI), [h] Assemblages A

Cyst concentration in surface waters is usually low or moderate, ranging from <1 to $2.5x10^2$cysts/L. Low concentrations (<1 to 50 cysts/L) are often reported in the United States and in some European countries, although significantly high number of cysts (from 2 to $7.22x10^2$cysts/L) were detected in river samples from Spain (Castro-Hermida et al., 2009). In developing countries, higher concentrations are more common (up to $3.24x10^4$cysts/L), as represented by studies of water from canals in Thailand (Anceno et al., 2007), and rivers in Malaysia (Lim et al., 2008), and Iran (Mahmoudi et al., 2013).

Furthermore, *Giardia* cysts have been detected in raw water samples from treatment plants, at levels ranging from <1 to 97 cysts/L. This includes studies in Taiwan (Hsu et al., 2001), Russia (Egorov et al., 2002), Italy (Briancesco & Bonadonna, 2005), Portugal (Lobo et al., 2009), and Brazil (Nishi et al., 2009; Razzolini et al., 2010, Sato et al., 2013).

Molecular methods have not been widely used to identify the *Giardia* species and genotypes present in contaminated water samples. Based on PCR, the presence of Assemblages AI, AII, B and E was demonstrated in surface waters (Anceno et al., 2007; Plutzer et al., 2008; Castro-Hermida et al., 2009; Lobo et al., 2009; Xiao et al., 2013; Prystajecky et al., 2014, Ehsan et al., 2015a), supporting the fact that both human and animal feces contribute to contaminating these waters.

Taken together, environmental data highlight the importance of establishing adequate protection of surface water worldwide, because most people are supplied by systems that use surface water. Indeed, the lack of, or inadequate, treatment of water sources is the most common deficiency reported from investigations of waterborne giardiasis outbreaks linked to drinking water (Karanis et al., 2007).

Giardia cysts have been found in natural recreational freshwaters such as lakes, rivers and streams (Table 5). In Europe, 15-98% of samples from rivers and lakes used for bathing or sporting activities contained *Giardia* cysts (Schets et al., 2008; Wicki et al., 2009; Júlio et al., 2012). Cyst concentration ranged from <1 to $3.27x10^2$cyst/L, with the highest level recorded during a very rainy season at a river beach in Portugal, which was close to agricultural lands, livestock farms, and wastewater discharges (Júlio et al., 2012). In developing countries, 30-78% of samples from recreational lakes and rivers in Malaysia contained *Giardia* cysts at concentrations ranging from <1 to 12 cyst/L (Azman et al., 2009; Lim et al., 2009). Low cyst concentrations (<1 cyst/L) were found in samples from recreational waters facilities, such as public swimming pools and water parks, in Italy, the United States, and Belgium (Oliveri et al., 2006; Shield et al., 2008; Ehsan et al., 2015b). The detection of cysts in filter backwash samples of swimming pools in USA and Belgium indicated a need to improve pool operation and maintenance (Shield et al., 2008; Ehsan et al., 2015).

Contamination of natural recreational fresh and marine waters is usually due to urban and non-urban runoff, storm waters, and human or animal wastes. Contamination of swimming pools is typically associated with accidental fecal contamination, particularly from toddlers in wading pools, but can also be caused by poorly constructed and/or maintained plumbing and disinfection processes (Karanis et al., 2006). Molecular analyses have identified *Giardia duodenalis* Assemblage A in samples from lakes in Malaysia (Lim et al., 2008), and Assemblages AI, AII, BIII and E in samples from lakes in Belgium (Ehsan et al., 2015), again indicating that both human and animal feces contribute to contamination.

2.2.3 Ground waters

A considerable number of people are supplied with ground water (springs, wells, and boreholes). Groundwater is supplied by waterworks or from single-household wells in some communities (Guzman-Herrador et al., 2015). For example, in Europe, ground water contributes to >99% of the daily water supply in Denmark, 85% in Sweden, 41% in Finland, and 39% in Norway (Guzman-Herrador et al., 2015). The way this water is provided for human consumption also differs among countries. In the United States, an estimated 114 million people receive their drinking water from public water systems that use a ground water source, whereas approximately 20 million people drink untreated water from public ground water systems (Wallender et al., 2014). In Nordic regions, ground water is usually not disinfected (Guzman-Herrador et al., 2015).

Studies on contamination of ground water with *Giardia* cysts are scarce; the available data are presented in Table 6. A study in Bulgaria found *Giardia* cysts in 11% of water samples from wells and springs, with concentrations ranging from 0.016 to $1.28x10^2$cysts/L (Karanis et al., 2006). In Germany, cysts were detected in only one of 66 wells, with a low level of contamination (< 0.1 cyst/L) (Gallas-Lindemann et al., 2013). In Spain, cysts were detected in 44.4% of boreholes and springs with concentrations ranging from 2 to 12 cysts/L, and were characterized at the molecular level as Assemblages AI and E, suggesting hoofed animals as the main contamination source (Castro-Hermida et al., 2015). Studies in Malaysia and Nepal found cysts in 17.9% and 22% of the wells tested, respectively, with higher cyst numbers in Malaysia (1.1 to 2.2 cysts/L) (Lim et al., 2008, Haramoto et al., 2011). Interestingly, a study in an important tourist city in Brazil found *Giardia* cysts in 25% of the samples taken from springs that is used for consumption by the local population and tourists, albeit contamination levels were low (0.07 to 0.1 cysts/L) this water is (Branco et al., 2012).

Table 6. Occurrence of Giardia cysts in ground water

Area	Sample Type	Percent Positive (# of samples)	Concentration Average (range), Cysts/L	Reference
Brazil	Springs	25.0% (3/12)	0.07 to 0.1	Branco et al., 2012
Bulgaria	Wells	11.1%[a] (2/18)	1.6E-02 to 1.3E+02	Karanis et al., 2006
Germany	Radial and vertical wells	1.5%[c] (1/66)	0 to 0.5	Gallas-Lindemann et al., 2013
Malaysia	Wells	17.9% (6/28)	0 to 0.25	Lim et al., 2008
Nepal	Shallow wells	22.0%[b] (2/9)	1.1 to 2.2	Haramoto et al., 2012
Spain	Boreholes	44.4%[b,d] (12/27)	2 to 12	Castro-Hermida et al., 2015

[a]IFA, [b]IMS-IFA, [c]sucrose centrifugation and IFA

[d]*G. duodenalis* Assemblages found A (AI) and E

Groundwater can pose a significant human health risk, particularly if acquired from poorly constructed or maintained wells, close proximity to a contaminant source, such as septic system or surface water source, contamination by animal feces or manure piles, or if locationed at vulnerable hydrogeological formations (Wallender et al., 2014; Painter et al, 2015). Recently a review of all drinking water outbreaks associated to untreated groundwater that occurred in the United States during 1971 to 2008 revealed that among the 172 outbreaks with contributing factor data available, the leading contamination sources included human sewage (33.1%), animal contamination (9.3%), and contamination entering via the distribution system (7.0%) (Wallender et al., 2014).

2.2.4 Drinking waters

Cysts have been detected in final treated drinking water from water works in different countries (Table 7). In general, surveys have reported prevalence rates between 6.5 and 46% and concentrations typically lesser than one cyst /L. However, higher concentrations were noticed in surveys carried out in Galicia, Spain (Castro-Hermida et al., 2008; 2010; 2015). In these surveys, cysts were detected both in conventional treatment plants and in small treatment facilities, at levels ranging from 0.5 to 18 cysts/L and mean concentrations of 0.5-4.0 cysts/L (Castro-Hermida et al., 2008), 1.0-1.5 cysts/L (Castro-Hermida et al., 2010) and 2.8-3.3 cysts/L (Castro-Hermida et al., 2015). The presence of high numbers of cysts in the final treated

water implies that the treatment processes applied are not fully effective (Castro-Hermida et al., 2015). In addition, genotyping analysis of the isolates revealed the presence of Assemblages A (AI and AII) and E in final treated water, suggesting contamination by human and hoofed animals (Castro-Hermida et al., 2008; 2015).

Table 7. Occurrence of Giardia cysts in treated drinking water

Area	Sample Type	Percent Positive (# of Samples)	Concentration Average (range), Cysts/L	Reference
Australia	Tap water from rainwater tanks	12.5%[a] (3/24)	110 to 140	Ahmed et al., 2012
Brazil	Finished water at a treatment plant	41.7%[b] (5/12)	0 to 0.06	Razzolini et al., 2010
Bulgaria	Tap water	6.5%[c] (3/46)	4.0E-3 to 0.5	Karanis et al., 2006
Hungary	Final water from treatment plants	26.7%[b] (12/45)	0.63 (Max)	Plutzer et al., 2007
Japan	Filtered water at treatment plant	12.0% (3/26)	5.0E-04 to 2.0E-03	Hashimoto et al., 2001
Portugal	Treated water at treatment plants	25.6%[b,d] (27/106)	NR	Lobo et al., 2009
Portugal	Water from treatment plants	15.5%[b,e] (26/167)	0.01 to 10.8	Almeida et al., 2010
Russia	Water treatment plant	7.1%[b] (5/70)	1.6E-04	Egorov et al., 2002
Spain	Treated water from facilities	19.2%[b] (5/26)	0 to 0.25	Carmena et al., 2007
Spain	Tap water	26.8%[b] (22/82)	0 to 0.62	Carmena et al., 2007
Spain	Final treated effluent water	100.0%[b,f] (16/16)	0.5 to 4	Castro-Hermida et al., 2008
Spain	Treated water at treatment plant	36.5%[b] (19/52)	1 to 5	Castro-Hermida et al., 2010
Spain	Treated water from treatment plants	45.7%[b,f] (58/127)	1 to 18	Castro-Hermida et al., 2015
Venezuela	Finished water at treatment plants	33.3%[b] (5/15)	0.05 to 0.25	Betancourt et al, 2012

NR - Not Reported

[a]qPCR, [b]IMS-IFA, [c]filtration, flocculation, sucrose gradient and IFA

G. duodenalis Assemblages [d]Assemblage A (AI), [e]Assemblages A (AII), B and E, [f]Assemblages A (AI, AII) and E

Very occasionally, *Giardia* cysts have been detected in tap water samples (Karanis et al., 2006; Bajer et al., 2012). In Bulgaria, cysts were found in 6.5% of samples, with concentration ranging from 0.004 to 0.5 cysts/L (Karanis et al., 2006).

2.2.5 Rainwater harvesting

In areas where potable water is scarce, communities are looking for alternative sources of water supply, such as rainwater collected from roofs in residential dwellings. A recent study of a sustainable community located in Australia, found that, among 24 households, 12.5% of water samples from rainwater tanks and connected household taps contained *Giardia* cysts at levels ranging from $1.1x10^2$ to 5.8 $x10^2$cysts/L and from $1.1x10^2$ to $1.4x10^2$cysts/L, respectively (Ahmed et al., 2012). Wild animals, such as birds, mammals, and reptiles, that have access to roofs, are the most likely sources of rainwater contamination. Since all households use captured rainwater as drinking water, residents can be at risk of infection.

2.2.6 Seawater and shellfish

Increased population density in coastal communities is contributing to seawater quality degradation, especially through sewage discharges and agricultural runoff. Studies on fecal contamination of coastal and estuarine waters with domestic sewage have highlighted the risk associated with exposure to various pathogens.

The occurrence of *Giardia* cysts has been reported in coastal and estuarine waters (Table 8). In the United States, *Giardia* cysts were found in 20% of water samples assessed, with concentrations ranging from 0 to 33 cysts/L (Graczyk et al., 2007b). Interestingly, a positive correlation between the number of bathers and the number of cysts in water was observed; indeed, samples collected during weekend days showed higher concentrations (mean, 9.1 cysts/L) than those collected on weekdays (mean, 0.6 cysts/L). Most probably, the higher number of bathers during weekends contributed to an increase in cysts via resuspension of bottom sediments and direct input (Graczyk et al., 2007b). More recently, *Giardia* cysts were detected in seawater samples from a shoreline region of Gaza City (Hilles et al., 2014). In this overpopulated area, the lack of appropriate wastewater treatment facilities leads to a huge discharge of untreated or only partially treated sewage directly into the seashore (Hilles et al., 2014).

Table 8. Occurrence of Giardia cysts in seawater

Area	Sample Type	Percent Positive (# of samples)	Concentration Average (range), Cysts/L	Reference
Brazil	Estuarine	25.0%[a] (1/4)	NR	Souza et al., 2012
Gaza Strip	Coastal water	2.3%[c] (1/52)	NR	Hilles et al., 2014
Mexico	Beaches	65.6%[a,d] (21/32)	1 to 30	Magana-Odorica et al., 2010
USA	Beaches	20.0%[b] (12/60)	4 to 33	Graczyk et al., 2007
Venezuela	Beaches	35.0%[a] (9/26)	0.2 to 17	Betancourt et al., 2014

NR - Not Reported

[a]IMS-IFA, [b]flotation and Fish, [c] microscopy

[d]Assemblage A

Surveys have also focused on tropical beach areas, which are visited year-round by many tourists. In Mexico, cysts were found in 65% of marine recreational water samples, with a concentration ranging from 1 to 30 cysts/L (Magana-Odorica et al., 2010). In Venezuelan marine beaches, cysts were detected in 85% of water samples collected in near-shore swimming areas at concentrations ranging from <0.2 to 17 cysts/L (Betancourt et al., 2014). While no outbreaks of giardiasis were linked to exposure to contaminated seawater, occurrence of *Giardia* cysts nevertheless suggests a notable risk to marine bathing waters.

Worldwide, consumption of seafood has increased in the

context of a healthy and sustainable lifestyle; however, marine water pollution raises safety issues. This is particularly the case for bivalve shellfish (oysters, clams, mussels) that are consumed raw or undercooked, and that are capable of accumulating waterborne pathogens by filter feeding. Moreover, some practices in aquaculture, particularly in use in developing countries, involve the direct addition of untreated or partially treated waste and contaminated surface waters as nutrient sources (WHO, 2006).

Giardia cysts have been recovered from clams, mussels and oysters harvested in different regions of the world (Table 9). In Spain, cysts were found in 42% of mussels from shellfish harvesting areas along the Galician coast, with concentrations ranging from 1 to 19 cysts/40 µl of tissue (Gómez-Couso et al., 2005). In the Netherlands, cysts have been isolated from both commercial and non-commercial oysters with an overall prevalence of 3.4%, as well as from sewage effluents that can reach the oyster-harvesting sites in the sea (Schets et al., 2007). In Ireland, wild mussels species from twelve sites in surface inland and coastal waters were examined, and cysts were found at eleven (92%) of these sites, including sites receiving effluents discharges from wastewater treatment plants and used for leisure (Lucy et al., 2008).

Table 9. Occurrence of Giardia cysts in fish and shellfish

Area	Sample Type	Percent positive (# of samples)	Concentration Average (range), Cysts/L	Reference
Australia	Marine and freshwater shellfishes and finger lings	3.8%[g,i] (27/709)	NR	Yang et al., 2010
Egypt	Fish	3.3%[b,f] (3/92)	NR	Ghoneim et al., 2012
Ireland	Mussels	91.7%[a,c] (11/12)	1 to 13	Lucy et al., 2008
Italy	Mussels	10.0%[g,h] (6/60)	NR	Giangaspero et al., 2014
Netherlands	Oysters (non-commercial)	6.7%[a] (9/133)	NR	Schets et al., 2007
Netherlands	Oysters (Commercial)	13.0%[a] (6/46)	NR	Schets et al., 2007
Spain	Mussels	41.8%[a] (77/184)	1 to 19	Gòmez-Couso et al., 2005
USA	Mussels	0.4%[e,b,j] (4/961)	NR	Adell et al., 2014

NR - Not Reported

[a]IFA, [b]PCR, [c]Fish, [d]Sucrose flotation, [e]IMS-IFA, [f]ELISA, [g] PCR and sequencing

G. duodenalis [h]Assemblage A, [i]Assemblages A, B and E, [j]Assemblages B, C and D

To date, in the few reports that investigated species and genotypes of *Giardia* in molluscan shellfish, the zoonotic Assemblages A and B, and the non-zoonotic Assemblages C, D and E were identified (Table 9). Recently, Assemblage A was detected in 23.3% (14/60) of the Mediterranean mussels (*Mytilus galloprovincialis*) purchased from markets in the city of Foggia, Italy (Giangaspero et al., 2014). In the United States, Assemblages B, C and D were detected in the hemolymph from mussels collected in Californian coastal areas near possible sources of pathogen pollution, adjacent to freshwater sources (Adell et al., 2014). Despite the proven occurrence of cysts in shellfish, there are no reported outbreaks of giardiasis due to the consumption of oysters, clams or mussels (Willis et al., 2013). This may be due to the strong underestimation of foodborne outbreaks of giardiasis.

Investigations for the presence of *Giardia* have been carried out also on fish (Table 9). In Australia, cultured fingerlings, wild freshwater and marine/estuarine fishes were tested for the presence of *Giardia* (Yang et al., 2010). Twenty-seven of the 709 fishes (3.8%) analyzed were positive by PCR, but the hatchery reared fingerlings had the highest prevalence (19/227, 8.4%). The genetic analysis revealed *G. duodenalis* Assemblages A, B and E, but also *Giardia microti*, a species infecting rodents (e.g., voles and muskrats). In a recent study in Egypt, *G. duodenalis* was detected in 3.4% of fecal samples from both farmed and

wild fish in the Nile River, and all isolates were genotyped as Assemblage A (Ghoneim et al., 2012).

2.3 Persistence

Despite many reports on the occurrence of *Giardia* in the environment, little is known about the actual viability of cysts and the potential to cause infection. The methods commonly applied for the detection of cysts in environmental samples do not differentiate between viable and nonviable organisms. Thus, one of the ongoing challenges is that the assessments of cyst survival require estimates of cyst viability. Procedures to evaluate viability include morphological criteria, inclusion/exclusion of fluorogenic vital dyes, such as 4', 6'- diamidino-2-phenyiindole (DAPI) and propidium iodide (PI), propidium monoazide (PMA), fluorescent in situ hibridisation (FISH), *in vitro* excystation, animal infectivity assays, and nucleic acid-base assays (Health Canada, 2012). The inclusion or exclusion vital dyes have been used extensively to assess viability; however, these methods are not sufficient to determine if cysts are infectious.

Cysts remain viable outside the host for extended periods of time depending on environmental factors such as temperature and relative humidity, but also some physical, chemical and biological characteristics of matrices (pH, dissolved oxygen, turbidity, etc). Temperature is considered the most important factor influencing the survival of cysts, but they are also susceptible to desiccation and direct sunlight, which are able to affect the persistence with a fast die-off of cysts (Percival et al., 2004; Alum et al., 2014).

Survival in water ranges from weeks to months, and it is primarily dependent on decreased environment temperature (USEPA, 1999). However, the occurrence of freeze-thaw cycles may be a relevant contributor to the decline in cyst viability (Robertson and Gjerde, 2006). Bingham et al. (1979) observed that *Giardia*cysts can survive for up to 77 days in tap water at 8°C, compared with 4 days at 37°C. A laboratory study using PI dye exclusion and mouse infectivity assays demonstrated that temperatures as low as -4°C are able to inactivate *Giardia* cysts in water, while infectivity is maintained for 11 weeks at 4°C (Olson et al., 1999). The viability of *Giardia* cysts (*Giardia muris* is used as a model) was assessed in lake, river, and tap water (de Regnier et al., 1989). By using PI dye exclusion and in vivo assay, this study found that cysts suspended at 30 ft in lake water remained viable for up to 56 days, whereas cysts stored at 15 ft were nonviable after day 28. Cysts exposed to river water remained viable up to 28 days as determined by in vivo assays. Additionally, cysts exposed to tap water were nonviable by day 14. In another study, the fate of *Giardia* cysts was investigated in an aquatic environment in Norway. The study was conducted during the winter season (temperature range, 1-7°C). Morphology and uptake of dyes were used as indicators of viability (Robertson and Gjerde, 2006). The results showed that no apparently viable cysts could be detected after 1 month, suggesting that *Giardia* cysts cannot survive during the winter.

In addition to the temperature, sunlight can be detrimental to cysts survival in the aquatic environment. Sunlight has germicidal effects as it provides both ultraviolet (UV) radiation and heat, which also depends on the exposure duration and intensity (Mtapuri-Zinyowera et al., 2009). In Zimbabwe, a study to assess the impact of natural sunlight on water contaminated with *Giardia* cysts and stored in PET (polyethylene terephthalate) containers demonstrated that solar radiation and heat produced by the sun have a synergistic effect in killing cysts when temperatures rise above 50°C, with complete death at 56°C (Mtapuri-Zinyowera et al., 2009).

In drinking water, *Giardia* cysts can be effectively killed after boiling for one minute.

Information on the survival of *Giardia*cysts in marine environments is limited and additional research is needed in this area. Survival of cysts in marine waters is important in relation to recreational water activities and through consumption of uncooked oysters, clams, or mussels (Nordic Council of Ministers, 2007). *Giardia* cysts are sensitive to both salinity and sunlight, and both factors decrease cysts survival time (Johnson et al., 1997). In the dark, cysts can survive up to 77 hours as compared to only 3 hours in the presence of light.

3.0 Reductions by Sanitation Management

3.1 Excreta and Wastewater Treatment

Wastewater treatment involves a number of types and various sequential steps in some cases, which may include a combination of physical (sedimentation, filtration, solar radiation and UV), biological (activated sludge, organic matter removal) and chemical (coagulation, oxidation) stages. Combination of these processes is meant to create a multi-barrier, present at all times, to minimize the health risk posed by the treated wastewater (Zhang et al., 2012).

3.1.1 On-Site sanitation

3.1.1.1 Dry onsite sanitation systems

The association of water, sanitation, and hygiene (WASH) on neglected tropical diseases is attracting interest, and this issue has been recently reviewed, with a focus on protozoa, including *Giardia* (Speich et al., 2016). In their meta-analysis, Speich et al. (2016) showed that people having access to, or using sanitation facilities, have significantly lower odds of being infected with *Giardia* (0.64) compared with their counterparts who lack access to, or do not use sanitation facilities. Likewise, lower odds (0.63) were observed among people who treat water before consumption. The fact that people without sanitation are at higher odds of being infected with soil-transmitted helminths compared with those with sanitation has been already discussed (Strunz et al, 2014), and strengthen the concept that improved sanitation facilities and safe water are crucial for combating a large number of infectious diseases.

3.1.1.2 Inactivation by storage

Cysts are able to survive for about one week in solid cattle faeces (manure), but the survival in slurry is longer. The study by Grit et al (2012) investigated the infectivity of *Giardia* cysts from slurry in two animal models (gerbil and lamb) and concluded that storing cattle slurry for 90 days reduced their number and viability by only 0.64 $\log_{10}$ and 1.52 $\log_{10}$, respectively.

No relevant data were found as per the effect of pit latrines, vault toilets and dry toilets, except for the study reported above (Speich et al., 2016).

3.1.1.3 Water-based onsite sanitation (septic tanks)

No relevant data on the use, and effect of, septic tanks were found.

3.1.2 Waste stabilization ponds

The performance of a stabilization pond in Morocco, consisting of a series of two circular basins, each of a superficial area of 2500 m^2 and a depth of 2.3 m for the first basin and 1.5 m for the second, was evaluated (Amahmid et al., 1999). In this study, wastewater samples were collected twice monthly for 24 months. Sampling points included the entrance of the first basin, the exit of the first basin, and the exit of the second basin. Sediments were also sampled monthly for 24 months. Results showed that *Giardia* cysts were detected in 50% (24 of 48) wastewater samples, with a geometric average concentration of 2.8 x 10^3cysts /L. However, only 2 of the 48 samples (4.2%) at the exit of the first basin contained *Giardia* cysts, with a mean number of 21 cysts /L, and no cysts were detected at the outlet of the second basin. For sediment samples, *Giardia* cysts were detected in 25% (18/72) of samples at the entrance, with an average of 1.3 x 10^3per gram dry weight. At the exit of the first basin, 5.6% (4/72) of samples contained *Giardia*cysts, whereas no cysts were detected in sediment samples taken at the entrance of the second basin.

A waste stabilization pond formed by two anaerobic ponds, a facultative pond, and a maturation pond was examined in Spain. The system operated with only 6 days of total retention time (Reinoso et al., 2011). All raw wastewater samples (10 liters) were positive, with a mean concentration of 67 cysts/L (range 17.2 to 2.17x10^2cysts/L). The study showed that anaerobic ponds were the most effective in removing parasites followed by the facultative pond and the maturation pond. The removal efficiency was higher during summer (4.3 $\log_{10}$cysts removed $m^{-2}day^{-1}$) than in winter (3.9 $\log_{10}$cysts removed $m^{-2}day^{-1}$). The authors further commented that predation and natural mortality are important in the removal of *Giardia* cysts.

Four waste stabilization pond systems, two located in subtropical South East Queensland, Australia, serving a population around 1500 inhabitants, and two in wet-dry tropic regions of the Northern Territory, serving 1000–2500 inhabitants, were compared (Sheludchenko et al 2016). Three of the four systems used baffles. Water samples from the inlet and the outlet of three ponds were tested for the presence of *Giardia* using the USEPA 1623 method. Highest removal rates were observed in the two WSP which used baffles (2.2 and 1.6 $\log_{10}$), compared to the one that did not (1.3 $\log_{10}$). This result indicates that retention time was a key factor in *Giardia* cyst removal.

3.1.2.1 Aerated lagoons

A single study compared the removal efficiency of two sewage treatment plants in Malaysia, one using extended aeration (EA, plant A) and the other using an aerated lagoon (AL, plant B) (Lim et al, 2007). Samples of 10 liters from the influent and the treated water were concentrated by sucrose flotation and *Giardia* cysts detected microscopically after staining with fluorophore conjugated monoclonal antibodies. All water samples (raw and treated) at both plants were positive for *Giardia*. At plant A, the number of cyst /L ranged from 18 to 5.24x10^3 in raw influent samples and from 1 to 5.0x10^2 in treated sewage effluents. At plant B, the number of cyst /L ranged from 55 to 8.48x10^3 in raw influent samples and from 28 to 1.46x10^3 in treated sewage effluents. Thus, removal rates were >1 $\log_{10}$ in both plants, but were higher in plant A (1.4 $\log_{10}$) compared to plant B (1.1 $\log_{10}$). This is due to the fact that in EA plants, fine bubbles, generated by submerged diffusers, can promote higher oxygen transfer efficiency compared to AL plants, which use surface aerators to provide air [45].

3.1.3 Constructed wetlands

Wetlands may provide an attractive low technology and low energy solutions for treating wastewater. Postulated removal mechanisms are settling and sorption of protozoa to wetland vegetation and substrate. Constructed wetlands (or planted soil filters) may be installed either as vertical or as horizontal flow filter constructions. Horizontal systems are further classified, depending on the pathway of water flow, as surface and subsurface flow systems. Removal of *Giardia* (and other pathogens) by the use of wetlands is influenced by many factors, including vegetation cover, turbidity, salinity, and temperature of the water, as well as disinfection from ultraviolet and solar radiation, and predation from filter feeding and grazing organisms, such as zoo-plankton and snails.

The removal of *Giardia* cysts at two plants in Germany that received municipal wastewater (population served, 100.000 at Plant A, and 300 at Plant B) was also evaluated (Redder et al., 2010). Samples of wastewater (influents) and effluents (first and second filter flow, and from a facultative pond) were tested by the USEPA 1623 method. The mean concentration of *Giardia* cysts in the influents was close to 150 cysts/100 L at both Plants, and a 2 to 3 $\log_{10}$reduction was observed. The authors commented that the use of a two-stage system consisting of a subsurface horizontal flow filter was essential to achieve an efficient removal of cysts.

Another study by Graczyk and colleagues (Graczyk et

al., 2009) examined four horizontal wetlands that received unchlorinated municipal wastewater subjected to secondary treatment after sewage sludge activation and secondary sedimentation. Plant A had two components: the first component (surface flow, SF) discharged to the second component (subsurface flow, SSF), with a final effluent released into the groundwater. Plants B, C and D were small-scale surface flow wetlands discharging to surface waters. Grab samples (2 liters) of influents and effluents were processed and *Giardia* cysts were detected by FISH and IFA. Concentrations of cysts in influents ranged from 8 to 241 cysts/L (mean, 69 ± 37.7), and from 11 to 140 cysts/L (mean, 88 ± 24.0) in effluents. Only Plant A achieved a significant removal efficiency (1.3 $\log_{10}$), while the number of cysts was higher in the effluents than in influents at the other three Plants. This was interpreted as the result of active deposition of cysts by wild animals.

The efficiency of a combined constructed wetland (facultative pond, FP, SF wetland and SSF wetland) that received domestic raw wastewater from a village (150 habitants) in the province of León, Spain was evaluated (Reinoso et al., 2008). Ten liter samples were collected monthly over a period of 1 year, and cysts were quantified by IFA. The arithmetic mean of *Giardia* cysts in the influent was 280.94 (SD, 99.14), while in the final effluent was <1 cyst; therefore the cumulative treatment system removal was 3.04 $\log_{10}$. The SSF wetland was significantly more efficient in the removal of cysts than SF and FP; the removal rates in SF wetland were significantly higher ($p<0.05$) in summer than in winter.

3.1.4 Combined sewer overflows – treatment of fecally-polluted stormwater

Combined sewer overflows (CSOs) occur in combined sewer systems when sewage and stormwater runoff are released into water bodies, potentially contaminating water sources. The use of CSO is necessary to accommodate hydraulic strain when the combined rain and sanitary flows exceed the system capacity. In recent years, the USEPA has identified CSOs as a significant source of pathogens and other pollutants and contaminants in surface water bodies.

A study of the Chicago Area Waterway System (CAWS) assessed the impact of CSO on the microbial quality of the CAWS (Rijal et al., 2011). Dry and wet weather samples were collected upstream and downstream of the three water reclamation plants. During dry weather events, cysts were detected in 32, 68 and 72% of samples taken from the plants. The concentration of cysts was usually low (<10 cysts/L), with the exception of one downstream sample (49.5 cysts/L); the detection of cysts in upstream samples (at concentrations of 0.1 to 5.4 cysts/L) suggests input of cysts in the CAWS from both WRP and non-WRP sources.

3.1.5 Wastewater Treatment and Resource Recovery Facilities

Table 10 presents a summary of results from studies conducted at different wastewater treatment plants. The following sections focus on the most relevant treatments and their efficacies in removal of *Giardia* cysts.

Table 10. A summary of studies on the removal efficiency of Giardia cysts in differently operated wastewater treatment plants

Area	Plant	Population served	Primary treatment	Secondary treatment	Tertiary treatment	Disinfection	Cyst/L influent Average	Cyst/L effluent Average	Log10 reduction	Reference
Brazil	Plant 1	NR	Screening, aeration	Activated sludge, secondary clarification	None	UV	1E+05 ± 8.7	1.1E+03 ± 1.0	1.96	Neto et al 2006
Brazil	Plant 1	NR	NR	Activated sludge	Sand filtration, membrane filtration	Chlorine	2.8E+03	18	2.19	Hachich et al., 2013
Brazil	Plant 2	NR	NR	Upflow anaerobic sludge blanket, membrane bioreactor	Ferric chloride coagulation, sedimentation	Chlorine	1.1E+03	6	2.26	Hachich et al., 2013
Brazil	Plant 3	NR	NR	Activated sludge	Sand filtration	Chlorine	3.9E+03	3	3.11	Hachich et al., 2013
Brazil	Plant 4	NR	NR	Anaerobic and facultative pond	Maturation pond, trickling filter	None	9.3E+03	<1	>3.97	Hachich et al., 2013
China	WTP-G	NR	Screening and grit removal	Activated sludge	Sand filtration	None	1.6E+03	0.53	3.02	Fu et al 2010
China	WTP-Q	NR	Screening and grit removal	Anaerobic-anoxic-oxic process	Membrane ultrafiltration	Ozone/chlorine	9.1E+02	<0.033	>2.94	Fu et al 2010
China	WTP-J	NR	Screening and grit removal	Oxidation ditch process	None	None	8.0E+02	2	2.60	Fu et al 2010
Germany	Plant 1	5.0E+04	Rake, grit chamber, sedimentation	Activated sludge	Sand filtration	None	3.73E+02	0.51	2.39	Kistemann et al 2008

Area	Plant	Population served	Primary treatment	Secondary treatment	Tertiary treatment	Disinfection	Cyst/L influent Average	Cyst/L effluent Average	Log10 reduction	Reference
Germany	Plant 2	2.7E+04	Rake, grit chamber, sedimentation	Activated sludge	Sand filtration	None	2.12E+02	0.22	2.24	Kistemann et al 2008
Germany	Plant 3	1.1E+04	Rake, grit chamber, sedimentation	Activated sludge, trickling filter	None	None	2.38E+02	0.34	2.25	Kistemann et al 2008
Germany	Plant 4	1.07E+04	Rake, grit chamber, sedimentation	Activated sludge, trickling filter	None	None	5.08E+02	2.37	2.33	Kistemann et al 2008
Germany	Plant 5	8E+02	Rake, grit chamber	Activated sludge	None	None	1.61E+02	0.21	2.12	Kistemann et al 2008
Germany	Plant 6	5E+02	Rake, grit chamber	Activated sludge	None	None	2.43E+02	4.5	1.73	Kistemann et al 2008
Ireland	Plant A	1.9E+03	Screening and grit separation	Sludge activation in oxidation ditch	None	None	3.20E+02 ±22.6	1±1.5	2.51	Cheng et al 2009
Ireland	Plant B	1 E+03	None	Sludge activation in extended aeration tanks	None	None	1.23E+02 ±9.2	3±1.5	1.61	Cheng et al 2009
Ireland	Plant C	2.5E+03	Screening and grit separation	Sludge activation in extended aeration tanks	None	None	7 ±4.1	<1	>0.85	Cheng et al 2009
Ireland	Plant D	2.1E+03	Screening and grit separation	Biofilm-coated percolating filter	None	None	NR	1±1.1	NR	Cheng et al 2009
Italy	Plant A	1.5E+05	Screening and grit separation	Oxidation with O2 and sedimentation	None	None	1.6E+05	1.56E+02	3.01	Cacciò et al 2003
Italy	Plant B	3.3E+05	Screening, grit separation and sedimentation	Activated sludge and sedimentation	None	Chlorination	1.16E+05	2.8E+04	0.62	Cacciò et al 2003
Italy	Plant C	3E+05	Screening, grit separation and sedimentation	Activated sludge and sedimentation	None	Chlorination	1.61E+05	6.44E+03	1.4	Cacciò et al 2003
Italy	Plant D	1E+05	Screening, grit separation and sedimentation	Activated sludge and sedimentation	None	Filtration and peracetic acid	3.61E+05	2.7E+03	2.13	Cacciò et al 2003
Spain	Plant 1	2.5E+05	Screening and grit separation, sedimentation	Anaerobic digestion, sedimentation	None	UV	NR	0 to 13.2	2.33	Rodriguez-Manzano et al, 2012
Spain	Plant 2	2.5E+05	Screening and grit separation, sedimentation	Anaerobic digestion, sedimentation	Sand filtration	UV	NR	0.4 to 2	2.98	[53] Rodriguez-Manzano et al, 2012
Tunisia	Plant 1	1.9E+04	Primary decantation	Oxidation channel	None	None	3.2E+02	<1	>2.51	Khouja et al 2010
Tunisia	Plant 2	6.3E+05	Primary decantation	Oxidation channel	None	None	2.6E+02	<1	>2.41	Khouja et al 2010
Tunisia	Plant 3	NR	Primary decantation	Activated sludge	None	UV	2E+02	<1	>2.3	Khouja et al 2010
Tunisia	Plant 4	9.8E+03	None	Aerated lagoon	None	None	1.06E+02	2	2.02	Khouja et al 2010
Tunisia	Plant 5	1E+06	Primary decantation	Oxidation channel	None	None	1.6E+02	2	1.9	Khouja et al 2010
USA	Plant A	5.0E+05	Not indicated	Activated sludge	None	Chlorination	4.8E+03	33	2.08 ±0.44	Kitajima et al 2014
USA	Plant B	2.5E+05	Not indicated	Trickling filter	None	Chlorination	6.4E+03	1.9E+02	1.52 ±0.62	Kitajima et al 2014
USA	Plant 1	NR	Not indicated	Activated sludge, lime treatment	Sand filtration, upflow carbon adsorption	Chlorination	4.9E+02	1.1	2.65	Rose et al, 2001

NR - Not Reported

3.1.5.1 Primary /preliminary treatment

Pretreatment may include a sand or grit channel or chamber, where the velocity of the incoming sewage is adjusted to allow the settlement of sand, grit, stones, and broken glass trough the process of sedimentation. Removal of these particles is necessary to prevent damage of pumps and other equipment. Primary treatment consists of temporarily holding the sewage in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface. The settled and floating materials are removed and the remaining liquid may be discharged or subjected to secondary treatment.

Enhanced primary includes coagulation and settling as well as disinfection and is often used when secondary treatment is not available. No data are available on removal of cysts by this method.

Enhanced coagulation is a process used to improve removal of disinfection-by-products precursors through modified conventional treatment that includes reduction of pH to levels of 5–6 and the use of higher doses of coagulants.

Dissolved air flotation (DAF) is a clarification process used to remove particles in membrane plants or in conventional type plants using granular media filtration. DAF allows removal of fragile floc particles found in water treatment via adherence to air bubbles. DAF can achieve 2–3 $\log_{10}$removals, compared to removals by sedimentation of 2 Log_{10}decreasing to 1 $\log_{10}$ or less for winter water temperatures (Edzwald, 2010).

3.1.5.2 Trickling filters

The $\log_{10}$reduction of *Giardia* cysts was compared at two wastewater treatment plants in Arizona, one that utilized a conventional activated sludge process (A) and the other that utilized a biological trickling filter process (B) (Kitajima et al., 2014). A total of 48 wastewater samples (12 influent and 12 effluent samples from each of the two plants) were collected, and cysts were concentrated using an electronegative filter method followed by IMS. Molecular assays were also employed to genotype cysts. *Giardia* cysts were detected in all influent samples with mean concentration of 4.8×10^3cysts/L and 6.4×10^3cysts/L at Plants A and B, respectively. Cysts were also detected in all effluent samples, except one, with mean concentration of 3.3×10^1cysts/L and 1.9×10^2cysts/L at Plants A and B, respectively. The concentration of *Giardia* cysts in effluent at Plant B was significantly higher than Plant A (t-test, P = 0.0003), suggesting that a conventional activated sludge process (2.2 $\log_{10}$removal) is slightly more efficient than a biological trickling filter process (1.5 $\log_{10}$removal) in removing *Giardia* cysts.

3.1.5.3 Activated sludge

A bench scale activated sludge reactor was used to simulate the performance of the secondary treatment process in the Bolivar WWTP (Adelaide, South Australia). Bolivar utilizes a conventional biological wastewater treatment process, including activated sludge and biological nutrient removal (Wen et al., 2009). The laboratory system was fed with primary settled wastewater from the Bolivar WWTP, and six samples were taken, including three influent and three effluent samples. The number of *Giardia* cysts in the influent varied from 2.5 to 8.2×10^3 /L, whereas in the effluent the number varied from 1.7 to 8.0×10^1 /L. Therefore, the average $\log_{10}$reduction was 2.49 (Wen et al, 2009). The removal performance at the Bolivar WWTP was investigated during June and July, and a $\log_{10}$reduction of 1.42 was estimated, much lower than that in the laboratory system. This difference was attributed to the lower temperature outdoors in winter compared to the constant temperature in the laboratory. Another study in Arizona was mentioned in the previous paragraph (Kitajima et al., 2014).

It is well known that during wastewater treatment, sanitation procedures can result in a significant reduction of *Giardia* cyst levels. However, information on parasite survival is deficient. In a project undertaken in Nordic countries, FISH was used to estimate cysts viability in raw sewage and in both untreated and treated sludge from treatment plants in Sweden (Nordic Council of Ministers, 2007). Results showed that the viability of cysts entering the sewage treatment varied considerably overtime and before settling into the sludge for further treatments. In two plants from Henriksdals in Stockholm and Tegeludden in Kalmar, viable cysts were detected in untreated sludge samples at every sampling using the FISH assay, with rates of 2.9-41.7% and 14.3-66.7%, respectively. After sludge treatment by mesophilic and thermophilic digestion, in Henriksdals plant 1.8% (9/500) cysts were still FISH-positive after mesophilic treatment with a retention time of 21 days. At the Tegeludden plant, 1.2% (1 of 81) cysts were FISH-positive after thermophilic digestion plus 14 days of retention time. In both anaerobic sanitation procedures, the time/temperature ratio was the key factor affecting cyst viability; however, higher temperatures adopted in thermophilic digestion (around 55°C) resulted in greater reduction of cyst viability (Nordic Council of Ministers, 2007; Dumontet et al., 2001).

3.1.5.4 Oxidation ditch

Giardia cyst removal was compared from three municipal wastewater treatment plants (WWTP) in Beijing, China (Fu et al., 2010). One of the WWTP used an oxidation ditch process as secondary treatment. It was found that oxidation ditch process had higher reduction efficiency for *Giardia* (2.60 $\log_{10}$) than anaerobic-anoxic-oxic process (2.04 $\log_{10}$) and conventional activated sludge process (1.68 $\log_{10}$). This was interpreted as the result of longer retention time and higher sludge concentration in these types of treatment processes.

3.1.5.5 Membrane bioreactors

A Membrane BioReactor (MBR) is the combination of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor, and is now widely used for municipal and industrial wastewater treatment

with plant sizes up to 80,000-population equivalent.

The overall water quality produced from 38 satellite MBR facilities located in various parts of the US was evaluated. In the majority of the facilities, submerged MBR configuration, hollow-fiber ultrafiltration membranes and backwashing, as a fouling control strategy, were used (Hirani et al., 2013). Using the USEPA 1623 method, the presence of *Giardia* cysts was assessed on 10 liter samples of MBR effluent. Only two filtrate samples from two satellite facilities contained cysts (3 cysts/10 liters and 3-18 cysts/10 liters, respectively); it was noted that the two facilities also had much higher particle and bacterial counts than the other satellite plants. However, *Giardia* cysts are not expected to pass through intact microfiltration or ultrafiltration membranes. Therefore, monitoring of MBR facilities for membrane integrity was suggested.

3.1.5.6 Anaerobic/ anoxic digestion and biogas

The removal efficiency at three WWTP using anaerobic/anoxic digestions were compared. Plant 1 used activated sludge and anaerobic/anoxic/oxic treatment, followed by sand filtration and chlorination, whereas Plant 2 used activated sludge and chlorination, and Plant 3 used trickling filter followed by activated sludge, chlorination and UV (Hatam-Nahavandi et al 2015). Unfortunately, the results were expressed for all protozoan combined. At Plant 1, the mean concentration of cysts was $1.3x10^3$ ($\pm$ $4.1 x10^2$) / L in raw water, and $2.5 x10^2$ ($\pm$ 59) / L in the treated water (0.72 $\log_{10}$reductions). At Plant 2, the mean concentration was $1.4 x10^3$ ($\pm$ $3.8 x10^2$) / L in raw water, and $2.4 x10^2$ ($\pm$ 89) / L in the treated water (0.79 $\log_{10}$reductions). At Plant 3, the mean concentration was $1.4 x10^3$ ($\pm$ $1.4 x10^2$) / L in raw water, and $2 x10^2$ ($\pm$ 70) / L in the treated water (0.85 $\log_{10}$reductions). Thus, no significant differences in reduction could be attributed to the different treatment processes.

3.1.5.7 Coagulation

The significant role played by chemical lime treatment barrier in the reduction of *Giardia* was highlighted during an investigation of the Water Reclamation Plant in Virginia (Rose at al,. 2001). This treatment was the most effective along the entire process, achieving a reduction of *Giardia* cysts by 4.6 $\log_{10}$.

3.1.5.8 Membranes

Membrane technologies have a strong potential in the removal of protozoa from wastewater, and ultrafiltration (UF; pore sizes of 0.002 to 0.1 µm) can achieve effective removal of protozoan cysts (4 to 15 µm) by physical sieving. Membrane filtration is also used for clarification and removal of inorganic and synthetic organic chemicals and control of disinfection by-products.

The removal efficiency at a pilot-scale membrane system, where wastewater tertiary treatment was carried out by UF using a submerged hollow-fiber system was investigated (Lonigro et al., 2006). Using direct IF microscopy, *Giardia* cysts were found in four of four wastewater samples at a concentration of $1.83 \times 10^3 \pm 1.81 \times 10^3$/L (mean ± standard deviation). UF resulted in a cyst removal of 3.6 $\log_{10}$.

3.1.5.9 Sludge management

The use of lime to reduce or eliminate pathogen content in sewage sludge represents a simple and inexpensive treatment. Lime stabilization is a process where calcium hydroxide ($Ca(OH)_2$) or calcium oxide (CaO) is added and the pH elevated to 12 for 2 or more hours. *Giardia* cysts, suspended in either water or sludge, and exposed to lime treatments for 24h, 48h, and 72h resulted in cyst inactivation, as demonstrated by lack of infection in experimentally challenged gerbils (Bean et al. 2007).

3.2 Disinfection

3.2.1 Chemical disinfection (chlorine, ozone)

A study has evaluated the efficiency of ozonation (and UV treatment) in reducing pathogens, including *Giardia*, in combined sewer overflows (Tondera et al., 2016). Artificial combined wastewater samples were prepared by pumping from the wastewater-settling tank through the pilot testing systems. In the ozone reactor, two doses of ozonation (10 mg and 15 mg /L) were tested with a contact time of 15 minutes. The USEPA 1623 method was used, and some samples were stained with propidium iodide to distinguish viable and dead cysts. It was found that the $\log_{10}$reduction was 1.3 ± 1.8 and 1.1 ± 1.9, respectively, for the two doses evaluated.

Thus, ozonation is effective against *Giardia*, but a 3 $\log_{10}$inactivation requires higher ozone exposure compared to bacteria. In turn, this raises concerns about the formation of by-products, such as bromides, a potential carcinogenic compound (Von Gunten, 2003). Another point of concern is the inverse correlation between cyst concentration and disinfection efficacy (Haas and Kaymak, 2003), which is of relevance considering that laboratory studies usually employed large number of cysts, a situation that do not represent the natural concentration of cysts in real world waters.

Alternative disinfection methods are being considered, and organic acids have gained attention because of disinfection-by- products. Per-acids such as peracetic acid (PAA) and performic acid (PFA) are organic peroxides. The suggested disinfection mechanisms of PAA are based on the release of highly reactive oxygen species (ROS) such as hydroxyl, alkoxyl and hydroperoxyl radicals and superoxide. A study evaluated the disinfection efficiency of PFA against various microbial contaminants, including *Giardia*, on secondary-treated wastewater (Karpova et al 2013). While most microorganisms were already efficiently inactivated with 2 mg PFA /L within 5 min, for *Giardia* cyst this value was 10 mg PFA /L.

3.2.2 Physical disinfection (Ultraviolet)

Ultraviolet disinfection has been introduced at many

water and wastewater treatment plants as a microorganism reduction method, with the majority employing monochromatic low-pressure (LP) UV lamps for irradiation.

Relatively low doses of UV (1–9 mJ/cm^2) have been shown to inactivate 2–4 log$_{10}$ of *Giardia* cysts in one study (Linden et al., 2002). Similar findings were obtained using the *G. duodenalis*, WB isolate, and a gerbil model of infection (Campbell and Wallis, 2002): up to 2 log$_{10}$ inactivation was observed at a UV dose of approximately 10 mJ/cm^2 (range 9.3-11.7 mJ/cm^2). Higher UV doses (between 20 and 40 mJ/cm^2) resulted in up to 3 log$_{10}$inactivation of the cysts. The most recent study indicates that cysts could not survive irradiation doses higher than 5 mJ/cm^2 (Einarsson et al., 2015). Cysts exposed even to the lowest dose (1 mJ/cm^2) of medium pressure (MP) UV irradiation failed to establish an infection in gerbils (Shin et al., 2010). The capability of *Giardia* cysts to repair UV-mediated DNA damage and maintain infectivity has also been investigated. A recent study showed that UV treatment did not actually kill the cell but damaged its DNA, in agreement with the hypothesis that the replication machinery is important for survival of *Giardia*(Einarsson et al., 2015). Indeed, mature cysts might not have had an active replication machinery at the time of irradiation, and are therefore unable to detect and repair the DNA lesions.

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