Chapter 9

Risk Assessment for *Giardia* in Environmental Samples

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Abstract

Giardia is a gastrointestinal parasite that causes infections in humans worldwide. In developing countries, giardiasis is an emerging infection because it plays an important role in diarrhea outbreaks linked to water and food consumption affecting the population in general. Giardiasis is referred to as zoonosis because its biological etiological agent is transmitted to humans through animal reservoirs by oral-fecal route. Detection and occurrences of *Giardia* cysts have been documented in water, food, soil, and air. The principal risk factors for developing giardiasis include environmental contamination associated with malnutrition and immunosuppression. The small size of cysts and their environmental resistance together with the small infection dose to produce the disease allow giardia dissemination especially in marginalized populations; however, parasitism is present in all countries and at different economic levels. This zoonotic illness contains several species of *Giardia duodenalis*, infecting mammals and humans with eight serotypes, of which A and B are of public health importance. Quantitative microbiological risk assessment (QMRA) is a methodology used for predicting health risk to establish regulations for permissible *Giardia* risk in water and food. This chapter focuses on worldwide reviews of *Giardia* incidence in environmental samples including giardiasis prevalence, serotypes, risk factors, and finally options for cyst reduction in the environment, emphasizing on QMRA.

Keywords: water, air, soil, food, giardiasis

1. Introduction

Gastrointestinal diseases have caused up to 871,000 deaths worldwide, which have been related to unsafe drinking water, health, and hygiene. Mortality rate is greater in African countries where death risk is 4.3×10^{-1} [1, 2]. Such data justify that the main risk factors are unsafe water and deficient cleaning linked to malnutrition and immunosuppression, invariable characteristics in marginalized communities. The microscopic parasite *Giardia* is among the main pathogens that cause gastrointestinal diseases at world level. In developing countries, 200 million people have been diagnosed with giardiasis symptoms, which are related to inadequate sanitation problems and access to safe drinking water. Giardiasis does not make a distinction between humans and animals by fecal-oral transmission using different routes: zoonotic, anthropogenic environmental, food, and water [3–5].

The strategy used in this research was assessed by analyzing different literature studies related to *Giardia* risk assessment; the search was performed in databases from October 2000 to October 2016, using the following terms: giardiasis risk factors; *Giardia* cysts in water, air, soil, and food; quantitative microbial risk assessment (QMRA); and cyst treatments. No restriction was found in language. This chapter focuses on reviewing world incidence of *Giardia* in water, soil, air, and food, including giardiasis prevalence, risk factors, and finally a system to reduce cysts in the environment, emphasizing on QMRA.

2. *Giardia* generalities

Giardia is one of the most primitive eukaryotic protozoa initially described by Leeuwenhoek in 1681; its taxonomy was confusing and complicated throughout the first half of the twentieth century. The name *Giardia lamblia* was well-known in the 1970s, but it was consolidated and changed to *Giardia duodenalis* or *Giardia intestinalis* in the 1990s. It is classified within the class Zoomastigophorea in the order Diplomaida and family Hexamitidae. Historically, 41 species have been described based on their hosts. To date, eight of these species have been detected in mammals: *duodenalis, enterica, canis, bovis, muris, cati, simondi*, and *microti* (**Table 1**) [6–9].

Giardia exists in two forms: an active form called a trophozoite and an inactive form called a cyst. The cyst measures 5 × 7 to 10 μm in diameter, containing four nuclei covered by a wall from

Species*	Host
G. intestinalis	Humans and mammals
G. agilis	Amphibian
G. muris	Rodents
G. ardeae	Birds
G. psittaci	Birds
G. microti	Rats and moles

Table 1. Giardia species.

0.3 to 0.5 μm in thickness; it is composed of an exterior filament layer formed by glycoproteins and an internal membrane layer that makes it very resistant providing an environmentally stable life cycle; it helps withstanding long periods in water at temperatures less than 25°C, and it even makes it invulnerable to chlorination processes. Trophozoites are pear-shaped and are approximately 12–15 mm in length and 5–9 mm in width with a cytoskeleton that includes a medium size body, four pairs of flagella, and a ventral disk; they have two nuclei without nucleoli in its interior, which are found in front and symmetrically located [10, 11].

The vital cycle of *Giardia* starts with the ingestion of cysts by the host whether found in food or water contaminated with feces of the infection carriers; once consumed, the cyst enters in contact with the gastric acid destroying itself and excystation occurs and trophozoites are released, which pass through the upper small intestine infecting the duodenum and the upper part of the intestine where they are reproduced by binary fission adhering to the intestinal epithelium surface and triggering symptomatology. The adaptation mechanism of *Giardia* known as encystment is essential to the parasite for their survival once out of the intestine of the host since trophozoites are extremely sensitive to changes in temperature, humidity, and the presence of chemical agents. In this process, trophozoites descend through the host intestine, and when they find a cholesterol-poor environment, their differentiation to cysts is induced and eliminated with feces. It has been reported that infected persons excrete from 10^8 to 10^9 cysts in only one evacuation and can continue discarding them from 50 days and including months later after diarrhea has subsided [10, 12].

For the parasite to survive within the host and avoid the immune response, *Giardia* shows what is known as antigenic variation that allows it to elude the immunologic system and produce chronic and recurrent infection. Giardiasis symptoms in human beings show variable degrees. Acute giardiasis shows acute diarrhea and urticaria or it can show itself asymptomatically; the acute form is usually self-limiting lasting for 2–7 days; it is also possible to evolve to a subacute or chronic stage lasting from months to years; malabsorption results as the most frequent and harmful complication difficult to solve from a therapeutic point of view because it causes malnutrition and low weight [13–15].

3. Giardiasis epidemiology

3.1. *Giardia* in the environment

The necessary dosage for giardiasis to start is from 10 cysts, which have been found in all environmental matrices: water, soil, air, and food. In drinking water, up to 24 cysts/L have been reported [16]; 87 cysts/L in soil; 0.0087 cysts/L in air [17]; and 40 cysts/L in leafy vegetables [18]. Most research has monitored *Giardia* cysts in water (**Table 2**). Giardiasis life cycle includes illness in several mammal species mediated by the environment, which is why climate factors such as humidity and temperature influence in cyst exposure, which are very persistent and their viability is preserved more in temperate and humid climates. *Giardia* cysts can maintain their infectious capacity from 15 days up to 2 months in water; 15 days to 1 month in soil; and from 2 to 10 days in vegetables; because of their small size and weight, they can be found suspended in air. Their resistance in the environment is a natural advantage to invade new hosts and expand their

Source	Location	Percent positive*	(oo)cyst 100 L^{-1}*	Reference
Waste water	EU, Italy, Ireland, Spain	25–100	3.2×10^3–4.2×10^4	[19–22]
Surface water	Belgium, Germany, Ireland, the Netherlands, Malaysia, Taiwan, EU, México, China	10–81	0.2–18.6×10^4	[23–27]
Drinking water	Bulgaria/Russia, Spain	5–27	0–62	[28, 29]
Ground water	Bulgaria/Russia, Brazil, France	8–62.5	6–3.61×10^3	[29–32]

Table 2. Occurrence and density of Giardia cyst in water.

progeny, which is why they have been identified as a potential danger to food products that are equally contaminated with animal feces or with contaminated water [17, 32]. Fecal runoff and vectors increase pathogen dissipation and thus the risk of acquiring the disease [33].

3.2. Genotypes

Giardia has many species characterized, but *G. intestinalis*, *lamblia*, or *duodenalis* is more recognized as a pathogen for human beings and a wide range of hosts including wild animal species. Currently, eight genetic groups from A to H (**Table 3**) are recognized; nonetheless, the species that are harmful for humans are divided into two genotypes: A (or Polish) and B (or Belgian), of which B is the most pathogenic in man [34]. Recent studies have revealed that genotype E has also been identified in humans [32].

The majority of research studies report that genotypes A and B have been found in clinical samples, and their distribution in the world is related to social and economic factors. The mix of both genetic groups (A and B) has also been reported in one sample, which suggests multiple infections [1] and confirms constant exposure to contaminated sources. It is common to find assemblages or genotypes A and E in superficial water [27].

Giardia genotypes can appear mixed as in the case of Scotland where both genotypes turn up, of which A is more prevalent [35]; the same case happened in Malaysia [36] and in Egypt, where A subtype I was the most prevalent [37]. In Latin America, particularly in Mexico, genotype A

Genotype	Hosts
A	Humans, cats, dogs, horses, calves, pigs, deers, lemurs, beavers, Guinea pigs, and sloths
B	Humans, dogs, monkeys, beavers, rabbits, guinea pigs, muskrats, and chinchilla
C, D	Dogs
E	Cows, goats, lams, and pigs
F	Cats
G	Rats
H	Marine vertebrates

Table 3. Recognized ***Giardia*** genotypes.

type II is only present [38]. While in Argentina, genotype A type II showed low seroprevalence, genotype B was found in high number of cases that included children and adults [39].

Genotype A is linked to diarrhea [40] and more in human origin than in zoonotic [1]; in disagreement, another study indicated that humans are the greatest source of assemblage B and that domestic animals are the greatest hosts of assemblage A [41].

3.3. Outbreaks and risk factors

An outbreak is a spontaneous increase of a disease occurrence. These cases are epidemiologically linked with at least one confirmed laboratory case. Numerous giardiasis outbreaks transmitted in water have occurred in the USA, Canada, England, France, Australia, Japan, and other industrialized nations due to contamination of water and food sources (**Table 4**). The factors that could be attributed to the increase of parasitic disease outbreaks produced by water and food are diverse. The increase of international travelers and migrants produces a rapid dissemination of the symptoms. Globalization of food sources, food imports as exotic fruits and vegetables are now necessary to satisfy consumption demands. Unfortunately, transportation conditions as controlled temperature have favored parasite survival in fruits and vegetables [50].

Two significant factors that contribute to the risk of contracting giardiasis are age and gender. Children from 1 to 5 years of age are more prone to the disease; in addition, infection incidence is greater in men than in women [38]. Divers have a high risk of contracting parasitosis even more than swimmers [51].

3.4. Impact in public health

Political, legal, economic, and public health is very committed to having reliable and safe drinking water sources for human consumption. An important concern is having them contaminated with pathogenic microorganisms such as *G. intestinalis*. The World Health Organization

Source	Location	Quantity of cases	Reference
Water sources	New Zealand	14	[42]
Swimming pool	Victoria, Australia	30	[43]
Drinking water	New Hampshire, EU	31	[44]
Water sources	New York, EU	36	[45]
Recreational water	California, EU	50	[46]
Water supply	Izmir; Turkey	196	[47]
Food/water	Scotland	185	[35]
Contaminated water	Bergen, Norwegian	2500	[48]
Foodborne/anthropogenic	All states in EU	19,140	[49]

Table 4. Giardiasis outbreaks.

(WHO) estimates that at least 10^9 cases of gastrointestinal diseases occur per year in one-third of the countries in the world, causing mortality of more than 5×10^6 persons at early age. The economic costs of diseases are alarming and cause financial losses. For this reason, social institutions have decided to work in developing better techniques for researching and controlling parasites in such a way that water turns out to be a safe liquid. Knowing the relative importance of specific transmission routes of intestinal protozoa, including potential sources of environmental contamination, constitutes fundamental aspects that allow understanding the epidemiology of parasitic diseases. In this manner, corrective measures can be applied to minimize prevalence and incidence of these diseases in the population. In developed countries, giardiasis is an emerging infection because it plays an important role in diarrhea outbreaks linked to water and food consumption that affect the population in general. As to developing countries in Asia, Africa, and Latin America, approximately 200 million people experience giardiasis symptoms [1, 52].

4. Detection methods in the environment

Detection techniques in environmental samples are diverse. Molecular biology methods are used to differentiate genotypes by using hybridization DNA probe DNA and polymerase chain reaction (PCR) techniques, starting from diverse fragments of nucleic acids as ribosomal RNA, los genes *hsp* 70, and random amplification of polymorphic DNA fragments (RAPD). The advantages of PCR techniques include high sensitivity, rapid analysis of several samples, relatively low cost, simultaneous detection of several pathogens, and the capacity to discriminate among several species of strains. These techniques are used mainly to differentiate *Giardia* species and genotypes [40].

The techniques to number *Giardia* cysts are those that use fluorescent antibodies; they can also differentiate viable and nonviable cysts by phase-contrast imaging [53]. Immunofluorescent microscopy techniques are used to detect *G. intestinalis* in water. The methods endorsed by the United States Environmental Protection Agency (USEPA) are the Information Collection Request (ICR) method protozoans (1999) and the 1623 method (2005). For water samples, this method is based on elution and purification of a filter stained with fluorescent monoclonal antibodies to then count the structures in a brilliant apple green color in an epifluorescence microscope (**Figure 1**). Many of these methods have already been adapted to recover cysts in food [54].

Fluorescent staining as acridine orange, propidium iodide (PI), and 4,6-diamidino-phenylindole (DAPI) are prone to have false positives and have variable stain characteristics depending on the viability state of the microorganism; nonetheless, the use of these stains, especially DAPI, can be very useful in identification when used together with other microscopy techniques such as fluorescence and phase contrast and differential interference contrast (DIC) [53].

Immunomagnetic separation (IMS) methods and the 1623 method have been developed to concentrate bacteria and protozoan pathogens. These methods use specific antibodies on the surface of paramagnetic particles to link target pathogens, followed by a magnet used to separate them

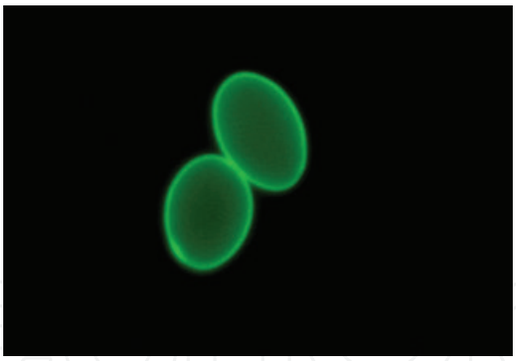

Figure 1. *Giardia intestinalis* cysts stained with fluorescent antibodies.

from the matrix sample [55]. The assay method of immunoabsorption linked to enzymes (ELISA) is more sensitive than the microscopy techniques for (oo)cyst detection [56].

Flux cytometry (FC) is a method by means of fluorescent activators capable of classifying cells according to their fluorescence and size. Detection and selective enumeration of *Giardia* cysts that are applied in FC consists of separating and observing the stained particles with immunofluorescent antibodies by the dispersion process. This method can potentially turn out to be the most precise in detecting and quantifying cysts [57].

Knowing cyst concentration in environmental samples and the necessary dosage when giardiasis starts allows us to estimate pathogen exposure; with this information and using the appropriate mathematic model, it is possible to calculate health risk. This methodology called quantitative microbial risk assessment (QMRA) is based on a series of steps that convey predicting daily and annual risks. In developed countries, QMRA has been adapted to assess permissible risk limits for *Giardia* in drinking water samples where the accepted risk worldwide is one infection for each 10,000 individuals.

5. Quantitative microbial risk assessment

The Codex Committee on Food Hygiene and the National Advisory Committee on Microbiological Criteria for Foods have proposed a framework for conducting QMRA. These guidelines also provide methods and approaches used to evaluate potential health effects and assess risks from contaminated source media, i.e., soil, air, and water. One of the key benefits of this method is the development of models describing the complex nature of pathogen populations in water or food supply [58].

Hazard identification involves pathogen detection in terms of concentration in water, for example. Next is exposure assessment where the quantity of water consumed for the people at risk is determined. In these two steps, one should take into account the recovery efficiency of the method, the characteristics of the people (age, sex, immune state, and customs), and pathogen survival. Then, the dose-response curve is calculated with the mathematical models described in the literature; finally, the integration of all the parameters provides the risk characterization that results in the likelihood of infection risk per day and year per person [59].

Quantitative microbial risk assessment has become a standard; the UK has pronounced a mandate that establishes that risk assessment be carried out by local government on many water supplies [60]. The US Environmental Protection Agency (EPA) handled permissible water *Giardia* risk values of <1:10,000 (10^{-4}) in a yearly exposure [61]. In the UK, the Water Supply Regulations 1999, and The New Dutch Drinking Water Decree state that for pathogenic microorganisms, health risk should be less than 1 infection/10,000 consumer/year [62]. These risk regulations are equal to those of EPA. Developed countries are in the position to provide guidance, training, information resources, and technical assistance to advance supports for water safety. Thus, greater cooperation and collaboration at all levels should be effective and ensure that QMRA, as a water safety tool, will be available to all countries.

6. Quantitative microbial risk assessment for *Giardia* in environmental samples

The QMRA is an approach that has been widely used around the world to estimate the risk of infection by giardiasis in different sources of exposure. Most research studies have been performed in water samples, but the method has been applied in food, soil, and air samples.

In the last few years, the most relevant studies where QMRA has been used to evaluate giardiasis infection risks are the following:

In New Jersey, USA, the risk by accidental water ingestion (50 mL) of the Lower Passaic River was assessed resulting in a probability of 1:1 [23]. In Amsterdam, risk probability was calculated from 9×10^{-4} to 1.2×10^{-2} in recreational waters [63], while in Eastern Europe a giardiasis risk was predicted from 3×10^{-1} for water consumption from a well contaminated with sewage water [64]. In Mexico, a risk of 1.09×10^{-2} was estimated by lettuce consumption [65].

In France and England, *Giardia* risks were assessed due to water consumption from private well with values from 5.8×10^{-1} and 5.7×10^{-1}, respectively [60]. Risk for swimming in recreational waters of the Great Lakes in the USA was 5×10^{-3} [66]. The risk for rainwater consumption was also calculated in Australia at 3.1×10^{-1} [67].

In Brazil, giardiasis risk for drinking water consumption was estimated at 1.92×10^{-2} [68]. In Venezuela, the risk for bathers swimming in seawater was 3.6×10^{-2} [69]. In Switzerland, the risk by indirect contact with water from a lagoon contaminated with residual water was 3.5×10^{-1} [70], whereas risk by joint exposure to soil and dust transported by air was assessed at 1:1 in a rural town in Mexico [17].

In all the previous studies (**Figure 2**), the risks were greater than those allowed by the regulating commissions (1×10^{-4}), which is why these studies show that the microorganism concentration is enough to produce the disease in a percentage of the populations.

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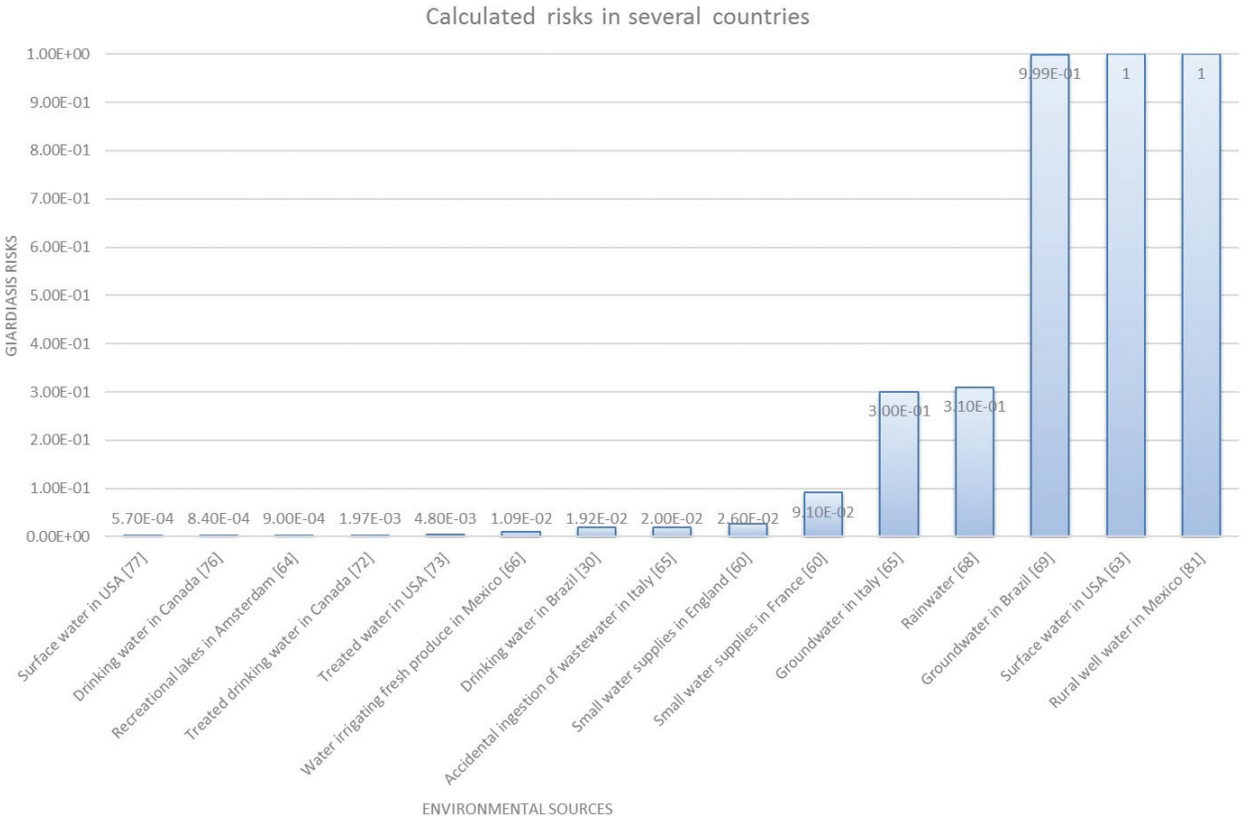

Figure 2. Risks by giardiasis calculated by quantitative microbial risk assessment in different countries.

Source	Location	Annual risk	Reference
Drinking water	Québec, Canada	8.4×10^{4}	[73]
Reclaimed water	California, EU	1.58×10^{-1}	[74]
Surface water	NJ, EU	1	[23]
Surface water	Arizona, EU	4.2×10^{-4}	[25]
Urban flooding	Netherlands	6×10^{-3}	[75]
Treated water	Saint Lawrence River, Canada	1.46×10^{-3}	[71], [73]
Tank water	Queensland, Australia	1.2×10^{-1}	[67]
Small water supplies	England	9.1×10^{-2}	[60]
	France	2.6×10^{-2}	
Well water	Sao Paulo, Brazil	9.9×10^{-1}	[30]
Drinking water	Zhejiang, China	6.25×10^{-6}	[76]
Drinking water	Sao Paulo, Brazil	1.92×10^{-2}	[69]
Tap water	Gorges Reservoir, China	1.3×10^{-1}	[27]
Reclaimed water	Tianjin, China	9.83×10^{-3}	[77]
Well water	Sonora, Mexico	9.9×10^{-1}	[17]
Small private systems	Canada	3.3×10^{-2}	[5]

Table 5. Annual risks of Giardia infections in different regions in the world.

Based on this information, it should be solved how to make these sources not harmful for humans and implement the necessary treatments for decreasing or eradicating giardiasis risk.

Using the concentrations reported in the literature, annual risk by giardiasis was calculated. To estimate infection probabilities (P_i), a consumption exposure of 1.46 L was taken into account [71], and then the exponential model equation $P_i = 1-\exp^{(-rN)}$ was used where $r = 0.0199$ [72] (**Table 5**).

7. Treatments decrease giardiasis risk

7.1. Water

It has been proven that the use of effective removal treatments for *Giardia* in water decreases the risk of acquiring the disease considerably. According to Surface Water Treatment Rule (SWTR), a series of requirements for superficial and underwater treatments were developed, which specify a removal or minimum inactivation of 3 log for *Giardia* [61].

The stabilization ponds are biological treatment systems that consist of excavated deposits with the sufficient surface and volume to provide the treatment periods; depending on oxygen requirements, the artificial lagoons can be aerobic, facultative, and anaerobic; it has been reported that these lagoons eliminate up to 2 logarithmic units of *Giardia* cysts. Stabilization ponds are the most conventional and it does not reach the minimum requirements for cyst removal according to the EPA; only does aerobic digestion reach 1.3 log of *Giardia* removal [11]; as to the water treatment with coagulation-flocculation only two logarithmic units of removal were achieved [78].

Currently, the best *Giardia* cyst removal treatments consist of using activated mud together with UV disinfection, with which 3.6 logarithmic units of cyst removal are reached [79]. The future bets on removing water contaminants by nanotechnological compounds, for example, nanocompounds of clay polymers and nanoadsorbents based on carbon and polymerics. Besides being effective, these compounds are economic [80].

7.2. Food

After water, the most important infection route with *Giardia* cysts is by food. The infections caused by these parasites are greatly the result of bad hygiene of the person responsible for food preparation. *Giardia* is always found coinfecting with other microorganisms, such as *Cryptosporidium*, *Vibrio cholerae*, and *Rotavirus* [81].

The consumption of raw food increases the risk of infection, which is why international recommendations exist to provide innocuousness in food preparation. It is especially important to (1) practice adequate hand hygiene for protection against this parasite; (2) buy food

from reliable providers; (3) maintain food packed or closed; (4) perform pest control frequently; (5) make sure refrigerator temperature is below 5°C; (6) avoid cross-contamination by surfaces and recipients; (7) separate cooked from raw food; (8) use purified or boiled water especially if food is consumed raw; and (9) make sure food is cooked at high temperatures (≥70°C).

One of the main regulators of food innocuousness is the system ISO 22000, which is a combination of preliminary programs, such as the hazard analysis and critical control point (HACCP) principles, the implementation steps defined by the *Codex Alimentarius* Commission (CAC) and the regulated components of the norm ISO 9001:2000 [82].

8. Impact of climate change on giardiasis epidemiology

Climatic change is actually being considered as a triggering infection risk factor of zoonotic diseases because certain temperature conditions may increase the pathogens' infective capacity. In the case of *Giardia* cysts, the temperature may be a determining factor in its propagation because an increase in temperature may promote transmission although at low temperatures the cysts viability remains stable [33]; it may be due to increased intake of contaminated water either for drinking or using it for recreational activities [43].

Escobedo et al. [83] in their ecological study verified statistically that giardiasis increases significantly during the climate change that occurs with the "El Niño" phenomenon by using nonlinear Poisson models similar to those in QMRA and proving that *Giardia* infections are sensitive to climate. This knowledge can be helpful to identify sources of infection and support in the prevention and control of these diseases. Besides temperature, other factors that can increase the risk of giardiasis and directly related with climate change are precipitation/humidity and wind/dust [84].

9. Conclusions

Giardiasis outbreak studies have been reported worldwide with occurrence of *Giardia* cysts values up to still 100%; however, a continuous environmental examination is expensive, and it does not offer the necessary information about giardiasis reduction. QMRA is an approach indicated for determining risk infection probability due to pollution with cysts in water, food, soil, and air. It permits researching about the probable cause of pollution and the adequate treatment process. The high capacity of *Giardia* to infect (because of the large number of oocysts in the environment and the low dose necessary to infect) turns it into a serious world health risk. Therefore, it is important to create correct worldwide regulations designed for developing safety measurements of water, soil, air, and food sources.

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