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Water for Human Consumption in Rural Areas Worldwide: A Systematic Review of the Presence of *Cryptosporidium* spp. and *Giardia* spp.

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ABSTRACT

Reports of gastrointestinal disease outbreaks caused by pathogenic protozoans in rural areas are still small in numbers. This study has evaluated, at global level, the parasitological contamination by *Cryptosporidium* spp. and *Giardia* spp., of water resources used as sources for human consumption in rural areas, spanning from 1994 to 2022. For such, a systematic review was conducted, following the PRISMA guidelines, applying a descriptive and statistical analysis to the data. A total of 29 eligible articles reported contamination in 90 different springs spread throughout six continents. It was possible to see a greater tendency of giardiasis outbreak occurrences in water coming from surface springs in countries with higher HDI (above 0.8), justified by the lack of data in countries with other HDIs. The water sources intended for human supply in rural areas are susceptible to parasitological contamination, especially in Asia and Oceania. In spite of the fact that there have been no reports regarding giardiasis outbreaks in the reviewed studies, it is important to notice that the concentration of pathogens may have been greater due to the low recovery of the methods of detection. Therefore, it is advisable to conduct more studies about the contamination of water resources in rural areas, as well as the adoption of prevention measures regarding waterborne diseases, such as boiling water.

Keywords: rural population, diarrhea, emergent, pathogen, risk, drinking water, community.

INTRODUCTION

In the scope of public health, infectious diseases related to potable and non-potable water, due to the presence of organisms such as bacteria, virus and parasites, are the most common cause of acute and chronic risk to health (Cheng et al., 2009; Hunter and Nichols, 2002; Ligon; Bartram, 2016; WHO, 2017), especially in more vulnerable populations, such as immuno-compromised people (Abubakar et al., 2007; Fayer, 2004; Xiao et al., 2004).

At global level, among the infectious agents such as *Cyclospora cayetanensis, Dientamoeba fragilis, Blastocystis hominis, Entamoeba histolytica, Microsporidia, Naegleria fowleri and Toxoplasma gondii,* the *Cryptosporidium* spp. and *Giardia* spp. protozoans were responsible for most of the waterborne disease outbreaks since 1950 (Table 1). It is noteworthy that there is underreporting in developing countries due to the diseases are not compulsory notification and the lack/inefficiency of the vigilance system to combat parasites (Baldursson and Karanis, 2011; Ma et al., 2022; Ogura and Sabogal-Paz, 2022; Sammarro and Sabogal-Paz, 2020).

The outbreaks related to *Cryptosporidium* spp. and *Giardia* spp. (Table 1) were attributed, in their majority, to recreational water, followed by inefficacy/failure in the water treatment and distribution processes, contaminated water resources (lakes, rivers and wells) and the use of untreated water, coming from surface and subterranean springs, being prevalent in developed countries (Baldursson and Karanis, 2011; Ma et al., 2022; Ogura and Sabogal-Paz, 2022; Sammarro and Sabogal-Paz, 2020).

Regarding means of transmission, these may occur by faecal-oral means, through the contact among people and among people and animals, drinking contaminated water (independent of its source), recreational activities, as well as the ingestion of infected food (Lanata, 2003; Plutzer and Karanis, 2016; Ryan et al., 2014). In this context, while water might not be the exclusive method of disease transmission, it serves as the primary mode of transportation, particularly for the protozoans *Cryptosporidium* spp. and *Giardia* spp. This is due to their high environmental resistance (Karanis, 2011). Additionally, the (oo)cysts have become smaller and less responsive to disinfection processes, posing challenges for retention within water treatment systems (Cunha et al., 2019; Slifki; Smith; Rose, 2000), alongside their heightened infectivity rate (Thompson, 2004; Toledo et al., 2017).

The risk of contamination is significantly higher in surface waters when compared to underground waters, because of the spilling of treated and untreated domestic sewage (Bonatti et al., 2023; Cheng et al., 2009; Toledo; Martins; Freire, 2017) and the superficial water outflow containing bovine faeces (Escobar et al., 2022; Imre et al., 2017).

The *Cryptosporidium* spp. protozoan, pertaining to the apicomplexan phylum, infects a wide range of vertebrate hosts through zoonotic and anthroponotic transmission of the gastrointestinal disease called cryptosporidiosis (Ryan et al., 2014; Xiao et al., 2004). The oocyst presents formats from spherical to oval, containing four sporozoites in its interior, and typical size of 4.0 to 6.0 μm in diameter. The species *C. hominis* and *C. parvum* are the main responsibles for causing the diarrheic disease in humans, transmitted through multiple means (Ryan et al., 2014; Xiao et al., 2004).

In what refers to the *Giardia* spp., it is a flagellate and binucleate protozoan, responsible for the giardiasis disease (Karanis, 2011; Thompson, 2004). The cyst presents a round to oval or ellipsoidal form, with dimensions varying from 8–18 μm in length by 5–15 μm in width (USEPA, 2012). In this stage of its life cycle, the cyst is highly resistant to the disinfection process with chlorine and ozone (Lane and Lloyd, 2002), commonly applied in the treatment of water supply.

Among the specimens reported in literature, the *Giardia duodenalis* (also named *Giardia intestinalis* or *Giardia lamblia*) presents itself as the most common cause of outbreaks of gastrointestinal disorders reported in humans living in developed countries (Lane and Lloyd, 2002).

The clinical manifestations of the cryptosporidiosis and giardiasis depend on the infecting species, the dose ingested (Karanis, 2011; Teunis et al., 2002), the virulence of the pathogen and the immunological state of the person (WHO, 2017). Therefore, the environmental contamination of water represents an important source of chronic infections on a large scale, responsible for the symptom of intense diarrhea, lasting for one to two weeks (Hunter and Nichols, 2002), and it may be lethal in immuno-compromised individuals (Abubakar et al., 2007; Efstratiou et al., 2017b; WHO, 2017; Xiao et al., 2004).

It is important to highlight that climate changes, including events of intense rains and extreme droughts, are increasing the propagation of diseases related to protozoans (Lal et al., 2013; Lobo et al., 2009), especially in countries with limited resources and population residing in rural areas (Dong et al., 2020; Liu et al., 2020). The social

Table 1. Number of outbreaks caused by waterborne protozoans in the world until 2020

Period	*Giardia* spp.	*Cryptosporidium* spp.	Continents[1]	Reference
1950 – 2004	40.62% (132/325) *Giardia duodenalis* outbreaks	50.77% (165/325) *Cryptosporidium parvum* outbreaks	North America, Europe, Asia, Oceania and South America	(Karanis et al., 2007)
2004 – 2010	35.18% (70/199) *Giardia lamblia* outbreaks	60.30% (120/199) *Cryptosporidium* spp. outbreaks	Oceania, North America, South America, Europe and Asia	(Baldursson and Karanis, 2011)
2011 – 2016	37.27% (142/381) *Giardia* spp. outbreaks	62.73% (239/381) *Cryptosporidium* spp. outbreaks	Oceania, North America, Europe and Asia	(Efstratiou et al., 2017b)
2017 – 2020	19.12% (48/251) *Giardia* spp. outbreaks	76.49% (192/251) *Cryptosporidium* spp. outbreaks	North America, Asia, Europe, Oceania and South America	(Ma et al., 2022)

Note: [1] The continents were listed in decreasing order of predominance of reported outbreaks.

and demographic factors also affected the disease burden, such as the changes in use and occupation of land, which may lead to the contamination of water resources (Lal et al., 2013).

In rural areas, the populations lack adequate basic sanitation infrastructure due to the inexistence of public policies of rural sanitation or the difficulty of access to the implemented instruments, intensifying the social vulnerability (FUNASA, 2019; Lee et al., 2017). As examples of rural groups, there are: 1) in Brazil, the rural population can be defined by several cultural references, being denominated as traditional peoples and communities by Article 3, item I of Decree n. 6,040 (Brasil, 2007); 2) in Colombia, the Politic Constitution, Article 7, recognizes and protects the rights of ethnic minorities (Semper, 2006), and 3) in the Northern Hemisphere, the Mexican sustainable rural development law promotes the sustainable rural and territorial development of the indigenous peoples and rural communities (Oliver and Santos, 2017).

There are studies which indicate contamination of water sources used for human consumption in rural regions, such as springs, rivers and subterranean wells (Castro-hermida et al., 2009; Efstratiou et al., 2017b; Franco et al., 2012; Silva et al., 2023). Some of these studies compare the contamination and occurrence levels between rural and urban waters, pointing that the rural communities present more incidence of *Cryptosporidium* spp. and *Giardia* spp. (Bryan et al., 2021). Therefore, it is crucial to comprehend the role of water as a means of disease transmission in the different communities, and the development of preventive strategies to guarantee water safety and avoid its contamination (Baracho et al., 2023; Karanis et al., 2007).

The diarrheal diseases were classified as the third cause of non-lethal diseases with the most occurrence cases worldwide (Vos et al., 2016). However, some studies suggest that the prevalence of cryptosporidiosis and giardiasis are underreported due to the lack of laboratory diagnosis, once that they conduct analysis only in specific cases upon receiving medical request (Cunha et al., 2019).

The difficulty of diagnosing environmental contamination caused by *Cryptosporidium* spp. and *Giardia* spp., it should be noted that arises due to the high costs associated with environmental analysis and the requirement for skilled labor (Khurana et al., 2021).

In general, the rural population uses untreated water for consumption, existing evidence of a risk up to four times higher of exposure to protozoans in rural areas in comparison to urban areas (Huang et al., 2023). This risk may be justified by the low salubrity rates in rural communities, related to the absence of water treatment, precariousness in sanitary sewage facilities and proper management of solid waste (Braga et al., 2021). This is an alarming situation for the health of the population that lives in rural areas, which reinforces the importances of evaluating the environmental contamination of the water resources used for human consumption, food and personal hygiene (Wohlsen et al., 2004)

Therefore, the goal of the present study was to evaluate, at global level, the presence of *Cryptosporidium* spp. and *Giardia* spp. in waters for human consumption in the rural areas, to investigate parasitological contamination and its interactions with the socioeconomic conditions of the countries and the type of water source.

MATERIALS AND METHODS

Systematic review

The protocol for this systematic review was created in accordance with the guidelines contained in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), consisting of three steps. The review protocol is described in S1 Protocol (http://prisma-statement.org/) (Moher et al., 2009). The research questions were formulated in accordance to the population/problem method, intervention, comparison and result (PICO) (Santos et al., 2007): 1) What is the incidence of *Cryptosporidium* spp. and *Giardia* spp. in aquatic environments used for consumption in the rural zone? 2) Are there differences in the prevalence of these protozoans between surface and subterranean waters? 3) What is the influence of protozoan identification methods? 4) What is the origin of contamination sources of water resources in rural zones?

The literary research was developed using the Scopus multidisciplinary database (www.scopus.com), applying a logical structure composed of descriptors (keywords) and boolean operators: Giardia OR Cryptosporidium OR Giardiasis OR Cryptosporidiosis OR Giardiase OR Cryptosporidiosis AND rural AND water. The words were searched in the research option "Article title, Abstract, Keywords". This step was conducted between September, 2021 and April 2023, without year, language and document type restriction. The relevant studies indexed by Scopus were tabulated in Excel, and the duplicated publishings were excluded.

During the initial triage, the articles' titles and abstracts were reviewed. The following eligibility criteria were considered: i) studies addressing the parasitological quality of water in relation to the protozoans *Cryptosporidium* spp. and *Giardia* spp., and ii) the examined water samples were intended for consumption by populations residing in rural areas. The study was classified as tracked (T), when related to the research subjects or did not present clear information in its abstract, and deleted (D) when it did not match the eligibility criteria

A thorough reading of the article was made, considering the presentation of the additional criterion about the sufficient details regarding to: (i) the water source type; (ii) the sample evaluation method, and (iii) the concentration of *Cryptosporidium* spp. and/or *Giardia* spp. oocysts detected in the water sample. Those articles with insufficient information or which have analysed only the occurrence of protozoans in the urban water were excluded.

Data organization

The relevant data of the articles obtained in the systematic review were organised in an electronic spreadsheet in Excel®, taking the following informations into consideration: country, continent, community name, Human Development Index (HDI) of the country, water type (surface, subterranean or rain water), type of water source (river, spring, well, cistern with rain water), water source protection level, research year, protozoan detection method, detection frequency, molecular results, oocyst concentration (average, minimum, maximum and standard deviation) and water treatment type.

Next, among the countries with reports of contamination of the water springs in rural areas, the Human Development Index was obtained through the United Nations Development Programme report referring to the year of 2021 (UNDP, 2022).

To classify the level of water source protection, three categories where defined:

- Protected spring: there was reported evidence related to the precautions taken by the local population to avoid the contamination of the water source used as source of supply.
- Unprotected spring: there were potential causes of water source contamination due to conditions of use and the region's land occupation, the presence of animals next to the source and absence of hygiene measures.
- Probably protected spring: there were no reports of contamination of this spring.

Statistical analysis

The statistical association among the independent qualitative variables (continent, spring type, water source type, water source preservation level, protozoan concentration and HDI) was conducted through a multiple correspondence analysis, starting with the program R 4.2.2 (R Core Team, 2023), using the FactoMineR package (for analysis) and factoextra (for data viewing). This exploratory technique allows the visualisation of association between different groups of variables in a dimensional graph (STHDA, 2022). To carry out the analysis, the variables were divided into the categories contained in Table 2. The HDI was classified in countries as low (< 0.8) and high (≥ 0.8), in accordance to what was applied by Rocha et al. (2022).

The mean concentration of *Giardia* spp. was tagged as "below to 0.05 cysts/L" and "above to 0.05 cysts/L", in conformity to the action level of 0.03 to 0.05 cysts/L (Wallis et al*.,* 1996) and "below to 0.3 oocysts/L" and "above to 0.3 oocysts/L" for concentration of *Cryptosporidium* spp., according to action level of 0.1-0.3 oocysts/L (Haas et al*.,* 1995) which represent the lowest doses of finished water at which infection was observed in human studies. The results of absence of concentration of these protozoa in fresh waters were classified with the lower limit of the action level, given that the recovery efficiency of the analytical methods vary widely between 10% and 80% (Efstratiou et al., 2017a).

RESULTS

Making use of the PRISMA protocol, 361 articles were identified and, among these, one was excluded from the identification step for

Table 2. Summary of the variables and its categories adopted at multiple correspondence analysis

Variables	Category
Continent	Africa
	Asia Europe
	North America
	Oceania
	South America
Type of water	Surface
	Groundwater
	Rain
Type of water source	Cistern with rain water
	River
	Spring
	Well
Preservation of water source	Probably protected
	Protected
	Unprotected
Concentration	< 0.3 oocysts/L
	> 0.3 oocysts/L
	< 0.05 cysts/L
	> 0.05 cysts/L
Human Development Index	HDI≥0.8
	HDI<0.8

being duplicated (Figure 1). Thus, 360 remained, from which 238 were excluded in the initial selection step because they analysed the presence of *Cryptosporidium* spp. and *Giardia* spp. in other types of samples (faeces, soil, vegetables and sewage) (Figure 1). In the sequence, 122 articles remained, of which 93 were excluded as they have not presented the type of water source or the concentration of pathogens in the aquatic environment, among other justifications listed in Figure 1. It resulted in 29 articles that were thoroughly analysed, as they have approached the parasitological contamination of water sources used for the rural population consumption from 1994 to 2022 (Figure 1).

Within these publications 90 different springs were found, of the types river, well, spring, lagoon or rainwater cistern. Among the water samples selected and presented on Table 3 and the Supplementary Materials Table S1, 71.1% (64/90) of the consumption sources are surface springs, 25.6% (23/90) are subterranean springs and 3.3% (3/90) rain water. Only the waters in 21 surface and/or subterranean springs receive some kind of treatment, including the disinfection with chloride step and, in some researches, the filtration process.

These water supplying sources are distributed among six continents (Table 3), and these sources are illustrated in Figure 2, indicating the data's country of origin. The biggest number of springs were identified as follows: in the African continent, with predominance of 31.1% (28/90); in the Asian continent, with 30.0% (27/90); in North America, with 24.4% (22/90); in Oceania, with 6.7% (6/90); in South America, with 4.4% (4/90), and in Europe, with 3.3% (3/90).

The greatest parasitological contamination rates were identified in surface springs, with maximum values of 416 oocysts/L in the African continent (Sente et al., 2016) and 3482 cysts/L in the Asian continent (Daniels et al., 2015), as presented in Table 3.

In underground sources , the largest number of (oo)cysts identified were of 7 oocysts/L in North America (Balderrama-Carmona et al., 2015) and 26 cysts/L in Asia (Daniels et al., 2015) (Table 3). In rain water collected and stored in recipients built with different materials, such as concrete, stones and bricks, the maximum prevalence was of 50 oocysts/L (Abo-Shehada et al., 2004) and 219 cysts/L (Ben Ayed et al., 2018).

In general, methods for detecting *Cryptosporidium* spp. and *Giardia* spp. in water were described in Table 4 using the filtration/elution and concentration/purification procedures adopted in each article. It appears that only 41.4% (12/29) of the articles carried out the quality control recommended by the USEPA (2012) and reported the frequency of recovery of the method used. In some studies, based on molecular methods, it was possible to identify the species of *C. hominis*, *C. andersoni*, *C. parvum*, *C. baileyi*, *C. skunk*, *C. muskrat* and *Cryptosporidium* which infected rodents (Füchslin et al., 2012; Pignata et al., 2019; Ono et al., 2001; Daniels et al., 2015; Ehsan et al., 2015; Keeley and Faulkner, 2008; Prystajecky et al., 2014). The genotypes A, AII, B, C and of *Giardia lamblia* were reported in the aquatic compartment in the rural environment (Ong et al., 1996; Daniels et al., 2015; Ehsan et al., 2015; Prystajecky et al., 2014)

Statistical analysis

The statistical similarity between the qualitative variables identified by the multiple correspondence analysis can be observed in nine

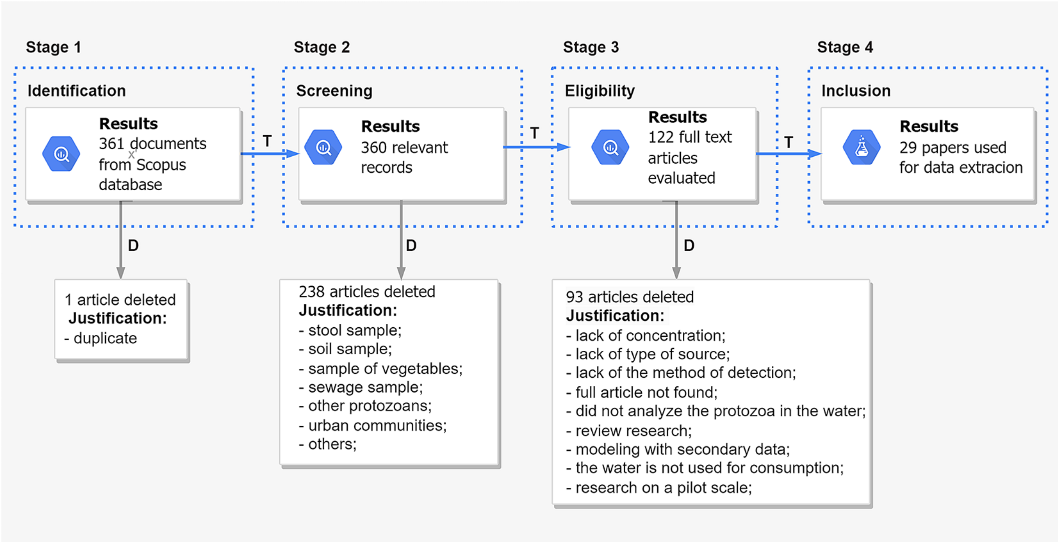

Figure 1. Flow chart, adapted from PRISMA (2009), containing the amount of tracked (T) and deleted (D) articles in each step of the systematic review

groups (Figure 3). Dimensions 1 and 2 of the multiple correspondence analysis were able to explain 46.4% of the variability of tabulated data (Figure 4), however, not all variables were well evidenced in these two dimensions. The representation quality is called "cosine squared" (cos2), which varies from 0 to 1 and, when the sum of "cos2" approaches one (1), the variable will be well represented by the adopted dimensions (STHDA, 2022).

In Figure 4 it is possible to see that the groundwater were negatively correlated with the concentration of *Giardia* spp. above the action level, as they are placed on opposite sides in the graphic, and in the surface sources they tend to be more contaminated with *Giardia* spp. Regarding the HDI, countries with a high index had water sources with a greater tendency to be contaminated by *Giardia* above 0.05 cysts/L, which may increase the risk of giardiasis infection in the rural population of these countries, if they consume untreated water. Whereas, countries with low HDI were negatively correlated with *Giardia* contamination because they were located on the opposite side of the graph (Figure 4).

Table 3. Prevalence interval of *Cryptosporidium* spp. and *Giardia* spp. in water supplying sources used in rural communities worldwide, summarised by continent

Continent	Type of water	Concentration range (minimum – maximum)	
		Cryptosporidium spp. (oocysts/L)	*Giardia* spp. (cysts/L)
Africa	surface	ND – 416.77	ND – 425.52
	groundwater	ND – 0.30	UR
	rain	ND	63.00 – 219.00
Asia	surface	ND – 298.4	ND – 3482.0
	groundwater	0.40 – 5.75	0.45 – 26.00
	rain	6.00 – 50.00	UR
North America	surface	ND – 182.00	ND – 22.15
	groundwater	ND – 7.00	0.52 – 5.25
South America	surface	0.20 – 2.00	ND – 0.10
	groundwater	ND	ND
Oceania	surface	ND – 0.60	ND – 0.70
Europe	surface	0.00203 – 0.127	UR
	groundwater	0.053 – 0.154	UR

Note: UR = unrealized; ND = not detected and below detection limit. Source: Drafted by the Authors.

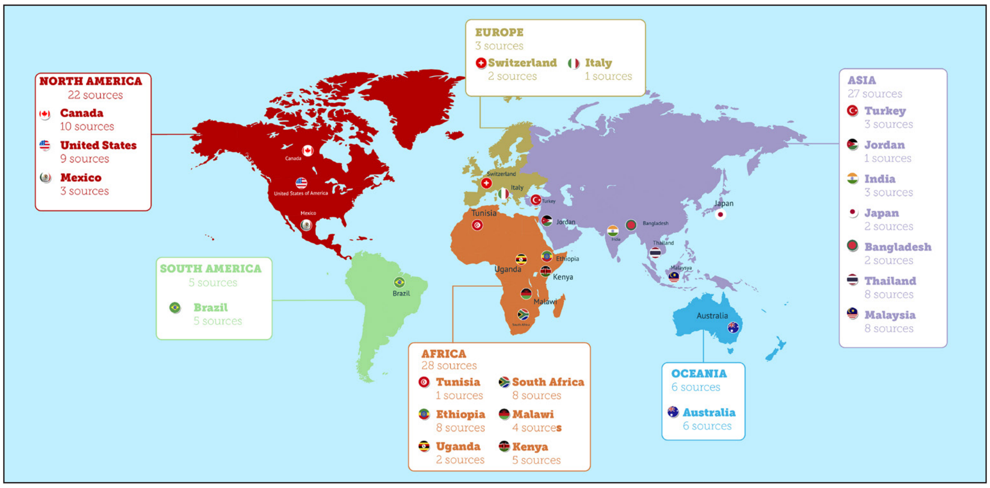

Figure 2. Indication of the continents, including their countries, where the parasitological contamination in the 90 springs in the scope of the current research were analysed

It is possible to observe, from the distance among the variables (Figure 4), that in Asia and Oceania there has been greater incidence in rural communities that consume water coming from rivers. Adding to that, the surface sources tend to overcome the action level for *Giardia*, with values above 0.05 cysts/L, as seen in group 5 in Figure 3.

In Figure 3, the water consumption of subterranean origin tends to be more prevalent in South America, and the rain water consumption was not associated with any continent, representing, thus, an outlier, due to the limitation of the quantity of data obtained and analysed, being a gap for future research.

In North America, within the sampling group analysed, the waters are less susceptible to contamination by *Giardia* due to the association to concentration below 0.05 cysts/L (Figure 3) (Wallis et al., 1996; Haas and Rose, 1995).

The contamination of water with *Cryptosporidium* spp. in varying levels was not associated with any continent (Figure 3). Similar results were identified in Figure 4, the group of variables located near the origin was not properly represented in this statistical analysis.

DISCUSSION

The water resources investigated in the rural zone were found to be contaminated with the *Cryptosporidium* spp. and *Giardia* spp. pathogens, in varying levels, creating risk to

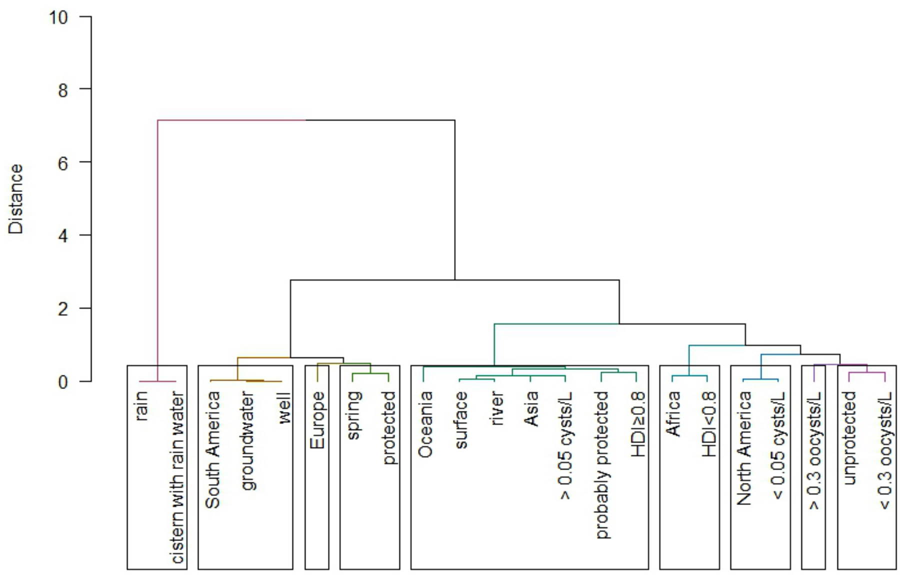

Figure 3. Cluster analysis dendrogram containing nine groups

Table 4. Methods for detecting *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts in water used in the articles selected by systematic review

Method (filtration/elution and concentration/purification procedures)	Recovery (R) of quality control test(%)	References
Filtration in membrane, without immunomagnetic separation technique	70.9% for *Cryptosporidium* and 48.3% for *Giardia*	(Franco et al., 2012)
	37.0% for *Cryptosporidium* and 53.0% for *Giardia*	(Tahar et al., 2022)
	UR	(Dworkin et al., 1996)
	UR	(Tiyo et al., 2015)
Filtration using PALL system with *Envirochek* ® capsules, with the immunomagnetic separation technique	99.0% for *Cryptosporidium*	(Pignata et al., 2019)
	UR	(Potgieter et al., 2020)
	57.0% for *Cryptosporidium* and 24.0% for *Giardia*	(Keeley and Faulkner, 2008)
	UR	(Dreelin et al., 2014)
Filtration in membrane, with immunomagnetic separation technique	UR	(Lee et al., 2017)
	UR	(Chambers et al., 2008)
	29.75% for *Cryptosporidium*	(Füchslin et al, 2012)
	30.0 – 40.0% for *Cryptosporidium* and 47.0 – 69.0% for *Giardia*	(Kifleyohannes and Robertson, 2020)
	UR	(Ono et al., 2001)
	UR	(Daniels et al., 2015)
	40.0% for *Cryptosporidium* and 41.0% for *Giardia*	(Ehsan et al., 2015)
	39.0% for *Cryptosporidium* and 45.0% for *Giardia*	(Chuah et al., 2016)
	UR	(Prystajecky et al., 2014)
Sheather's Flotation Technique	UR	(Chaidez et al., 2016)
Filtration in membrane through polypropylene string cartridge, with immunomagnetic separation technique	33.9 – 42.1% for *Cryptosporidium* and 43.1 – 50.8% for *Giardia*	(Balderrama-Carmona et al., 2015)
Flocculation with aluminium sulfate, without immunomagnetic separation	UR	(Koloren and Tas, 2012)
Modified Bailenger's Technique, flocculation with zinc sulfate	UR	(Ben Ayed et al., 2018)
Ultrafiltration in hollow fiber, with immunomagnetic separation	UR	(Morris et al., 2018)
Modified Ziehl-Neelsen staining	UR	(Sente et al., 2016)
Flotation with calcium carbonate, with immunomagnetic separation technique	59.0% for *Cryptosporidium*	(Robison et al., 2015)
Sedimentation followed of the immunomagnetic separation technique	UR	(Morse et al, 2008)
Filtration in membrane, staining with modified resistant acid	UR	(Abo-shehada et al, 2004)
Flotation with calcium carbonate, without the immunomagnetic separation technique	29.2 – 69.2% for *Cryptosporidium* and 31.4 – 74.0% for *Giardia*	(Thurman et al., 1998)
Filtration in membrane, flotation with Percoll-sucrose solution, without immunomagnetic separation technique	37.9% for *Giardia*	(Isaac-renton et al 1996)
	UR	(Ong et al., 1996)

Note: UR = unrealized. Source: Drafted by the Authors.

the population's health (Table 3). The risk of occurrence of giardiasis has shown greater tendency of incidence in the rural zone of the Asian and Oceania continents (Figure 3). In Asia, there was a greater percentage of population inhabiting rural areas, as well as in Africa (Worldatlas, 2023). North America has presented lower risk of infection by *Giardia* (Figures 3 and 4), however, there are possibilities of occurrence of outbreaks even if the concentration is below the action level, given that the ingestion of low doses of infectious cysts may trigger the infection in susceptible individuals (Karanis et al., 2007), such as the elderly, children, pregnant women and HIV carriers (Abubakar et al., 2007). It is necessary to highlight that our analysis was restricted to a small quantitative of studies and may not have revealed the risk in other continents (Africa, Europe, America).

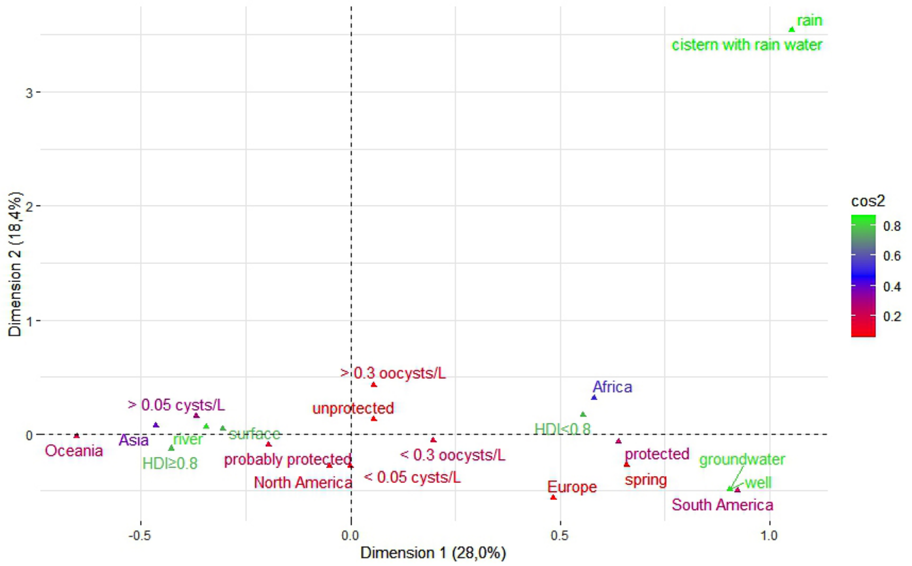

Figure 4. Map of factors containing the degree of association among the variables continent, spring type, water source type, water source situation and concentration obtained through multiple correspondence analysis

The data sources about risk to public health may be of epidemiologic origin or obtained through mathematical models of microbiological risk quantification (WHO, 2017). This systematic review found a single report of a cryptosporidiosis outbreak in the rural area in Washington (USA), associated to the consumption of rural well water contaminated by the irrigation system, which used residual treated water due to the damaged coating, in which 15 cases were confirmed (Dworkin et al., 1996). No evidence in the studies of giardiasis outbreaks linked to the aquatic environment, whether symptomatic or asymptomatic, within rural areas.

This does not mean that rural areas are being able to control the diseases, but reflects the underreporting of outbreaks in countries and regions with precarious access to sanitation infrastructure, as well as inefficiency of the health surveillance system (Baldursson and Karanis, 2011; Samarro and Sabogal-Paz, 2020; Ogura and Sabogal-Paz, 2022), as studies which validated the risk of parasitic diseases in rural areas is four times greater comparing to the risk in urban areas (Huang et al., 2023), considering that a majority of individuals in these areas live under conditions of isolation and geographical dispersion (Ngobeni et al., 2022) with financial and operational limitations of these rural communities in managing treatment systems (Chuah et al., 2016), putting them at high risk of exposure to waterborne pathogens (Potgieter et al., 2020).

Pitkänen et al. (2015) also did not report giardiasis outbreaks in small subterranean rural supplying systems in Finland (Europe), given that most cases of diarrhea diseases cause light symptoms, making it so that the ill person does not seek the health system for a treatment and, therefore, it is not reported (Brasil, 2021). There is also the possibility of individuals in these regions to have acquired protective immunity (Balderrama-Carmona et al., 2015). The unreported parasitic diseases may be the cause of several morbidities and silent mortalities in the rural environment (Sente et al., 2016), necessitating further in-depth studies to arrive at more assertive conclusions.

In this research the socioeconomic variable may be indicative of giardiasis incidence, because the greater risk was associated with countries with high HDI. These results are in accord with Baldursson and Karanis (2011), once that the developed countries have presented greatest occurrence of waterborne outbreaks due to the existence of an effective health vigilance system, as well as their own regulations for the control of these protozoans in aquatic environments (Dreelin et al., 2014). This scenario may be due to the high costs of the available methods for the detection of *Cryptosporidium* and *Giardia*, as the technique of filtration in membrane without immunomagnetic separation costing around US$ 137.50, and US$ 362.50 being the price of the filtration in membrane with the Filta Max (IDEXX®) system proposed in USEPA (2012), with the immunomagnetic separation step. These values were based on commercial values of the year of 2019 in Brazil.

The regulations for the management of human health risk, caused by the *Cryptosporidium* and *Giardia* pathogens have been elaborated as a reaction of the managers of developed countries to the outbreaks related to water (Dreelin et al., 2014). However, in the rural zones, there are low regulatory standards applied (Robison et al., 2015), or the absence thereof, and a minority of available data, given that few studies are directed towards the analysis of water sources used for drinking in rural regions in the world. That way, to prevent the negative effects of parasitic diseases in the rural population, more predominant than in urban environments, it is imperative that there is continuous monitoring of the parasitological contamination of the aquatic compartment, to offer managers information that helps on decision-taking, while expensive analytical methods are an obstacle (Dreelin et al., 2014).

The rural environments throughout the world represent a problematic of public health related to cryptosporidiosis and giardiasis, primarily due to the prevalent consumption of untreated surface and subterranean water sources (Huang et al., 2023; Dreelin et al., 2014). In exceptional way, the communities of Distrito Bau (Tahar et al., 2022), the small Italian villages (Pignata et al., 2019), the United States rural community (Robison et al., 2015), the Swiss Alpine Hamlet (Füchslin et al., 2012), the Turkey Ilica Village (Koloren and Tas, 2012), the United States Alaskan Community (Chambers et al., 2008) and the Canadian Black Mountain Irrigation District (Isaac-Renton et al., 1996; Ong et al., 1996) have water with chlorine. However, these protozoans show high infectivity, can survive for long periods of time in the environment and are resistant to the process of traditional disinfection of water with chlorine (Karanis, 2011; Thompson, 2004; USEPA, 1992).

An indoor protection measure recommended by WHO (2017) against the exposure to the waterborne pathogens consists in boiling the water before consumption, and such measure is widely adopted in the rural region of the City of Galway (Ireland) (Bresnihan and Hesse, 2021), because of its efficiency of removal of protozoans, virus and bacteria of 6 log (WHO, 2017). The emission of alerts for highly vulnerable populations is recommended, in cases of acute health risk, when the *Cryptosporidium* spp. and *Giardia* spp. pathogens are detected in the water (Hunter, 2002; WHO, 2017).

To reduce impacts on the public health of the rural population, investments are needed in the implementation of water treatment solutions capable of removing *Cryptosporidium* and *Giardia*, such as the use of activated carbon filters for individual treatment (WHO, 2017). It is essential, also, that there is a diagnostic of the regional specificities of each community, of the demographic and socioeconomic conditions, the population dispersion and the conditions of sanitation, in order to propose an appropriate treatment technology (Balderrama-Carmona et al., 2015).

Karanis et al. (2007) reported that the giardiasis outbreaks in urban areas have been more frequent in water consumed without treatment from surface sources (6.1%), when compared to the risk of groundwater (3.8%). In rural areas, Dreelin et al. (2014) revealed that there is a greater disease burden linked to the consumption of surface waters. Similar results were identified in Figure 4, it was possible to perceive a tendency of association between the surface sources and water contamination above the action level for *Giardia* spp.

Among the methodologies presented in Table 4 and used by the 29 articles analyzed, it was noticed that some studies did not adopt the immunomagnetic separation technique endorsed by the USEPA (2012), in order to amplify the parasite's recovery capacity, or did not carry out control of quality of the method. The absence of these informations of technical characteristic, underestimates the contamination of water resources and may propitiate false negative results (Pitkänen et al., 2015). USEPA establishes the method's acceptance criteria, in which the average recovery must vary from 38–100% for *Cryptosporidium* and 27–100% for *Giardia* (USEPA, 2012). Therefore, the *Cryptosporidium* spp. recovery (Franco et al., 2012; Robison et al., 2015; Pignata et al., 2019; Kifleyohannes and Robertson, 2020; Ehsan et al., 2015; Chuah et al., 2016; Keeley; Faulkner, 2008; Balderrama-Carmona et al., 2015; Thurman et al., 1998) and *Giardia* spp. (Franco et al., 2012; Tahar et al., 2022; Kifleyohannes and Robertson, 2020; Ehsan et al., 2015; Chuah et al., 2016; Balderrama-Carmona et al., 2015; Thurman et al., 1998) recovery is in accordance with the international guide (Table 4).

It is needed to highlight that the realistic evaluation of pathogens concentration is related to the recovery rate of the method adopted (Efstratiou et al., 2017a). Facing that, the contamination of water resources may surpass the values reported in this research in the sources in which there was no quality control of laboratory procedures (Table 4). That is why the real contamination level is unknown.

The low concentration of pathogens reported in surface and subterranean waters might be related to the low concentration in the water source or the method's restrictions (Morris et al., 2018).

The analysis of the 90 aquatic environments revealed a tendency that the surface waters in rural areas may present a concentration of *Giardia* spp. cysts above the action level of 0.05 cysts/L (Figure 3). The parasitological contamination of surface waters of the communities in Malaysia (Lee et al., 2017; Tahar et al., 2022), South Africa (Potgieter et al., 2020), Turkey (Koloren and Tas, 2012) and Japan (Ono et al., 2001) was associated to diffuse pollution from farming outflow, transportation of faecal matter which got contaminated during the rain events and inadequate sanitation conditions.

It is verified that there is a wide diversification of the sources of water pollution, which ranges from domestic and wild animal faeces, confined or not, human faeces and failures in the local wastewater treatment system (Wyer et al., 1996; Jones and Obiri-Dans, 1998; Goss and Richards, 2008). In the present systematic review, it was found that the *C. hominis* species was isolated in four surface waters (Daniels et al., 2015; Ehsan et al., 2015; Prystajecky et al., 2014), along with the *C. parvum*, isolated in five surface waters (Füchslin et al., 2012; Keeley and Faulkner, 2008; Prystajecky et al., 2014) and two groundwater (Füchslin et al., 2012; Prystajecky et al., 2014). These species represent the most common causes of outbreaks of gastrointestinal diseases in humans (Xiao et al., 2004; Ryan et al., 2014), including those supplied by private sources or small treatment systems (Craun et al., 1998), indicating, thus, a human, bovine and mixed (Human and bovine) source of faecal contamination in the water (Dreelin et al., 2014).

The *Giardia duodenalis,* genotypes A and B, was isolated from six surface waters (Ong et al., 1996; Daniels et al., 2015; Prystajecky et al., 2014) and a groundwater (Prystajecky et al., 2014). It is worth highlighting that only the subgenotypes AI and BIII have zoonotic potential (Thompson, 2004), being that these were not isolated in waters of rural regions. No evidence of zoonotic transmission of the *Cryptosporidium* spp. and *Giardia* spp. protozoans were identified in this research. The adequate manure handling represents an important measure in the rural environment, where there is predominance of agriculture and cattle farming activities, to prevent the zoonotic transmission (Dreelin et al., 2014; Goss and Richards, 2008). Notably, the composting with high temperatures is effective in inactivating (oo)cysts, as opposed to treatment involving lime supplementation (Van-Herk et al., 2004).

In the subterranean water sources, the shallow well of the Potam Community, located in Sonora, Mexico, was the groundwater with the greatest rate of environmental load of the *Cryptosporidium* spp., due to the contamination by infiltration of domestic effluents coming from precarious septic tanks (Balderrama-Carmona et al., 2015). This solution is very common in the rural area (Vale et al. 2022). The contamination of wells with *C. parvum,* in Switzerland, was caused by the presence of bovines in the catchment zone during the sample collection (Füchslin et al., 2012).

The need for works focused on the quality of subterranean waters in rural area, in order to understand the pathogen load, the sources of contamination and the routes of transportation of the (oo) cysts, as well as the limited movement in water and land should be highlighted (Dreelin et al., 2014).

Tendencies

Water sources intended for rural supply tend to be unsafe if consumed without treatment, due to parasitological contamination. This result reinforces the need for interventions on the environmental, economic, social and political fronts that aim at universal access to drinking water, a basic human right capable of contributing to the reduction of poverty in rural communities (Ribolzi et al., 2011), as established in the Goal of Sustainable Development 6 by the ONU (2011).

There is a tendency of giardiasis incidence in surface waters and in countries with high Human Development Index, but, even then, it is not possible to discard the risk of cryptosporidiosis facing the limitation of different methods of detecting protozoan,which present discrepant recovery rates. That said, there is a need to alert the managers so that they implement preventive measures for the control of gastrointestinal diseases in rural regions. There are few studies directed towards the conditions of sanitation and health in rural areas, focused on the protozoan *Cryptosporidium* spp. and *Giardia* spp. in water, due to the high cost of the analytical method. Therefore, it is necessary to increase the studies aimed at rural populations, especially those in conditions of social vulnerability, without access to sanitation.

CONCLUSIONS

From the obtained results, it is possible to conclude that:

1. At worldwide level, the surface and subterranean water sources used in rural areas present parasitological contamination at varied levels. There is a higher tendency of health risk for the rural population residing in Asia and Oceania regarding giardiasis, particularly in countries with HDI above 0.8, if the water is consumed without treatment.
2. There are few studies of environmental contamination of the water resources destined to the supplying of rural areas when compared to studies focused on the urban area, identifying more predominance of studies in the African and Asian continents, due to the high percentage of people living in rural areas on these two continents.
3. In the articles selected in this research, there were no giardiasis outbreaks; however, the fact that the concentration of pathogens may have been higher than reported due to the low recovery of the protozoan detection methods must not be discarded.
4. Outbreak of cryptosporidiosis reported in the US reinforces the necessity of research focused on the quality of subterranean water in rural areas and the possible sources of contamination.
5. The surface water destined to the supplying of rural areas tends to present contamination *Giardia* spp. above the action level of 0.05 cysts/L.
6. No evidence of zoonotic transmission has been reported in the eligible articles, but zoonotic specimens were isolated, such as the *C. parvum* and *C. hominis*.
7. The *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts are resistant to the methods of disinfection commonly applied, making a boil water order the most recommended approach for mitigating the incidence of waterborne parasitic infections in rural settings.
8. Despite the problems associated with analyzing water samples for *Giardia* and *Cryptosporidium*, the data has the potential to provide support to health and sanitation authorities.

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