Food and Waterborne Parasitology 19 (2020) e00073

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

journal homepage: www.elsevier.com/locate/fawpar

Preliminary insights regarding water as a transmission vehicle for *Cryptosporidium* and *Giardia* in Tigray, Ethiopia

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ARTICLE INFO

Article history:
Received 30 September 2019
Received in revised form 3 January 2020
Accepted 7 January 2020

Keywords:
Cryptosporidium
Cyst
Drinking water
Ethiopia
Giardia
Oocyst

ABSTRACT

This study was part of a larger One Health project with the aim of investigating the epidemiology of *Cryptosporidium* and *Giardia* infections among humans and animals in rural areas of Tigray, Ethiopia. Here we report on the contamination of different drinking water sources in four locations of this region with these *Cryptosporidium* oocysts and *Giardia* cysts; 19 samples were from unprotected surface water sources and 18 from protected water sources. A modified version of the standard ISO 15553 technique was used for analysis, and *Giardia* cysts were detected in 6 of the samples (16%) and *Cryptosporidium* in two (5%), with one of these samples containing both parasites. The number of *Giardia* cysts in positive samples ranged from 3 to 22 cysts per 10 L sample, and the number of *Cryptosporidium* oocysts in positive samples ranged from 1 to 3 oocysts per 10 L sample. Low numbers of parasites and absence of nuclei, as indicated by the absence of DAPI staining, precluded further molecular analyses. We found no association with contamination and whether the water source was protected or not, but there was an association with location, with one location more likely to have a contaminated sample than the others. These preliminary data suggest that this location should be in focus for further parts of this study.

1. Introduction

Deaths due to diarrhoeal disease among children in Ethiopia below the age of 5 years is calculated to have decreased by over 60% between 2005 and 2015. However, as the mortality rate in this age group due to diarrhoea is still at around 100.1 per 100,000 children, it remains considerably over the equivalent global mortality rate, which is estimated at 74.3 per 100,000 (GBD, 2017). Indeed, diarrhoea is considered to be the leading cause of mortality in Ethiopian children younger than 5-years of age, accounting for 23% of all deaths in this age group – more than 70,000 children a year (https://www.unicef.org/ethiopia/water-sanitation-and-hygiene-wash). Among the aetiologies associated with mortality due to diarrhoea in children under 5 years in Ethiopia, cryptosporidiosis was found to be responsible for 12% of cases, only surpassed by shigellosis and rotaviral enteritis, responsible for approximately 20% and 18% of cases, respectively (GBD, 2017). Studies on the occurrence of *Cryptosporidium* infections in Ethiopia

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https://doi.org/10.1016/j.fawpar.2020.e00073

have reported prevalence ranging from as low as 1% to as high as over 26% (Squire and Ryan, 2017); these estimates are presumably affected by patient characteristics (age group, clinical symptoms, immune status) as well as diagnostic technique used.

Another intestinal protozoan parasite that is often considered together with *Cryptosporidium*, is *Giardia duodenalis*. Although infection with *Giardia* is known as a cause of diarrhoea, the Global Enteric Multicentre Study (GEMS) that investigated aetiologies of moderate-to-severe paediatric diarrhoea (Kotloff et al., 2013), found no association between *Giardia* infection and these symptoms in young children; *Giardia* infection in children aged between 1-5 years was more commonly identified in children without diarrhoea. However, *Giardia* infection has been associated with persistent diarrhoea in school-age children in developing countries (Muhsen and Levine, 2012), and studies in Ethiopia have reported a prevalence of *Giardia* infection ranging between below 5% to over 50% (Squire and Ryan, 2017).

Cryptosporidium and *Giardia* are both transmitted to susceptible individuals via ingestion of infectious stages that have been excreted in faeces of infected people (or animals – some species or genotypes may be zoonotic). The infection route may be direct, hand to mouth, but ingestion via a contaminated transmission vehicle (food or water) also occurs commonly, and may result in large outbreaks (e.g., Widerström et al., 2014), particularly if the drinking water supply is contaminated. Although lower-income countries are more likely to have less sophisticated infrastructure regarding sewage disposal and water treatment, and thus may be expected to report more waterborne outbreaks of disease (e.g., Widerström et al., 2014), most waterborne outbreaks have been reported from developed countries (Omarova et al., 2018). Indeed, according to Aldeyarbi et al. (2016), no outbreaks of cryptosporidiosis have been reported from Africa (by any transmission route), leading these authors to question whether such outbreaks occur; furthermore, according to Ahmed et al. (2018), no outbreaks of protozoan disease transmitted by water has been reported in Africa either. As outbreaks of diarrhoeal disease are usually identified by medical practitioners identifying a rise of cases in diarrhoeal disease above background levels, when diarrhoeal disease occurs frequently, from different aetiologies, recognising an outbreak may be challenging. However, as noted by Ahmed et al. (2018), many risk factors associated with waterborne transmission occur in African countries and the few studies that have been conducted regarding occurrence of parasites in water sources in Africa indicate that they do occur; an overview of some of these studies and their results is provided in Squire and Ryan (2017). However, it should be noted that sampling and detection methodologies varies widely between studies, from basic microscopy studies to molecular methods, and it seems likely that not all data are equally reliable.

From Ethiopia, the only article that we were able to identify regarding analysing water for these parasites reports investigation of 115 samples of drinking water collected from various sources around Addis Ababa (Atnafu et al., 2012) using the standard US EPA methodology, with detection by immunofluorescent antibody testing (IFAT). Among 72 tap water samples analysed, 15 (21%) were considered to contain *Cryptosporidium* oocysts and 12 (17%) were considered to contain *Giardia* cysts; among 17 storage tank samples, 6 (35%) were considered to contain *Cryptosporidium* oocysts and 5 (29%) were considered to contain *Giardia* cysts; two raw surface water samples were positive for both parasites; and the single well water sample was positive for *Cryptosporidium* oocysts but negative for *Giardia* cysts (Atnafu et al., 2012). However, no attempt was made at molecular characterisation of the parasites.

Although other studies from Ethiopia have attempted to associate infection risk of these parasites with water source (e.g., Tigabu et al., 2010; Ayalew et al., 2008) with discrepant results, to the best of our knowledge further surveys for these parasites in drinking water in Ethiopia have not been conducted.

As part of a larger One Health study on the epidemiology of cryptosporidiosis and giardiasis among humans and animals in rural areas of Tigray, Ethiopia, we investigated the contamination of different drinking water sources in four locations.

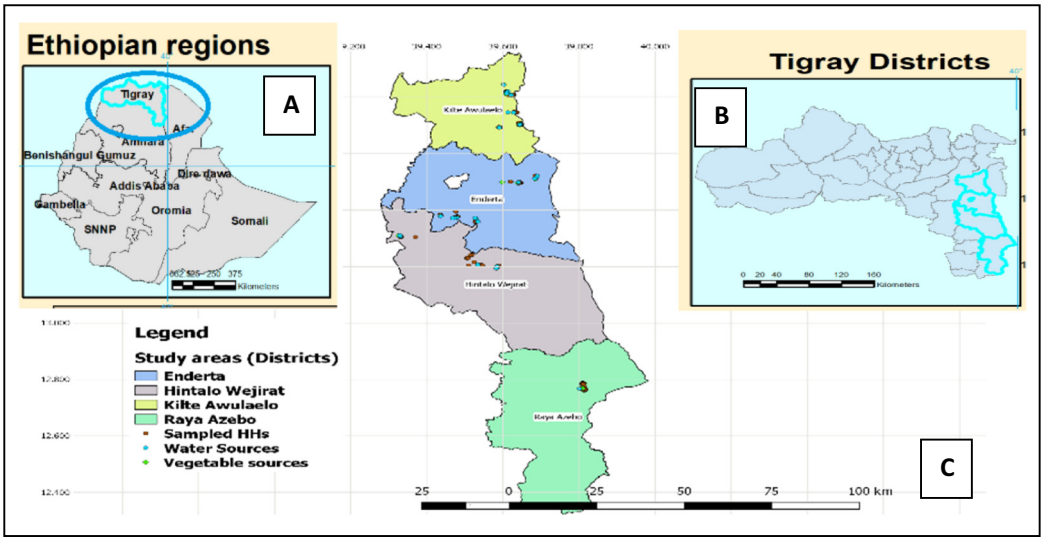

Fig. 1. Maps showing location of sampling areas. A: Map indicating location of Tigray in Ethiopia; B: Map indicating districts in Tigray; C (main map) indicating the four sampling areas.

2. Materials and methods

2.1. Sampling locations and sample collection

This project was based on four main sampling regions, Enderta, Hintalo Wejirat, Kilte Awulaelo, and Raya Azebo (see Fig. 1).

The rural area of Enderta district has around 24,600 households and an approximate total population of 114,300 (CSA, 2007). The average annual temperature in Mekelle, the main city in Enderta, is 19.1 °C and the average rainfall is 581 mm. The rural part of Hintalo Wejirat has approximately 31,000 households and the total population is approximately 141,000. The average annual temperature in Adi Gudom, the main town of Hintalo Wejirat, is 19.1 °C, and the average annual rainfall is 520 mm. The rural area of Kilte Awulaelo district has 20,222 households with a total population of 94,900. The average annual temperature in Agula (a village in Kilte Awulaelo) is 19.2 °C, and the average annual rainfall is 595 mm (https://en.climate-data.org/africa/Ethiopia/tigray). The rural area of Raya Azebo district has 27,582 households with a total population of 119,814. The area receives rainfall ranging from 300 to 750 mm, and the mean annual maximum and minimum temperatures are 25 °C and 18 °C, respectively (http://www.eiar.gov.et/mehoni).

The majority of people living in this area obtain their drinking water from unprotected water sources (streams/rivers/ponds etc.) or via communal hand pumps. In one of the districts, Raya Azebo, more water sources were unprotected (47%) than in the other three regions (21–25%) (CSA, 2007). Regarding sanitation in this area, most people do not have any access to toilet facilities and are defecating in open spaces (Table 1; CSA, 2007).

All samples were collected during the period from October 2018 until January 2019. A total of 37 samples were collected for analysis, 15 from Enderta, 7 from Hintalo Wejirat, 13 from Kilte Awulaelo, and 2 from Raya Azebo. Samples were collected into clean 10 L plastic containers and transported immediately to Mekelle University for the initial steps in the analysis. Weather during collection of all samples was consistently dry. Samples were collected from drinking water sources according to convenience sampling (sites available and accessible for sampling) and categorised as either tap water, well water, handpump, pond, or river/stream. Water sources were grouped as either untreated surface water (rivers/streams, well water, or ponds) or as protected water sources (tap water or handpump water). It should be noted that water from handpumps and tap water could have originated from unprotected surface water sources. Among the 37 samples, 14 were from rivers or streams, 4 were from ponds, and 1 was from a well (i.e., total of 19 samples grouped as untreated surface water), 15 were from handpumps, 3 were tap water, (i.e., 18 grouped as protected water sources). See Table 2 for overview of sample distribution.

2.2. Sample analysis

Standard methods for the analysis of water for contamination with *Cryptosporidium* and/or *Giardia* cysts have been developed, validated, and adopted globally. In Europe, the ISO 15553 standardized method for analyses of potable water for contamination with *Cryptosporidium* and *Giardia* is probably the most commonly used approach (ISO, 2006), and is very similar to the US EPA 1623.1 methodology (US EPA, 2012). Both these standard methods include the following steps: filtration, elution, concentration by centrifugation, separation and purification of target parasites by immunomagnetic separation (IMS), and finally detection and enumeration using (IFAT), using the fluorogenic DNA intercalator 4′,6-diamidino-2-phenylindole (DAPI) as an adjunct stage for detection of nuclei. As IMS is extremely expensive, this method is not possible for many research projects and certainly prohibitively expensive for routine monitoring in developing countries. However, a reduced cost approach has been developed and evaluated by intra-laboratory spiking studies, as well as being used with success in a project in which water samples from Northern India were analysed (Utaaker et al., 2019). The same approach was used here with the 5–7 ml sample concentrate obtained after filtration and centrifugation being further processed with IMS using a reduced volume 20 μl of each bead type (Dynabeads®: Cryptosporidium/Giardia Combo Kit, Idexx Laboratories), and the kit buffers are modified by augmenting with buffers as described in Utaaker et al. (2015). Prior to analysis of the samples, spiking experiments using this procedure were conducted with flow-sorted, commercially available *Cryptosporidium* oocysts and *Giardia* cysts (EasySeed™, TCS Biosciences, UK). Recovery efficiencies were between 30 to 40% for *Cryptosporidium* and 47 to 69% for *Giardia* (7 replicates). This is within the range considered acceptable using the ISO or US EPA methods.

For the samples in this project, the samples were filtered through Millipore Isopore membrane filters with a pore size of 2 μm at Mekelle University, using a Watson-Marlow 520 Bp Profibus pump. Following filtration, the filters were placed in 50 ml

Table 1
Sanitation facilities in sampling area (derived from: CSA, 2007).

District	Percentage of households without any toilet facility	Percentage of households with shared toilet facility (flush or latrine)	Percentage of households with private toilet facility
Kilete Awulaelo	70	6	24
Enderta	82	2	16
Hintalo Wejirat	73	3	24
Raya Azebo	78	8	14

Table 2
Sample distribution (number of samples) by location and water source.

	Enderta	Hintalo Wejirat	Kilte Awulaelo	Raya Azebo	Total
Unprotected surface water					
Ponds	0	2	2	0	4
Rivers streams	10	1	2	1	14
Well	0	0	1	0	1
Group total	10	3	5	1	19
Protected water source					
Handpump	5	3	6	1	15
Tap water	0	1	2	0	3
Group total	5	4	8	1	18
Overall total	15	7	13	2	37

centrifuge tubes that were then filled with sample water and stored at 4 °C. These sample tubes containing filters were then transported to the NMBU parasitology laboratory in Norway for the final stages of the analyses (IMS and IFAT).

In Norway, the filters were washed as according to the 15,553 protocol (ISO, 2006). The eluate was collected into 50 ml conical-base centrifuge tubes that were then centrifuged at 1690 rcf (relative centrifugal force) for 10 min. The supernatant was aspirated and the pellets concentrated into a single tube. IMS was performed as described by Utaaker et al. (2019), and the beads and any captured parasites subsequently dissociated by vigorous vortexing under acidic (0.1 M HCl) conditions. The final suspension of 50 μl was pipetted onto a single-well slide (Novakemi ab, Sweden), neutralised with sodium hydroxide, and air-dried at room temperature. The dried samples were methanol fixed before staining with FITC-conjugated monoclonal antibodies (mAbs) against *Cryptosporidium* oocyst walls and *Giardia* cyst walls (Aqua-Glo™, Waterborne™, Inc., USA). DAPI-staining was used for highlighting nuclei, both to assist in identification and for identifying samples for proposed molecular analysis for species and/or genotype identification. Samples were mounted with 1,4 diazabicyclo-octane (DABCO) antifade Mounting Medium, then each slide was covered by a glass coverslip and viewed immediately by fluorescence microscopy using a Leica DCMB microscope (20×, 40×, and 100× objectives), equipped with Nomarski differential interference contrast (DIC) optics. A blue filter block (480 nm excitation, 520 nm emission) was used for screening for *Giardia* cysts and *Cryptosporidium* oocysts labelled with the mAbs, and a UV filter block (350 nm excitation, 450 nm emission) was used for investigating DAPI staining. Morphological traits of objects reacting with the mAb were investigated further by light microscopy using DIC optics.

Each slide was scanned systematically at 20× objective, and suspect objects examined more closely at higher magnification. *Cryptosporidium* oocysts and *Giardia* cysts were enumerated according to staining characteristics and morphology.

According to laboratory protocols, samples were considered "confirmed" positive if (oo)cyst(s) exhibited typical fluorescence, with correct shape and size, and internal contents such as characteristic nuclear staining, were apparent. If the morphometry was correct and the structure had typical fluorescence, but DAPI-staining was absent and there were no discernible contents, the (oo) cysts were described as 'putative'.

2.3. Data handling

A database was created in excel and results included continuously during analysis. Contingency table analysis was used to investigate associations between occurrence of parasites in water samples and regional location and type of water source.

3. Results

3.1. Overall occurrence of Cryptosporidium and Giardia in the water samples

Among the 37 samples, parasites were detected in 7 (overall prevalence of 19%), with 5 samples being positive for only *Giardia*, 1 sample being positive only for *Cryptosporidium*, and 1 sample being positive for both parasites (16% prevalence for *Giardia*, 5% prevalence for *Cryptosporidium*). Among the samples in which *Giardia* cysts were detected, the number of cysts detected ranged from 3 cysts per sample up to 22 cysts per sample (median of 9 *Giardia* cysts per 10 L), and all of the parasites were considered putative; that is, although their morphology was correct in terms of size and shape, and their walls demonstrated characteristic apple-green fluorescence indication reaction with the mAb, none of them had visible internal contents or nuclei with DAPI staining. For the two samples positive for *Cryptosporidium* oocysts, one sample (from a river/stream in Enderta) contained a single oocyst and the other, from well water in Kilte Awulaelo, contained 3 oocysts.

The possibility of further analysis for species or genotype using molecular methods was precluded by the absence of intact *Giardia* cysts and the low numbers of nucleated *Cryptosporidium* oocysts.

3.2. Association of parasite occurrence with water source

Among the positive samples, 6 were from Enderta and 1 (*Cryptosporidium* alone in a well water sample) was from Kilte Awulaelo. Parasites were not detected among the 9 samples analysed from the other two locations (Hintalo Wejirat and Raya

Azebo). Comparison of positive samples from Enderta with those from all other locations indicates a significant association ($p = 0.0113$) between this location and the occurrence of parasites in the water supply.

As more samples from Enderta were classified as unprotected surface water (rivers/streams or ponds) than protected water sources (10 of 15 samples from Enderta; see Table 1), and the majority of the unprotected surface water samples were from Enderta (10 of 19 unprotected surface water samples; see Table 1), we investigated whether water source could be associated with whether a sample was positive or not, but no association was detected either in just Enderta or combining all locations.

4. Discussion

These data provide a preliminary snapshot of the extent of water contamination with *Cryptosporidium* oocysts and *Giardia* cysts in rural Tigray in northern Ethiopia. Although the proportion of samples positive for *Cryptosporidium* was considerably lower than previously reported from a similar study in Addis Ababa, being over 20% in Addis Ababa (Atnafu et al., 2012) compared with just 5% in our study, for *Giardia* the prevalence was similar in both studies, at around 16%. The number of parasites detected in each positive sample was not provided in the study of Atnafu et al. (2012), but as it appears that viability testing was conducted (based on exclusion and inclusion of vital dyes), it seems probable that more parasites were detected in the Addis Ababa samples than in ours. With so few data and some differences in analytical technique (for example, sucrose flotation rather than IMS was used for purification in the Addis Ababa survey), it is difficult to reach any conclusions about these differences.

Although we were unable to detect any apparent difference between the likelihood of water from different sources being contaminated, previous studies have indicated that children drinking from "protected" water supplies (handpumps) were less likely to be infected with *Giardia* than children drinking from "unprotected" water supplies (hand-dug well, spring or dam) in the Benishangul-Gumuz Region of Northwestern Ethiopia (Tigabu et al., 2010). However, unexpectedly, the same study indicated that more cases of cryptosporidiosis were detected in children using protected water sources than in children non-protected water sources (Tigabu et al., 2010). This may indicate that in this region, the transmission route for *Cryptosporidium* tends not to involve water, but may be more associated with direct transmission. Another or additional reason for this could be that the "protected" water sources are nevertheless contaminated by infectious *Cryptosporidium* oocysts. Indeed, in the Benishangul-Gumuz study, however, the water supplies themselves were not tested for contamination.

Another study from Ethiopia, this time in the Dire Dawa district of Eastern Ethiopia, found no association between the occurrence of *Cryptosporidium* or *Giardia* infections in children and whether their drinking water source was protected (springs, boreholes, protected wells) or unprotected (surface water, rivers, seepage and unprotected wells) (Ayalew et al., 2008). However, as all water sources in the study area (including that of protected sources) had previously been found to be contaminated with faecal matter (Ayalew et al., 2008), perhaps this is not surprising. Indeed, in our study, both protected and unprotected water sources seemed to have a similar likelihood of being contaminated with these parasites. Again, in the study from Dire Dawa, the water itself was not analysed.

In our study, one district seemed to be particularly associated with contaminated water, and as a further arm of our study, this area seems to have a higher prevalence of these infections among livestock (Kifleyohannes, unpublished work). This may have resulted in the greater prevalence of parasites, due to contamination, but, conversely the greater contamination could contribute to greater levels of infection. Further analyses, including molecular analyses of the parasites in faecal samples from people and their animals, should be able to provide more information on the epidemiology of these parasites in this region. It is worth noting that in this area, Enderta, fewer households have access to toilet facilities, and this may lead to greater contamination of the environment (see Table 1; CSA, 2007). This could be a contributory factor.

The relatively few samples taken in our study over a limited period are clear limitations of the study, as the results provide only an overview of the situation at a specific time point. It is possible that the contamination would be higher (samples more likely to be positive with higher numbers of parasites) immediately after precipitation. Heavy precipitation occurs more frequently in the summer months, and sampling during this time period may have provided a different picture. However, other studies that have tried to investigate whether water sources are more or less likely to be contaminated with *Giardia* and *Cryptosporidium* during rainy seasons tend to find relatively weak correlations, or correlations with only one of the parasites (e.g., Carmena et al., 2007; Mons et al., 2009; Utaaker et al., 2019). A further limitation was the period of storage and shipping between filtration of the water samples and completion of the analysis (between 2-6 months); this may have affected the oocyst integrity and hence the possibility of genetic characterisation for which the presence of sporozoite nuclei is essential. A further limitation was that sampling in the various regions was restricted by pragmatic considerations; unfortunately, the area where data from the CSA (2007) indicates that unprotected water sources are most often used as drinking water supply was actually the sampling area from which fewest samples were collected.

In conclusion, although *Cryptosporidium* contamination of water was not found to be extensive in this study, contamination of drinking water with *Giardia* appeared to occur relatively frequently but was associated with particular districts. Our results indicate the importance of the One Health approach when considering transmission routes and epidemiology of such infections, with environmental sampling providing further information to results obtained for analysis of faecal samples from both humans and animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded under the MU-HU-NMBU Institutional Collaboration Phase IV, Improved Livelihood of the Rural Communities in the Arid Highlands of Northern Ethiopia. Additional support was provided by Dr. Habtamu Taddele, Dr. Yohannes Hagos, Mr. Tadesse Teferi, Mr. Haftay Abraha, district vet technicians, and drivers from Mekelle University who participated during water collection. Dr. Kjersti Selstad Utaaker provided the water pump and technical support in the lab when setting up the water analysis, and Dr. Getachew Terefe, Dr. Kristoffer Tysnes, Dr. John James Debenham and Professor Ane C. W. Nødtvedt are gratefully acknowledged for their support and general supervision in this project.

References

Ahmed, S.A., Guerrero Flórez, M., Karanis, P., 2018. The impact of water crises and climate changes on the transmission of protozoan parasites in Africa. Pathog. Glob. Health 112 (6), 281–293.
Aldeyarbi, H.M., Abu El-Ezz, N.M., Karanis, P., 2016. *Cryptosporidium* and cryptosporidiosis: the African perspective. Environ. Sci. Pollut. Res. Int. 23 (14), 13811–13821.
Atnafu, T., Kassa, H., Keil, C., Fikrie, N., Letalan, S., Keil, I., 2012. Presence, viability and determinants of *Cryptosporidium* oocysts and *Giardia* cysts in the Addis Ababa water supply and distribution system. Water Qual Expo Health 4 (1), 55–65.
Ayalew, D., Boelee, E., Endeshaw, T., Petros, B., 2008. *Cryptosporidium* and *Giardia* infection and drinking water sources among children in Lege Dini, Ethiopia. Tropical Med. Int. Health 13 (4), 472–475.
Carmena, D., Aguinagalde, X., Zigorraga, C., Fernández-Crespo, J.C., Ocio, J.A., 2007. Presence of *Giardia* cysts and *Cryptosporidium* oocysts in drinking water supplies in northern Spain. J. Appl. Microbiol. 102 (3), 619–629.
CSA, 2007. Population and Housing Census of Ethiopia (Central Statistical Agency) Addis Ababa, Ethiopia.
GBD Diarrhoeal Diseases Collaborators, 2017. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect. Dis. 17 (9), 909–948.
ISO (International Organization for Standardization), 2006. ISO Standard 15553:2006 Water Quality—Isolation and Identification of *Cryptosporidium* Oocysts and *Giardia* Cysts from Water. International Organization for Standardization, Geneva, Switzerland.
Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., et al., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382 (9888), 209–222 2013.
Mons, C., Dumètre, A., Gosselin, S., Galliot, C., Moulin, L., 2009. Monitoring of *Cryptosporidium* and *Giardia* river contamination in Paris area. Water Res. 43 (1), 211–217.
Muhsen, K., Levine, M.M., 2012. A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. Clin. Infect. Dis. 55 (Suppl. 4), S271–S293.
Omarova, A., Tussupova, K., Berndtsson, R., Kalishev, M., Sharapatova, K., 2018. Protozoan parasites in drinking water: a system approach for improved water, sanitation and hygiene in developing countries. Int. J. Environ. Res. Public Health 15 (3). https://doi.org/10.3390/ijerph15030495 pii: E495.
Squire, S.A., Ryan, U., 2017. *Cryptosporidium* and *Giardia* in Africa: current and future challenges. Parasit. Vectors 10 (1), 195.
Tigabu, E., Petros, B., Endeshaw, T., 2010. Prevalence of giardiasis and cryptosporidiosis among children in relation to water sources in Selected Village of Pawi Special District in Benishangul-Gumuz Region, Northwestern Ethiopia. Ethiop. J. Health Dev. 24 (3), 205–213.
US EPA (United States Environmental Protection Agency, Office of Water), 2012. Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA.
Utaaker, K.S., Huang, Q., Robertson, L.J., 2015. A reduced-cost approach for analyzing fresh produce for contamination with *Cryptosporidium* oocysts and/or *Giardia* cysts. Food Res. Int. 77, 326–332.
Utaaker, K.S., Joshi, H., Kumar, A., Chaudhary, S., Robertson, L.J., 2019. Occurrence of *Cryptosporidium* and *Giardia* in potable water sources in Chandigarh, Northern India. J. Water Supply Res. Technol. AQUA 68 (6), 483–494.
Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., Ferm, M., Björkholm, B., Hansen, A., Hiltula, J., Långmark, J., Löfdahl, M., Omberg, M., Reuterwall, C., Samuelsson, E., Widgren, K., Wallensten, A., Lindh, J., 2014. Large outbreak of Cryptosporidium hominis infection transmitted through the public water supply, Sweden. Emerg. Infect. Dis. 20 (4), 581–589.

Web references

https://www.unicef.org/ethiopia/water-sanitation-and-hygiene-wash Accessed on September 10, 2019.
http://www.eiar.gov.et/mehoni Accessed on September 14, 2019.
https://en.climate-data.org/africa/Ethiopia/tigray Accessed on September 14, 2019.