Original article

Human parasitic protozoa in drinking water sources in rural Zimbabwe and their link to HIV infection

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Abstract

Objective We aimed to perform a risk assessment in a rural setting, where drinking water is obtained from both protected and unprotected deep or shallow wells, boreholes and springs. Water is consumed untreated and this poses a risk of acquiring waterborne infections that may cause diarrhea.

Methods The study included 113 study participants who volunteered in Chiweshe rural community (Musarara village) in Mashonaland Central Province in Zimbabwe. There were 34 (30%) males and 79 (70%) females with ages ranging from 2 to 89 years. HIV counseling was carried out at the communal meeting and testing was done at home visits. Stool and drinking water samples were collected from 104 subjects. Routine laboratory methods were used to examine for parasitic infections.

Results Only 29 (25.7%) of participants were confirmed HIV positive using 2 rapid serology tests; eighty-four (74.3%) were negative. Diarrheic stool samples were observed in 17 (16.3%) participants and of these 5 (29.4%) were HIV seropositive. Several parasites were isolated from stool samples: *G. duodenalis* 6 (5.7%), *E. histolytica/dispar* 19 (18.2%), *C. parvum*, 8 (7.6%) and *C. cayetanensis* 23 (22.1%). Eleven out of 30 (36.6%) water bodies had protozoan parasites: *G. duodenalis* 2 (6.6%), *E. histolytica* 4 (13.3%), *C. parvum* 1 (3.3%), *C. cayetanensis* 3 (10%), *E. coli* 1 (3.3%).

Conclusion The water sources were being used without treatment and were shown to pose a risk for acquiring diarrheagenic protozoan parasites.

Keywords Diarrhea, parasitic protozoa, drinking water, HIV/AIDS

Introduction

The World Health Organization (WHO) estimated that 88% of all diarrheal cases can be attributed to unsafe water supply.[1] It is also reported that 80% of all illness in developing countries are related to water and sanitation.[2] This affects mostly children and those that are immune-compromised, increasing their morbidity and mortality.[3,4] More than 25 million people in Sub-Saharan Africa are living with HIV.[5] Water is an indispensable commodity to everyone especially for people living with HIV/AIDS (PLWHA) as they have to take their medications with it.

Waterborne diseases are the most important concern when it comes to the quality of water.

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The pathogens involved include a wide variety of viruses, bacteria and protozoan parasites. Outbreaks of waterborne protozoan parasitic infections have been linked to contamination from sewage, wastewater effluent, etc. There are also other potential sources for protozoan oocysts and cysts alike, such as pasturing infected livestock, watering of infected animals, and disposal of infected feces of human or animal origin. Authorities deliver well-informed interventions and respond properly and timely to health threats when there is a proper surveillance system of waterborne protozoan infectious diseases. Surveillance systems should target water treatment processes, design of waste management systems, sanitation, and hygiene practices in communities, to name a few.

There have been recorded outbreaks of infection with *Cryptosporidium parvum, Giardia duodenalis* and *Entamoeba histolytica* due to treated tap water in several developed countries such as the United States, United Kingdom, Canada and Sweden, as chlorine that is commonly used to disinfect water does not kill protozoan parasites.[6-11] Multicentre studies on protozoan parasite outbreaks reported that 50.8% of these outbreaks were caused by *C. parvum*, followed by *Giardia duodenalis* with 40.6% and in smaller percentages by *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Isospora belli*, etc.[12] In developing countries, infection with *Cryptosporidium parvum* is highly prevalent during the first two years of life, when up to 45% of children experience the disease.[13] In Sub-Saharan Africa cryptosporidiosis has been shown to be correlated with HIV infection, the prevalence of HIV-positive children with diarrhea ranging from 13% to 73.5%.[14,15]

In rural settings such as those found in Zimbabwe or South Africa, drinking water is obtained from both protected and unprotected deep or shallow wells, boreholes or springs.[16] Water is consumed untreated and this represents a risk factor for acquiring waterborne infections that may cause diarrhea. Several studies have highlighted *Cryptosporidium* as the most common pathogen associated with diarrhea in patients with HIV infection.[17,18]

Coliform bacteria are used as indicators of fecal contamination of drinking water[1,19] but their persistence in water does not correlate with parasites and viruses.[19] Therefore specific tests have to be done for identification of these organisms and these are not routinely performed in developing countries due to lack of resources. The gold standard for detecting environmental protozoa such as *Giardia* and/or *Cryptosporidium* in source waters is represented by US Method 1622 and US Method 1623.[20,21] These methods are too costly in developing countries like Zimbabwe. Therefore we performed a study to ascertain the presence of protozoan parasites in a variety of water bodies in a rural setting using the limited resources available.

Methods

Ethics approval and informed consent

Approval was obtained from the Medical Research Council of Zimbabwe. Consent was also obtained from the stakeholders of Mashonaland Central Province, Director, the District Medical Officer, the District Administrator, the two traditional chiefs of that area, the village headman and the villagers themselves. Counsellors from the local hospital did the pre- and the post-counseling services for HIV for the study population. The objective of the study was explained to the people.

Study site and sample collection

The study site was Chiweshe rural area, 70 km from Harare, lying on a longitude of 310° 00', latitude of 17° 14' and altitude of 1250 m. Stool and drinking water samples were collected from 113 people, 34 males and 79 females, with an age range of 2 to 89 years.

HIV testing

Home visits were done and HIV testing using 2 rapid tests kits, namely SD BIOLINE HIV 1/2 (Standard Diagnostics, INC, Korea) and Determine HIV I /2 kit (Abbot Laboratories, Tokyo, Japan) was carried out on the spot although results were not disclosed as this would be done by the counsellors at the hospital.

Water testing

The physiochemical analysis of water was also done on the spot. Measurement of turbidity was done using the HACH Portable 2100P Turbidometer (Hach company, Loveland Colorado, USA), pH and temperature were determined using the Boeco Germany PT370 pH meter and conductivity was measured using the HI98311 conductivity meter (Hanna Instruments, Mauritius). Specimens were collected separately in wide mouthed plastic bottles for different tests such as for physicochemical tests and 1000 mL of water were used for parasitological analysis. The samples were stored in cooler boxes, and then rushed to the laboratory for processing within 4 to 6 hours.

Parasitological processing of stool samples

Stool specimens were macroscopically described and wet preparations were done using saline.[22] Laboratory tests included the formol-ether technique and the Gomori thrichome staining. The cold Ziehl-Neelsen method was carried out to identify intestinal sporozoites such as oocysts of *Cryptosporidium*.[22]

Parasitological processing of water samples

Water was collected in 1 liter amounts for parasitological analyses. The samples were spun at 500 G for 3 minutes. Wet preparations were also prepared. The zinc sulphate floatation technique was then carried out to identify intestinal sporozoites as described by Cheesbrough, 2005.[22]

Data management and analysis

The data was analyzed using SPSS 8. Descriptive statistics on parasite species in humans versus their HIV status were determined at 5% significance level and a stratified analysis was used to control for confounding factors. Chi-square test was used to detect association between drinking water sources and protozoa.

Results

Demographic data and HIV status of participants

Out of the 113 participants, 29 (25.7%) of the study subjects were HIV-positive and 84 (74.3%) were HIV-negative by two rapid serology tests. One hundred and four (92%) submitted stool specimens whilst 90 (79.6%) submitted both stool and water samples. Seventeen participants (16.3%) produced diarrheic stool samples; of these 12 (70.5%) had parasitic infections and 5 (29.4%) were HIV seropositive (see Table).

Table. Identified human parasites and health status

Identified parasites	Infected people, no. (%)	HIV-positive		HIV-negative	
		Diarrhea	No diarrhea	Diarrhea	No diarrhea
Entamoeba coli	45 (43.3%)	0	6	7	32
Cyclospora cayetanensis	23 (22.1%)	0	1	1	1
Entamoeba histolytica	19 (18.2%)	1	2	3	13
Iodamoeba bütschlii	15 (14.4%)	0	1	3	11
Chilomastix mesnili	11 (10.6%)	0	0	2	9
Cryptosporidium parvum	8 (7.6%)	2	3	2	1
Giardia duodenalis	6 (5.7%)	0	2	0	4
Endolimax nana	4 (3.8%)	1	0	0	3
Blastocystis hominis	3 (2.8%)	1	0	1	1
Trichomonas hominis	1 (0.9%)	0	0	0	1
Schistosoma mansoni	1 (0.9%)	0	1	0	0
No parasites	25 (24%)	1	6	1	16

Note: where values do not add up to the total, there were missing values regarding the patient's HIV status.

Polyparasitism was evident, with 20 patients (22.2%) harboring 2 different species of protozoan parasites, 15 (16.6%) harboring 3 species and 7 (7.7%) harboring 4 species. Four of them obtained their water from deep protected wells. Two of these four were positive for *E. histolytica*, 1 for *C. parvum* and the other harbored both *G. duodenalis* and *C. cayetanensis*. The participant whose water source was unprotected was infected with *E. histolytica* and the same parasite was also identified in the source of water.

Water source usage and parasitic contamination

Water samples were collected from thirty different water bodies. The most common water sources in the study area were deep protected wells (14, 46.6%) which were used by 34 (33.66%) of the study population. The second most common source of water was represented by deep unprotected wells (5, 16.6%) but it was utilized by only 3 people (2.97%) of the study population. Safe sources of water were very few in this setting, only one communal water tap (3.3%) which was accessible to only 4 people (3.96%) of the study population. There were only two boreholes (2.6%) in the study area and this source was used by 49 (48.51%) of the participants.

Thirty six percent (11/30) of the water bodies had protozoan parasites. Two (6.6%) of the water bodies had *G. duodenalis,* 4 (13.3%) had *E. histolytica*, 1 (3.3%) had *C. parvum*, 3 (10%) had *C. cayetanensis,* 1 (3.3%) had *E. coli*; 2 (6.6%) of the small water treatment plants after treatment had no parasites identified. Other parasites present in the water bodies included the *Paramecium*, a ciliate found in 6 (20%) of the water bodies, while 12 (40%) had unidentified water flagellates. Water samples from deep protected wells that were being used by 34 (33.6%) of the people yielded a significant association ($p<0.05$) with *G. duodenalis*, *E. histolytica/dispar, C. cayetanensis* and unidentified water flagellates.

Discussion

Water is essential for life. Coverage for both improved water supply and sanitation lags behind in the poorest communities such as in rural areas and in urban/peri-urban slums.[19] Developing countries occasionally experience outbreaks of intestinal protozoan parasites in treated tap water. This is regardless of having achieved their water treatment standards. There is a lot that needs to be done for water treatment plants in developing countries where resources for identification of these parasites are limited. Furthermore parasitic infections and HIV prevalence are high amongst the people living in the developing world who consume both treated and untreated water. The water could possibly be contaminated by protozoan parasites, raising the possibilities of mortality and morbidity associated with these waterborne parasites.

In our study, stool parasitology results indicate that there is high prevalence of parasitic infections in this area as 83% of participants harbored protozoan intestinal parasites regardless of their HIV status. This is actually an underestimation, given that a minimum of 3 stool samples need to be taken on alternate days to determine parasitic infection as parasites are not excreted every day. It was by chance that they were identified. A study by Omar et al.[23] in rural Saudi Arabia indicated that the source of domestic water was the sole factor significantly associated with high prevalence rates of parasitic infections. In our study water was one of the sources of infection since 11 (36.6%) of the water bodies had parasites identified in them. Our study also shows that the population is highly infected with *C. cayetanensis,* which is an emerging parasite, causing prolonged *Cryptosporidium*-like diarrhea in the immune-compromised population. A study done by Nimri[24] in rural Jordan indicated that risk factors for acquiring cryptosporidiosis and cyclosporiasis were: source of drinking water, contact with animals, and eating unwashed vegetables. The same risks are most probably applicable to the study population presented in this manuscript.

Several publications even elsewhere demonstrated that the acquisition of intestinal parasitic infections could be due to ingestion of contaminated food or water. In our study chances are very high that these people acquired the parasites from their drinking water. Cifuentes et al.[25] identified *G. duodenalis* cysts in ground water used by some Mexico City dwellers. A review done by Karanis et al.[26] showed that at least 325 associated outbreaks of parasitic protozoan diseases have been reported worldwide. *G. duodenalis* accounted for 40.6% and *C. parvum* accounted for 50.8% of the outbreaks whilst *E. histolytica* and *C. cayetanensis* were etiological agents for 2.8% of the outbreaks respectively.[26] From our study it was noted that C. *parvum* and C. *cayetanensis* were detected in source waters before and during treatment by small water treatment plants. The types and number of parasites identified in our study could be an underestimation since the Ziehl-Neelsen stain used is less sensitive than the Method 1623 developed and used by USEPA.[27] There is therefore a probability that the water treated by small water treatment plants was contaminated with parasites.

One of the important findings was that five participants had similar parasites being identified in both their stools and water samples regardless of their HIV status and two of these had diarrhea.

Conclusions

Deep protected wells were shown to pose a risk in acquiring diarrheagenic protozoan parasites irrespective of HIV status. Some people are drinking water contaminated with protozoan parasites, therefore they are at risk of infection. As a result their water needs to be disinfected. Health education is required to inform the community about the safety of the sources of their drinking water.

Notes

This paper has been presented in part at the 13th International Congress on Infectious Diseases held during June 19-22, 2008 in Kuala Lumpur, Malaysia.

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Conflicts of interest

All authors – none to declare

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