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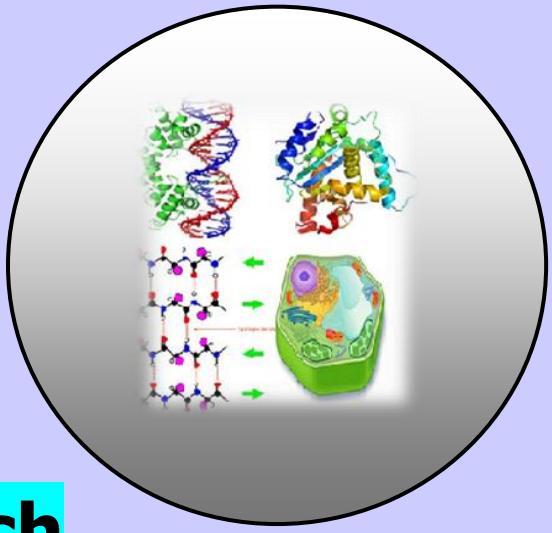

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RESEARCH PAPER

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Parasitological Quality of Hand Washing Water in Hotels and Restaurants of Ilu Aba Bora Zone, Oromia Region, Southwestern Ethiopia

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ABSTRACT

Giardia cysts and Cryptosporidium oocysts are (protozoan single cell) causative agent for most of enteric diarrhea and resistant to most of disinfectants. The main purpose of this research was to determine and enumerate Giardia cyst and Cryptosporidium oocysts in hand washing water from Hotels and Restaurants of Ilu Aba Bora Zone, Ethiopia since February - June, 2014.Even though different scientific study was undertaken on drinking water but hand washing water is neglected and less attention is given. A total of 48handwashing water samples were collected randomly from different sinks/bowls that stand in front of 48 Hotels and Restaurants (one sample from each).All the water samples were collected in triplicate. The presences of (oo) cysts were assessed by immune-fluorescence with monoclonal antibodies and the numbers of (oo) cysts were calculated for samples. Among the collected total samples about 24% were found positive for Giardia cysts and Cryptosporidium oocysts. Cryptosporidium oocysts and Giardiacysts were detected in all sampling site with variable concentration level. The concentrations of Giardia cysts and Cryptosporidium oocysts were dissimilar for different sources, where the highest 20 cysts/L of Giardia cyst and 30 oocysts/L of Cryptosporidium oocysts were detected from hand washing water samples collected from Darimu study site. On the contrary, the lowest concentration of Giardia cysts and Cryptosporidium oocysts were detected in town area, Mettu town with the concentration level of 2 cysts/L and 3 oocysts per liter respectively. In conclusion, the hand washing water at different sinks/bowls of different Hotels and Restaurants were highly contaminated with protozoan parasites. Therefore, we would like to recommend the proper sanitary survey and regular parasitological assessment of hand washing water should be planned and conducted.
Keywords: Hand-washing Water, Hotels and Restaurants, Hygiene, Parasitological Quality

INTRODUCTION

Environmental pollution is becoming a global concern and issues like water contamination and lack of safe and sufficient drinking water are problems that can lead to serious public health and life treat consequences. The environmentally robust *Cryptosporidium* oocysts and *Giardia* cysts are very persistent in the water system (De Reignier *et al*., 1989). They are extremely resistant to most disinfectants and are smaller enough to penetrate most drinking water treatment systems (Finch *et al*., 1993). Recently, there has been a dramatic incidence of waterborne disease outbreaks caused by the protozoan parasites, *Cryptosporidium* and *Giardia* spp. transmission is sustained both by zoonotic and anthroponotic cycles (Thompson, 2000). Cryptosporidium accounts for 165 (50.8%) and Giardia accounts for 132 (40.6%) of the 325 waterborne outbreaks of parasitic protozoan diseases, which have been reported worldwide so far (Karanis et al., 2007).In Ethiopia, where water supply and sanitation services are inadequate, only 32% of the total population has reasonable access to adequate water supply (MoWR/UNESCO/ WWAP, 2004). In Addis Ababa, nearly 80% of the population is dependent on public water points and in-house storage of water (Crampton, 2005). Previous studies conducted in Addis Ababa reported high prevalence of cryptosporidiosis and giardiasis among HIV/AIDS patients and diarrheic children (Fisseha *et al*., 1998). *Cryptosporidium* and *Giardia* causes diarrhea in a wide range of vertebrate organisms including humans and this is significant in immune compromised individuals (Watanabe*et al.*, 2005). *Cryptosporidium* is the main cause of diarrhea around the world (Clark, 1999). Especially in developing countries diarrhea is the main cause of mortality and morbidity (Kosek*et al*., 2003). *Cryptosporidium* accounts 1-10% of diarrheal disease in the world (Xian-Ming and Larusso, 1999). Studies indicate that *Cryptosporidium* sero prevalence in developed nations covers 25-35% where in developing nations the figure is higher ranging from 60-90% (Chen*et al.*, 2002; Xian-Ming and Larusso, 1999).*Cryptosporidiosis* is one of the main causes of mortality for infants and young children in developing countries. In children the prevalence of *Cryptosporidium* in developing nations is 1.3-22% where as in developed nations it is about 0.3-4.3% (Casemore, 1990). Similar to other developing countries in Ethiopia diarrhea and HIV/AIDS are a major cause of mortality (UNAIDS/WHO, 2005; Kosek *et al*., 2003). *Cryptosporidiosis* accounts about 12% and 7% of children disease respectively in developing and developed nations (Chen *et al.*, 2002). An improved hygiene-education programme appears to need to change deep-rooted inherent behaviors such as hand washing prior to water handling, as well as proper protection of container-stored water from personal, domestic and environmental contamination. Sources of protozoan parasites can be from hand washing sinks because they usually contain stagnant water that supports the growth of microorganisms, which can be transferred to hands during hand washing practices. Research hypothesis: hand washing water was contaminated with resistant infectious waterborne disease causing protozaons such as *Giardia* cysts and *Cryptosporidium* oocysts.

MATERIALS AND METHODS

The study was conducted in five districts (woreda) including Mettu town. These are: Bedele, Yayo, Hurumu, Alge, and Darimu. Mettu is a capital town for Ilu Aba Bora Zone. In general the study sites were located approximately 600 km southwest of Addis Ababa, Ethiopia.

Water sample collection, filtration, elution, centrifugation, and microscopic observation were conducted based on USEPA method 1623 with certain modification for identification, determination and enumeration of *Giardia* cysts and *Cryptosporidium* oocysts.

Sample collection

A total of 48 water samples were collected randomly from Mettu town and its neighboring five (5) woredas (Bedele, Yayo, Hurumu, Darimu and Alge) were collected. That means, from Mettu (n=12), Bedele (n=9), Hurumu (n=6) and seven samples (n=7) from each Yayo, Darimu and Alge, over six (6) months (November up to April, 2013). Triplicate samples were taken for all points. These sampling distributions, were designed depend on their weight proportion of Hotels and Restaurant's numbers in each study site.

Water samples were collected using sterilized white plastic containers. Before sample was taken from sinks the plastic containers were pre-sterilized using ethanol and marked from where it was collected including date, and time of sampling. Before the second sample was collected the plastic containers were pre-sterilized using sodium hypochlorite (NaOCl), and rinsed using distilled water.

Sample analysis

Finally, the collected water samples shipped to the laboratory for filtration, elution, centrifugation and examination. **Concentration**: Filter papers with the pore size more than 1µm can pass oocyst. A cellulose acetate filter paper with the pore size of 1µm and diameter of 47 mm, white in color filter paper was used (Pall filter paper). Vacuum pump (Thomas model number TA 1061, Monroe, USA and Edwards's vacuum pump) was used to filter water sample through the filter paper with the negative pressure of -0.4 up to -1bar pressure. This is around 2-4L/minute. The filtration cap was Gellman science using a bottle and tin barrel for sucking the air from the flask. One hundred liters of water (100L) for each sample of treated water was flittered.

Elution: The surface of the filter paper scraped using a smooth edge plastic loop and detergent of 0.01% Tween 80 and Phosphate Buffer Saline solution (PBS) (both PBS and Tween 80, Sigma Chemical Co., St. Louis, Mo.). The filter paper elute was transferred to 15ml conical polyethylene centrifuge tubes for centrifugation.

Centrifugation of (oo) cysts: The eluted material was centrifuged at 1,500 x G for 15 minutes and marked with the respective sample site for the purpose of identification. Supernatant discarded and pellets washed with distilled water. Purified clear (oo) cysts transferred to sterile Ependorf tube. In which Potassium Buffered Saline solution (PBS, pH=7.2) and disinfectants, Procancillin penicillin G (100U/ml), were added to make the media sterile till observation and staining was done. Potassium dichromate solution (PDS) with 2.5% added to the final stored sample for preservation.

Staining: Identification of *Giardia* cysts and *Cryptosporidium* oocysts was done using mixture of fluorescein-labeled mouse monoclonal antibody reagents for outer wall antigen sites (epitopes) of *Giardia lamblia* and *Cryptosporidium parvum* (Waterborne Inc., 2007). Slide staining was conducted according to the manufacturer instruction (Waterborne Inc.), using Aqua-Glo™ G/C direct comprehensive kit (Waterborne™, Inc. Hurst Street, New Orleans, and L.A 70118 USA).

Fluorescent microscope examination of *Giardia* and *Crypto:* The stained material for both the control and sample was observed through Olympus BX51 (BX51TF, Japan) fluorescent microscope for the determination of the existence of the *Giardia* cyst and *Cryptosporidium* oocyst. The *Cryptosporidium* oocysts was identified as oval to round shape with the diameter of 8-13µm in length and 7-10µm in width and the *Giardia* cyst was identified as 3-5µm in diameter (Waterborne™, Inc.). Analysis Softimage software (windows version 3) coupled with CC-12 camera on Olympus BX51 fluorescent microscope was used to measure the size of the (oo) cysts and to capture images.

Data analysis

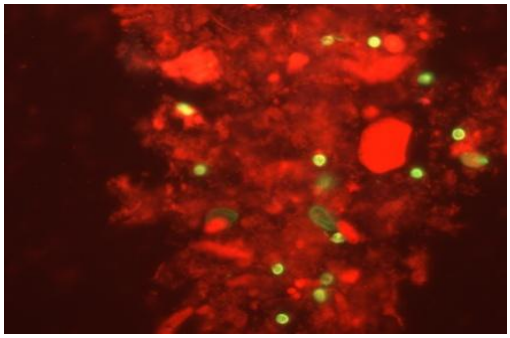
All analysis were conducted using SPSS windows version 17 (SPSS Inc., Chicago, IL, USA) software. Chi-Square (χ^2) test was used and *p*-values less than 0.05 ($P<0.05$) considered as statistically significant. In addition, for determining the correlation between the occurrence of *Giardia* cyst and *Cryptosporidium* oocyst detected in the distribution system liner regression test was applied. Pearson correlation coefficient and non-parametric Spearman's rho were used to determine the relationship of cyst and oocyst with residual chlorine and temperature.

RESULTS

The distribution of *Giardia* cyst and *Cryptosporidium* oocysts prevalence were different depending on the sampling sites. Among the collected total samples about 24% were found positive for *Giardia* cysts and *Cryptosporidium* oocysts. However, the pattern of contamination increases in woreda rather than town areas.

Table 1.*Giarida* cysts and *Cryptosporidium* oocysts detected in Hand washing water from Hotels and Restaurants, 2014.

Sampling site	*Giardia* cysts (%)	*Cryptosporidium*oocysts (%)
Bedele	11.5%	5%
Mettu	7%	3%
Yayo	9%	6%
Hurumu	12%	4.8%
Alge	15%	9%
Darimu	15.2%	7.6%



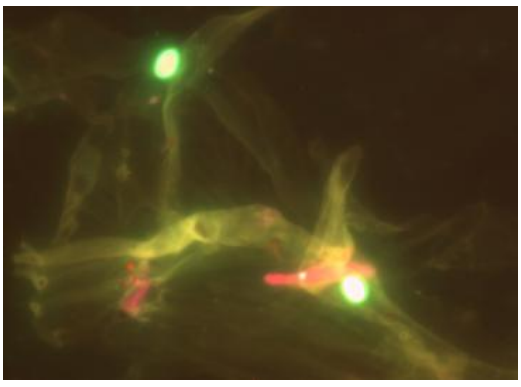


Figure 1. (a) Cryptosporidium oocysts (green small circles against red background) observed under florescent microscope.

(b)Cryptosporidium oocysts (green small circles against red background) observed under florescent microscope.

Cryptosporidium oocysts and *Giardiacysts* were found in all sampling site with variable concentration levelas shown in the results for the presence of cysts and oocysts were summarized in Tables 3.1. This research finding indicated that about 11.5%, 7%, 9%, 12%, 15% and 15.2% of the collected samples were positive for Giardia cysts in Bedele, Mettu, Yayo, Hurumu, Alge and Darimu respectively. In similar manner, the concentration of *Cryptosporidium oocysts* from Hand wash samples collected from Hotels and Restaurants of Bedele (5%), Mettu (3%), Yayo (6%), Alge (9%) Darimu (7.6%) and Hurumu (4.8%) were identified.

Table 2. Overall *Giardia* and *Crypto*/liter (mean ± SE) in the distribution system.

Study area	*Giardia* cysts/liter			*Cryptosporidium* oocysts/liter		
	Range	SD[3]	(mean ± SE)	Range	SD	(mean ± SE)
Mettu	0[1-]2	0.71	0.25±0.11	0-3	1.32	0.4±0.35
Bedele	0-3	0.61	0.15±0.11	0-6	1.52	0.5±0.26
Yayo	0-6	1.35	0.47 ±0.16	0-8	1.91	0.72±0.23
Hurumu	0-20	11.56	6.67±6.67	0-25	15.28	13.3±8.82
Alge	0-15	7.64	8.3±4.4	0-24	13.23	15±7.64
Darimu	0-20	7.91	5±2.64	0-30	12.05	9.89±4.02

The concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were different for different sources, where the highest 20 cysts/L of *Giardia* cyst and 30 oocysts/L of *Cryptosporidium* oocysts were detected from hand washing water samples collected from Darimu study site. In contrast, the lowest concentration of *Giardia* cysts and *Cryptosporidium* oocysts were detected in town area, Mettu town with the concentration level of 2 cysts/L and 3 oocysts per liter respectively (table 3.2).

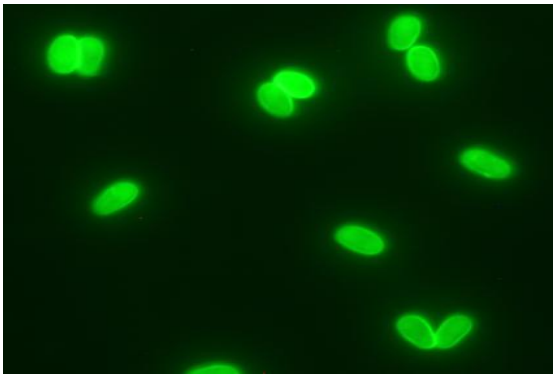


Figure 2. Giardia cysts green colored (FITC+) under fluorescent microscope.

DISCUSSION

Giardia and *Cryptosporidium* were identified in this study. In similar studies *Giardia* and *Cryptosporidium* were reported by different scholars in several part of the world. For instance *Cryptosporidium* oocyst and *Giardia* cyst were detected in surface or drinking water of Spain (Carmena *et al.*, 2007), Russia and Bulgaria (Karanis *et al.*, 2006b), Canada (LeChevallier *et al.*, 1991), USA (Le Chevallier *et al.*, 1991), and India (Anbazhagi *et al.*, 2007).

But hand washing water was neglected; while it could have significant public health importance.

From the total collected samples *Giardia* were detected in highest concentration from Darimu 15.2 %, and lowest level from Mettutown 7% of the collected hand washing water. This result was comparatively closer to drinking water studies conducted around the globe. For example a study conducted by Wallis *et al.* (1996) and his co-researchers detected *Giardia* in 18.2% of treated water samples. And a detection of *Crypto in* 18.2% in treated water samples in Southern Russia and Bulgaria was reported by Karanis *et al*. (2006). Likewise, the investigation on a treated drinking water in Spain detected *Giardia* in 19.2% of SWTF samples and *Cryptosporidium* in 15.4% of CWTF samples (Carmena *et al.*, 2007). Contrary to these, there are studies that in which either or both *Giardia* cysts and *Cryptosporidium* were not detected in treated and untreated raw water (Bakir *et al.*, 2003).

Giardia and *Cryptosporidium* range concentrations were lowest 0-2 cysts/liter and 0-3 oocysts per liter both from hand washing water samples collected from Mettu Town. These findings were nearly similar to the study of Le Chevallier *et al.* (1995) that reported the average concentration of 2.1 cysts/liter (range 0.4-6.3) and 2.0 oocysts/liter (range 0.3 - 9.8). The findings of this study were much lower that the finding of Sigudu *et al.* (2008) that reported the concentration of more than 1,400 oocysts/10 liter and 2,700 cysts/10 liter but higher than the mean concentration of 0.15 oocysts/l and 0.2 cysts/l recorded by Nishi *et al.* (2008).

Giardia (33.3%) and *Cryptosporidium* (55.6%) found at the highest concentration in Darimuworeda 20cysts/liter and 30 oocysts per-liter (Table 3.2). In addition, the statistical analysis result demonstrates there is significant difference between the concentration level of protozaons between town and districts (woreda) hand washing water collected from hotels and restaurants ($p<0.05$).We identified the major cause for such escalated level of difference were the source of the water used for hand washing; in most cases the hand washing water were from properly cleaned and designed sinks in town areas although it is unhygienic status in district areas.

In agreement with this investigation, Tesfalem *et al.* 2012 detected *Giardia* and *Cryptosporidium* were detected in highest concentration in untreated water sources like river than treated water. Similarly Carmena *et al.* (2007) detected *Giardia* in 45.2% samples from SWTF and 92.3% in samples from river and *Cryptosporidium* in 22.6% of samples from SWTF and in 63.5% samples from river. A study conducted in South Africa by Sigudu *et al.* in 2008 detected *Giardia* and *Cryptosporidium* in all (100%) raw water samples collected from selected catchments. *Giardia* cysts was found in 3/6 (50%) of samples from river water while no *Giardia* and *Cryptosporidium* were reported both in untreated dam raw water and municipal drinking water (Bakir*et al.*, 2003). Robertson *et al.* (2001) study in Norway raw water demonstrates the presence of *Cryptosporidium* in 13.5%, *Giardia* in 9% and both parasites in 2.5% samples. Intern Nishi *et al.* (2007) found *Giardia, Cryptosporidium* and both in 6.66%, 26.66% and 13.33% samples from raw water respectively. Karanis *et al.* (2005) also detected *Giardia* and *Cryptosporidium* in 81.81% of samples from river water. Wallis *et al.* (1996) detected *Giardia* in 21% of raw water samples. In addition Karanis *et al.* (2002) identified *Giardia* in 20% of samples from river water and 55.56% from lake water.

The overall status of this study indicated that hand washing water from hotels and restaurants were contaminated with *Giardia* cysts and *Cryptosporidium* oocyst shaving serious health concern Although sanitation aspect was very poor; handling of hand washing water, availability of soap; and source of the water. Protozoans were also detected in hand washing water samples from ground water source.

Giardia cyst and *Cryptosporidium* oocyst were detected in hand washing water from Restaurants and Hotels of Mettu town including other woreda as of the study sites. The possible source of contamination of the hand washing water can be zoonotic and human activities such as storage tankers for hand washing were not cleaned regular basis; source water used for hand washing were from untreated water such as river water and sometimes treated water in which agricultural and waste dumping around the water catchment area. This demonstrates the need for continuous monitoring of *Giardia* and *Cryptosporidium* in hand washing water; which is by far neglected and given less attention by government as well policy makers.

The concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were highest at woreda (20 cysts/l and 30oocysts/l) as compared to Mettu town (0.15 cysts/l and 0.32 oocysts/l). We found that, the occurrence of *Cryptosporidium* was more frequent than that of *Giardia* cysts in the hand washing water. The statistical test showed significant differences between the concentrations of *Giardia* and *Cryptosporidium* in hand washing water from rural to town ($P<0.05$).

This may be responsible need to be made aware the high-quality of hand washing water amongst owners and workers of the hotels and restaurants. Finally, this study suggested that further investigations were needed to verify the possible parasitical transmission from bowls/sink during hand-washing.

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REFERENCES

Adams, R.D. (2001). Biology of *Giardia lamblia*. *Clinical Microbiol. Rev*. **14**(3): 447-475.

Anbazhagi, M., Loganathan, D., Tamilselvan, S., Jayabalou, R., Kamatchiammal, S. and Kumar, R .(2007). *Cryptosporidium* Oocysts in Drinking Water Supply of Chennai City, Southern India.*Clean*.**35** (2): 167–171.

Appelbee, A.J., Thompson, R.C. and Olson, M. (2005). *Giardia* and *Cryptosporidium* in mammalian wildlife-current status and future needs.*TrendsParasitol*.**21**: 370–376.

Carmena, D., Aguinagalde, X., Zigorraga, C., Fernandez-Crespo, J.C. and Ocio, J.A. (2007). Presence of *Giardia* cysts and *Cryptosporidium* oocysts in drinking water supplies in northern Spain. *Journal of Applied Microbiology*.**102**: 619–629.

Casemore, D.P. (1990). Epidemiological aspect of human *Cryptosporidiosis*. *Epidemio linfect*. **104**: 1-28.

Chen, X.M., Keithly, J.S., Paya, C.V. and Larusso, N.F. (2002). *Cryptosporidiosis*. *N Engl J Med.* **346 (**22): 1723-1731.

Clark, D. P. (1999). New insights into human *Cryptosporidiosis*. *Clin. Microbiol. Rev.* **12:** 554–563.

Crampton, J. (2005). Maintaining clean water: contamination during water collection and storage in Addis Ababa. *Wateraid Ethiopia*. Pp :1-5.

DeRegnier, D.P., Lary, C., Schupp, D.G. and Erlandsen, S.L. (1989). Viability of *Giardia* cysts suspended in lake, river and tap water. *Appl. Environ. Microbiol*.**55**: 1223–1229.

Dupont, H.L., Chappell, C.L., Sterling, C.I., Okhuysen, P.C., Rose, J.B. and Jakubowski, W. (1995). The infectivity of *Cryptosporidium* in health volunteers. *N. Eng. J. Med*. **332**(13): 855-859.

Finch, G.R. and Belosevic, M. (2002). Controlling *Giardia* spp. and *Cryptosporidium* spp. in drinking water by microbial reduction processes. *J. Environ. Eng. Sci*. **1**: 17–31.

Finch, G.R., Black, E.K., Gyurek, L.L. and Belosevic, M. (1993). Ozone inactivation of *Cryptosporidium parvum* in demand-free phosphate buffer determined by in vitro excystation and animal infectivity. *Appl. Environ. Microbiol*. **59**: 4203–4210.

Fisseha, B., Petros, B. and Woldemichael, T. (1998). *Cryptosporidium* and other parasites in Ethiopian AIDS patients with chronic diarrhoea. *East Afr. Med. J.***75**: 100-101.

Guilherme, A. L. (2008). Investigation of *Cryptosporidium* spp. and *Giardia* spp. in a Public Water-Treatment System. *Zoonoses and Public Health*. Pp: 1-8.

Haigh, J. (1999). A day in the life of *Cryptosporidium*. Application report *Cryptosporidium*. Pp: 1-3.

Karanis, P. (2006a). A review of an emerging waterborne medical important parasitic protozoan. *Jpn. J. Protozool*. **39**(1): 5-19.

Karanis, P., Kourenti, C. and Smith, H. (2007). Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. *Journal of Water and Health*. **5**(1): 1-38.

Karanis, P., Sotiriadoua, I., Kartashevc, V., Kourentia C., Tsvetkovad, N. and Stojanova, K. (2006b). Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. *Environmental Research.***102**: 260–271.

Karanis, P., Sotiriadoua, I., Kartashevc, V., Kourentia C., Tsvetkovad, N. and Stojanova, K. (2006b). Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. *Environmental Research.***102**: 260–271.

Kosek, M., Bern, C. and Guerrant, R.L. (2003). The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ*. **81**: 197-204.

LeChevallier, M.W., Norton, W. D. and Atherholt, T. (1995). Survey of surface source waters for *Giardia* and *Cryptosporidium* and water treatment efficiency evaluation. *American Water Works Service Company, Inc.* Pp: 1-8.

LeChevallier, M.W., Norton, W.D. and Lee, R.G. (1991). Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl. Environ. Microbiol*. **57** (9): 2610-2616.

MoWR/ UNESCO/ WWAP. (2004). Generations integrated rural development consultants. National water development for Ethiopia, Addis Ababa Ethiopia.

Rendtorff, R.C. (1954). The experimental transmission of human intestinal protozoan parasites II. *Giardialamblia* cysts given in capsules. *Am. J. Hyg*. **59**: 209–220.

Sigudu, M.V., Booysen, M. G., Mtetwa, L. M. and Grundlingh, M. G. (2008). Monitoring of *Cryptosporidium (*oocysts) and *Giardia* (cysts) in the selected Vaal river catchment areas using the U.S. EPA METHOD 1623.*WISA*.Pp: 1-4

Tesfalem Atnafu, Hailu Kassa, Chris Keil, Nigus Fikrie, Seyoum Leta and Ian Keil. Presence, Viability and Determinants of Cryptosporidium Oocysts and Giardia Cysts in the Addis Ababa Water Supplyand Distribution System.DOI 10.1007/s12403-012-0065-z.Water Qual Expo Health.

Thompson, R.C.A. (2000). *Giardiasis* as a reemerging infectious disease and its zoonotic potential. *In. Journal of Parasitology*.**30**: 1259-1267.

Thompson, R.C.A. (2004). The zoonotic significance and molecular epidemiology of *Giardia* and *Giardiasis*.*Vet*.*Parasitology*.**126**: 15–35.

UNAIDS/WHO. (2005). Joint United Nations Program on HIV/AIDS/ World Health Organization (UNAIDS/WHO). AIDS epidemic update. Geneva.

USEPA (2005). Method 1623:*Cryptosporidium*and *Giardia* in water by Filtration/IMS/FA. United States Environmental Protection Agency, Office of water.

Wallis, P.M., Erlandsen, S.L., Isaac-Renton, J.L., Olson, M.E., Robertson, W.J. and Van Keulen, H. (1996). Prevalence of *Giardia* Cysts and *Cryptosporidium* Oocysts and Characterization of *Giardia* spp. isolated from drinking water in Canada. *Appl. Environ. Microbiol*. **62** (8): 2789–2797.

Watanabe, Y., Kimura, K., Yang, C. H. and Ooi, H.K. (2005). Detection of *Cryptosporidium* sp. Oocysts and *Giardia* sp. in faucet water samples from cattle and goat farms in Taiwan. *J. Vet. Med. Sci*. **67**(12): 1285-1287.

Xian-Ming, C. and Larusso, N.F. (1999). Human intestinal and biliary *Cryptosporidiosis*. *World Journal of Gastroenterology.* **5**(5):424-429.

Xiao, L., Sulaiman, I., Fayer, R. and Lal, A.A. (1998). Species and Strain-specific Typing of *Cryptosporidium* Parasites in Clinical and Environmental Samples. *MemInst Oswaldo Cruz, Rio de Janeiro*. **93** (5):687-692.

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