Protozoan parasites in a wastewater treatment plant of Bangladesh

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Abstract: Parasitic infection is a global health problem especially in developing countries. Municipal wastewaters always contain cysts of parasitic protozoans at some level. The present study was conducted to detect protozoan parasites in different stages of the treatment plant to check its efficacy. Wastewaters were collected from 3 points of the Pagla Sewage Treatent Plant (PSTP) of Dhaka, Bangladesh, throughout the year, 2007-08 at fortnight intervals. *Giardia* spp., *Entamoeba* spp., *Entamoeba coli*, *Endolimax nana, Idoamoeba butschlii* and *Balantidium coli* were detected at different times in different stages of the treatment plant. Among these *Giardia* and *Entamoeba* spp. were found most frequently than others*.* Both the prevalence and dominance of protozoan parasites were reduced gradually with the sampling point of the treatment plant which means that the treatment plant was effective in reducing protozoan parasites but not too effective to eliminate them completely.

Key words: Protozoan parasites, Wastewater treatment plant, Dhaka.

Introduction

Waterborne pathogens can be broadly characterised as parasitic protozoa, helminths, bacteria or viruses. Several protozoan infectious agents have been recognized as waterborne pathogens: *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora*, *Entamoeba histolytica* (Steiner *et al.*, 1997). To date, there have been at least 325 water associated outbreaks of parasitic protozoan disease documented worldwide (Karanis *et al.* 2007). Most are responsible for opportunistic infections in immunocompromised hosts and belong to the phylum Apicomplexa, with an exception of Microsporidia which are characterised by a unique mode of cellular infection and spore production (Levine *et al.*, 1980). Presently, the role of these emerging protozoan parasites in waterborne infections remains unclear (Marshall *et al.* 1997). Other protozoa, such as *Isospora belli*, *Cyclospora* and *Enterocytozoon bieneusi* have also been implicated in waterborne outbreaks, although the presence of these parasites in water has rarely been documented (Miegeville *et al.*, 2003). The frequent contamination of surface water by *Cryptosporidium* and *Giardia* is well established (; Fayer *et al.* 2004; Yoder *et al.*, 2004).

As documented, most waterborne protozoan parasites are causative agent for gastroenteritis, diarrhoea and others related to cellular or tissue infections (Stuart *et al.* 2003 and Roy *et al.* 2004). *C. parvum* and *G. lamblia* are protozoan parasites causing human gastrointestinal illness worldwide, with *C. parvum* reported to cause approximately 2% and *G. lamblia* between 2%-7% of all diarrhoeal illness in developed countries (Griffiths, 1998). For children under 5 years of age in developing areas and countries, there was a median of 3.2 episods of diarrhoea per childyear. Estimates of mortality revealed that 4.9 children per 1000 per year in developing countries died as a result of diarrhoeal illness in the first 5 years of life. Despite improving trends in mortality rates, diarrhoea accounted for a median of 21% of all deaths of children under 5 years in developing countries like Bangladesh, India, Brazil, Pakistan, Nigeria and including China, being responsible for 2.5 million deaths per year (Kosek *et al.*, 2003).

Sewage wastewater discharges are worldwide risk factors for the introduction of human protozoan enteropathogens into surface waters. The demand for microbiologically safe reclaimed waters grows exponentially owing to the global demographic rise of the population. Improvements in reclaimed water quality by lowering faecal coliform counts are not a sound solution for human protozoan enteropathogens (Hespanhol, 1997). In urban areas the use of treated wastewater can be considered in irrigation of public parks and recreational centres, sports fields, school gardens, landscaped areas, residential gardens, for commercial uses such as car and glass washing, for decorative purposes such as fountains and waterfalls, for dust control and building projects, to combat fires, in industrial and commercial constructions including bathroom flushing (United States Environmental Protection Agecy, 1992).

The possibility of an outbreak of diseases increases when there is a treatment plant malfunction that enables these parasites to penetrate the treatment processes more easily. Sewage treatment plants have the potential to be a source of contamination to our watershed if the treatment processes employed do not sufficiently treat the effluents before being discharged into nearby waterbody (Lim *et al.* 2007).

The objective of this research was to identify protozoan parasites in the treatment plant. This study was conducted to provide a quantitative basis for risk assessment studies and development of mitigation strategies, such as improving wastewater treatment efficiency.

Materials and Methods

Samples were collected from three points of the Pagla Sewage Treatent Plant (PSTP) which is situated in the eastern part of Dhaka, the capital of Bangladesh. The three sampling points were: Raw Sample from Grit Chamber, Reservoir Sample from Measuring Chamber and Outlet Sample from Outlet Lagoon. A volume of two liters of sample water from each sample site was collected at fortnight intervals during the period of 13 February, 2007 to 3 March, 2008. All the samples were immediately transferred to the laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) for parasitological examination. All the samples were processed by Formol-Ether Concentration Method (Cheesbrough, 2004) following Light Microscopy to observe and identified with the help of Bench aids of WHO (1994), then counted the number of protozoan parasites. However, the method utilized to detect the protozoan parasites does not differentiate between viable and nonviable organisms. Data were analyzed by using Microsoft Excel and SPSS software and the ecological terms considered in this study were according to Margolis *et al.* (1982) and Bush *et al.* (1997).

Results and Discussion

A total of 72 (= 24 samples X 3 sites) samples were collected from PSTP throughout the year. Six protozoan parasites were recognized distinctly by microscopy and these were: *Giardia* spp., *Entamoeba* spp., *Entamoeba coli*, *Endolimax nana, Idoamoeba butschlii* and *Balantidium coli*. Among these waterborne protozoans *Giardia* spp. and *Entamoeba* spp. were found in most of the samples throughout the year. Other parasites were comparatively less frequent; especially *B. coli* were very seldom observed (Table-I). The mean intensity, abundance, dominance and standard deviation (SD) were determined andh has been presented in the Table-II.

Table-I: Prevalence of protozoan parasites in different sampling point of the PSTP

Parasitic Protozoa	Prevalence (%)		
	Grit Chamber	Measuring Chamber	Outlet Lagoon
Giardia spp.	100	75	20.83
Entamoeba spp.	83.33	83.33	8.33
E. coli	41.67	41.67	16.67
E. nana	37.5	37.5	0
I. butschlii	54.17	33.33	4.17
B. coli	8.33	0	4.17

The mean intensity, abundance and dominance were found higher for *Giardia* spp. than all other protozoan parasites detected. *B. coli* was absent in Measuring Chamber and *E. nana* was absent in the Outlet Lagoon (Table-II). The mean intensity and abundance of identified parasites were very high. The dominance was the highest (44%) for *Giardia* spp. all the time and absent for *B. coli* and *E. nana* were absent some times.

Table-II: Mean intensity, abundance and dominance of protozoan parasites in different sampling points.

Sampling Site	Parasitic Protozoa	No. of contaminated samples	Mean intensity±SD (No. of parasite/L)	Abundance±SD (No. of parasite/L)	Dominance
Grit Chamber	*Giardia* spp.	24	$(2.23\pm1.44)X\ 10^5$	$(2.23\pm1.44)X\ 10^5$	44.03%
	Entamoeba spp.	20	$(1.40\pm0.75)X\ 10^5$	$(1.17\pm0.87)X\ 10^5$	23.05%
	E. coli	10	$(1.40\pm0.70)X\ 10^5$	$(5.83\pm8.30)X\ 10^4$	11.52%
	E. nana	9	$(1.39\pm1.11)X\ 10^5$	$(5.21\pm9.50)X\ 10^4$	10.29%
	I. butschlii	13	$(9.23\pm5.34)X\ 10^4$	$(5.00\pm6.08)X\ 10^4$	9.88%
	B. coli	2	$(7.50\pm3.54)X\ 10^4$	$(6.25\pm22.4)X\ 10^3$	1.23%
Measuring Chamber	*Giardia* spp.	18	$(1.14\pm0.78)X\ 10^5$	$(8.54\pm8.40)X\ 10^4$	33.88%
	Entamoeba spp.	20	$(6.25\pm4.98)X\ 10^4$	$(5.21\pm6.34)X\ 10^4$	20.66%
	E. coli	10	$(9.00\pm4.62)X\ 10^4$	$(3.75\pm5.16)X\ 10^4$	14.88%
	E. nana	9	$(6.67\pm2.50)X\ 10^4$	$(2.50\pm3.61)X\ 10^4$	9.92%
	I. butschlii	8	$(1.56\pm1.24)X\ 10^5$	$(5.21\pm10.2)X\ 10^4$	20.66%
	B. coli	0	0	0	0.00%
Outlet Lagoon	*Giardia* spp.	5	$(7.00\pm2.74)X\ 10^4$	$(1.46\pm3.12)X\ 10^4$	38.89%
	Entamoeba spp.	2	$(1.00\pm0.00)X\ 10^5$	$(8.33\pm28.2)X\ 10^3$	22.22%
	E. coli	4	$(6.25\pm2.50)X\ 10^4$	$(1.04\pm2.54)X\ 10^4$	27.78%
	E. nana	0	0	0	0.00%
	I. butschlii	1	$(5.00\pm0.00)X\ 10^4$	$(2.08\pm10.2)X\ 10^3$	5.56%
	B. coli	1	$(5.00\pm0.00)X\ 10^4$	$(2.08\pm10.2)X\ 10^3$	5.56%

Both the prevalence and dominance of protozoan parasites were reduced gradually which means that the treatment plant was effective in reducing protozoan parasites. However, the plant could not eliminate them. The number of protozoan parasites has been demonstrated by the Figure-I, II and III. A number of assessments on the efficiency of wastewater treatment plants by activated sludge have done by using the bacterial population as the indicator organism.
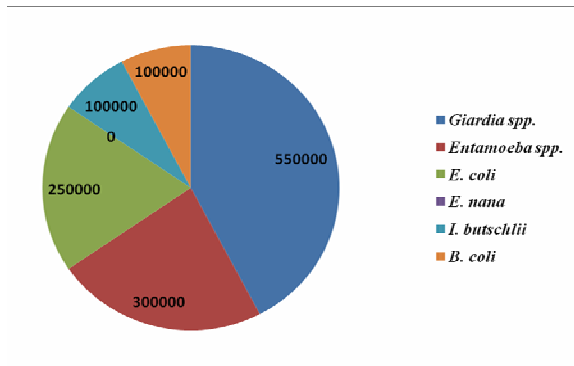
Figure-I. The number of protozoan parasites in Grit Chamber (Cysts/Litre) of the PSTP.
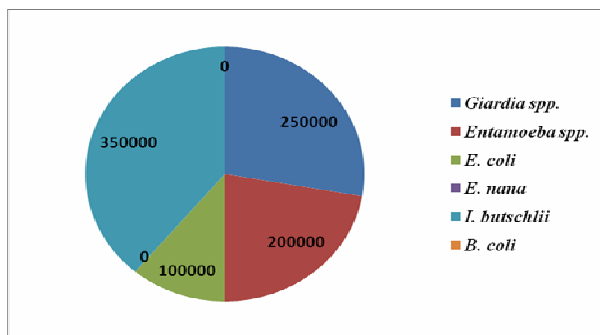

Figure-II. The number of protozoan parasites in Measuring Chamber (Cysts/Litre) of the PSTP.
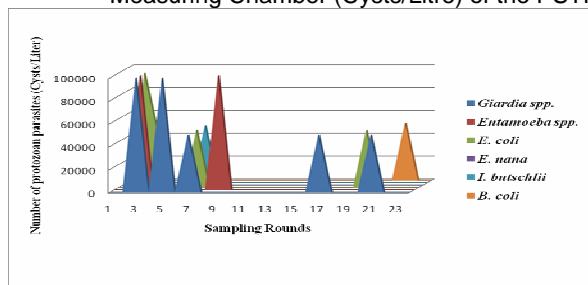

Figure-III. The number of protozoan parasites in Outlet Lagoon (Cysts/Litre) of the PSTP.

Environmental conditions make human beings vulnerable to parasites, and thousands of people in developing countries live under inadequate conditions without proper water supplies and sanitation. Waterborne diseases are common due to the shortage of drinking water, and conditions of storage and manipulation of foods contaminated by human and animal excrement (World Health Organization, 1991). The importance of monitoring and controlling the quality of residual water for reuse in irrigation and aquaculture has been highlighted by the World Health Organization (1989). The contamination of water bodies by discharged treated wastewater may contain emerging pathogens such as *C. parvum* oocysts and *G. lamblia* cysts (Cutolo *et al.,* 2006).

No strong correlation was found for any indicator-pathogen combination. Public health is not adequately protected by simple monitoring schemes based on detection of a single indicator (Harwood *et al.* 2005). The study revealed the occurrence and quantity of various parasitic protozoans in different stages of the treatment of the PSTP. The PSTP covers about 18% of the city population. No previous record concerning sewage treatment plant has been found and hence the present study seems to be very significant to assess the health risk of the Dhaka city. All the identified parasites are endemic in this country where *Giardia* and *Entamoeba* spp. were found most frequently than others. Raw and treated wastewater samples were analyzed for parasites and an array of parasites were detected, namely *Giardia* sp., *E. histolytica*/*dispar*, *E. coli*, *Ascaris* sp., *Enterobius vermicularis*, *Taenia* sp. by Lim *et al.* (2007) in Malaysia.

In the present study the count of parasitic protozoans' cysts varied per litre which is similar to the findings of Lim *et al.* (2007). Other studies conducted in Sweden, Norway and Canada also report the constant detection of *Giardia* in sewage (Ottoson *et al.* 2005). But there was a reduction in the number of cysts *E. nana* which may be due to the treatment of the plant. The results of the present study suggest that the treatment of wastewater promoted a reduction of infectious cysts of parasites but it could not remove all protozoan parasites properly, reflecting a constant risk of infection.

Conclusion
From the study a better understanding of the abundant protozoan parasites and the role of treatment process in removal of these parasites was obtained. As the treated sewage water from the PSTP is directly discharged into Buriganga River which embanks Dhaka city, the present study was designed to assess the Plant's role in meeting the public health criteria. The study discloses the occurrence of a variety of protozoan parasites, which represents a certain range of health risk existing in Dhaka city. The study also provides recommendations to improve the performance of the treatment plants in terms of parasites elimination.

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