Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

Research Journal of Parasitology 4 (2): 63-69, 2009
ISSN 1816-4943

Detection of Parasitic Contaminants in Sewage Water in Al-Ahsa, Saudi Arabia

M.S. Shathele and E.M. EL Hassan
Department of Microbiology, King Faisal University, P.O. Box 1757,
Al-Ahsa 31982, Kingdom of Saudi Arabia

Abstract: The study was carried to detect parasitic contaminants such as cysts of *Giardia* and entamoeba, *Cryptosporidium* oocysts and taenia eggs in sewage water from small lakes receiving sewage effluents from cabin toilets or directly from sewage treatment plants. There were no parasitic contaminants in the sewage water in the lake as determined by wet mount and floatation methods in Al-Ahsa. The absence of any parasite eggs in the sewage water could be attributed to the small volume of water samples taken for experimentation and to the chlorination treatment of the effluent by the sewage treatment plants. The study provided an excellent opportunity for further investigation to determine different types of water contaminants by applying other methods (specific polymerase chain reaction, PCR-RFLP methods, wound fiberglass cartridge filters and reverse transcription-PCR and commercial enzyme-Immunosorbent assay) in the sewage water in Al-Ahsa Oasis.

Key words: Sewage water, parasites, cysts, *Cryptosporidium* oocysts, wet mount, floatation, *Giardia*, Taenia eggs

INTRODUCTION

Land disposal of sewage effluent and its subsequent use for different purposes including irrigation is an important environmental issue. Sewage water contains organic and inorganic contaminants. Among the various contaminants, water based parasitic diseases are caused by a number of aquatic parasites those spend part of their life cycle in the water and another part as parasites in animals and man. They include guinea worm (dracunculiasis), paragonimiasis, colonrchiasis and schistosomiasis. On the other hand, parasicitic water-borne diseases which are transmitted primarily by fecal-oral route are the major source of contamination for water through contact with human and animal fecal pollution. These include giardiasis, taeniasis, amoebiasis as well as coccidiosis. Since, these contaminants are usually present in low concentrations in contaminated water, they have to be concentrated from large volumes of water before detection. Methods of concentration include filtration through yarn-wound filters or cellulose acetate filters and retained particulates are then eluted from the filters and reconcentrated by centrifugation. The polluted parasitic stages are then separated from particulate debris by floatation (Schaefer, 1996). Subsequent detection methods, particularly for parasitic protozoa, involve immunofluorescent staining of filtered sample concentrates (Schaefer, 1996), PCR (Mahbubani *et al*., 1991, 1992; Webster *et al*., 1993) and ELISA (Da la Cruz and Sivaganesan, 1995).

Corresponding Author: M.S. Shathele, Department of Microbiology, King Faisal University, P.O. Box 1757, Al-Ahsa 31982, Kingdom of Saudi Arabia

Uneke and Uneke (2008) found oocysts of *Cryptosporidium* species in all the sampling sites and there was significant variations` in the concentration of oocysts (F-ratio = 3.367, $p<0.05$) with the highest mean oocyst concentration of 183.3 L^{-1} of water, while the least mean oocyst concentration of 120.6 L^{-1} of water recorded. The differences in the mean monthly oocysts concentration per litre of water was statistically significant (F-ratio = 3.23, $p<0.05$).

Oocysts are present in many environmental waters because *Cryptosporidium* is not only a human pathogen but also a zoonotic pathogen infecting livestock, as well as feral animals, in many watersheds used as sources of drinking water (Simmons III *et al*., 2001). Surveys of surface water, groundwater, estuaries and seawater have dispelled the assumption that *Cryptosporidium* oocysts are present infrequently and in geographically isolated locations and indicate that water is a major vehicle for transmission of cryptosporidiosis (Fayer, 2004; Nwachcuku and Gerba, 2004).

Numerous studies have reported the contamination of surface water by *Cryptosporidium*. Sources of contamination of surface waters include sewage effluent overflows, waste-water discharges, abattoir waste, direct animal faecal deposition in waterways, indirect deposition via runoff from land grazed by livestock and wildlife, manure and effluent spreading and storm water run (Gary *et al*., 1983; Graczyk *et al*., 2000; Jellison *et al*., 2002; Xioa *et al*., 2001).

Sewage effluent may contain *Cryptosporidium* oocysts or *Giardia* cysts. The sewage contamination of water was implicated in a number of outbreaks of cryptosporidiosis and giardiasis (Craun, 1979; D'Antonio *et al*., 1985; Davies and Ritchie, 1948). Analysis of sewage for parasites of public health significance including *Cryptosporidium* and *Giardia* were the subject of several studies throughout the world (Carrington and Gray, 1993; Parker *et al*., 1993; Rose *et al*., 1986; Smith *et al*., 1994; Sykora *et al*., 1991). Significantly more *Giardia* cysts were detected than *Cryptosporidium* oocysts in our survey of sewage influents. Enriquez *et al.* (1995) investigated efficiency of tertiary sand and coal filtration and chlorination on removal of both *Giardia* cysts and *Cryptosporidium* oocysts. The concentration of *Giardia* cysts and *Cryptosporidium* oocysts detected in sewage influent is affected by the numbers of contributors (i.e., number of infected humans and animals in the community served by the STW), intensity of infection and dilution by other waste discharging to the STW. Also, attempts were made to correlate diagnosed cases of giardiasis and cryptosporidiosis with numbers of cysts and oocysts in sewage influent.

Madore *et al.* (1987) found average numbers of oocysts detected in raw water and treated sewage effluent were $5.18n\times10^{3}$ and 1.30×10^{3} L^{-1}, respectively. Filtered sewage effluents had significantly lower number of oocysts (10.0 L^{-1}). The results indicated that sand filtration may reduce the concentration of this parasite in waste waters. Highly variable oocyst numbers were encountered in surface waters. McHarry (1984) found *Giardia* sp. cysts at levels of 4,000-450,000/ 378,500 L (100,000 gallons) in sewage effluents from three of seven sewage treatment plants in sangamon county, illinois, in June, July and August 1981. Robertson *et al*. (2000) conducted a 3 year study on *Cryptosporidium* oocysts and *Giardia* cysts in sewage. The results demonstrated that, over 1 week, none of the environments tested had a deleterious effect on oocyst viability, with the exception of the sludge-holding tank. Similar studies were also conducted to detect oocysts of *Cryptosporidium* in sewage effluents (Robertson *et al*., 1992; 1993a, b, 1995).

Kaucner and Stinear (1998) described a Reverse Transcription-PCR (RT-PCR) for detecting low numbers of viable *Cryptosporidium parvum* oocysts spiked into clarified

environmental water concentrates. Xiao *et al.* (2004) reported the presence of *Cryptosporidium* oocysts in 67-100% of wastewaters, 24-100% of surface waters and 17-26.8% of drinking waters. Farm animals and human sewage discharge are generally considered to be the major sources of surface water contamination with *C. parvum* (Da la Cruez and Sivaganesan, 1995). Because *Cryptosporidium* infection is common in wildlife, it is conceivable that wildlife can also be a source for *Cryptosporidium* oocysts in waters (Webster *et al.*, 1993).

The information on parasitic contaminants in sewage water is inadequate for establishing criteria on the use of treated or untreated sewage effluent in Al-Ahsa Oasis, Saudi Arabia. The main objective of this investigation was to detect parasitic contaminants such as cysts of *Giardia* and Entamoeba, *Cryptosporidium* oocysts and Taenia eggs in the sewage water from Al-Ahsa Oasis, using Wet Mount and Floatation methods.

MATERIALS AND METHODS

A total of twenty water samples were collected from a small lake in Al-Ahsa Oasis receiving the sewage water from Hofuf city during 2007-2008. The site was selected because Al-Ahsa Oasis in the largest Oasis in the Arabian Peninsula having surface irrigation network. The Oasis has two main lakes (D-1 and D-2) which receive drainage effluent (agricultural drainage water, sewage water, gray water, industrial effluent etc) from the Oasis. The lakes are in an open areas and are good source of environmental pollution. Also, the drainage water after preliminary treatment is used for irrigation. The samples were randomly taken from five different locations in the lake. Four different specimens namely wet soil, mud, turbid water and clean water were collected from each sample location. Each sample was subjected to direct wet mount and centrifugation floatation techniques (McHarry, 1984; Webster *et al.*, 1993; Xiao *et al.*, 2004). The parasitic contaminants such as cysts of *Giardia* and entamoeba and *Cryptosporidium* oocysts were detected by following the methods of Abbaszadegan *et al.* (1991) and Campbell *et al.* (1992). Analysis of water samples was done using the techniques described in earlier studies with slight modifications (Lechevallier *et al.*, 1991; Wohlsen *et al.*, 2004; Njoku *et al.*, 2005).

Filtration

The water samples were filtered immediately after collection using a white khaki cloth (cotton) as filters (mesh size 250-300 μm) with the aid of a vacuum pump and a Buckner filter flask. The cloth was cut into 8 cm^3 pieces and to enhance an effective trapping of particles and the organisms during the filtration process; 5 of each piece was placed together in the funnel thus forming 5 layers. When the pores are blocked during the filtration process as indicated by the slow flow rate of filtrate into the Buckner filter flask, the topmost layer of the cloth was removed and another added at the bottom thus, maintaining the five layers at any point in time as the filtration continued. All the filters, which had trapped minute particles, were collected into marked polythene bags and stored in the refrigerator to keep them moist.

Backwashing

The filters were eluted by several backwashing with 200 mL of distilled water in which 10% wash solution concentration had been incorporated so as to enhance the recovery of oocysts. The wash solution was prepared using distilled water and Tween 20 (Sigma Chemical Co., St. Louis, Mo.).

Concentration

The sucrose gradient sedimentation technique was used for the concentration of oocysts. After the backwashing, 9 mL of the water sample was placed in a conical centrifuge tube and 2 mL of Sheather's sugar solution was added. This was stirred vigorously and centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed until about 2 mL is left with the sediment in the centrifuge tube. The sediment was transferred to the microscope slides and smears made.

Microscopic Examination

Demonstration of *Cryptosporidium* oocysts was by microscopic examination of smears made after the concentration of water samples, fixed with 70% methanol and stained using the modified Ziehl-Neelsen staining technique (Henriksen and Pholenz, 1981).

Visual Oocyst Count

The number of oocysts was determined by scanning through each slide randomly. This was done by moving three different parts of he slide each at a time across the ×10 objective and looking out for the pinkish stained oocyst. Oocyste encountered were confirmed using the ×40 objective, by their oval or round shape and pink-red coloration. These were counted and their numbers recorded.

RESULTS AND DISCUSSION

No parasite eggs or cysts were detected by the Wet Mount and floatation method in the sewage water from Al-Ahsa Oasis (Table 1).

Lakes receiving sewage effluents from cabin toilets or directly from small sewage water treatment plants are expected to harbour protozoan infective stages, such as cysts of *Giardia* and Entamoeba, oocysts of *Cryptosporidium* and Taenia eggs. Also, protozoan cysts deposited on soil from animals can contaminate such lakes during rainy season. Furthermore, aquatic helminthes, the causative agents of water based diseases, are expected to be present in such lakes. Although, the lake is formed mainly from different types of

Table 1: Parasitological examination of water samples from lake

Sample No.	Sample nature	Wet mount	Flotation method
10k-1	Turbid water	-ve	-ve
10k-8	Clean water	-ve	-ve
10k-14	Wet soil	-ve	-ve
10k-16	Mud	-ve	-ve
15k-5	Turbid water	-ve	-ve
15k-10	Clean water	-ve	-ve
15k-15	Wet soil	-ve	-ve
15k-19	Mud	-ve	-ve
20k-2	Turbid water	-ve	-ve
20k-9	Clean water	-ve	-ve
20k-11	Wet soil	-ve	-ve
20k-18	Mud	-ve	-ve
25k-3	Turbid water	-ve	-ve
25k-6	Clean water	-ve	-ve
25k-12	Wet soil	-ve	-ve
25k-20	Mud	-ve	-ve
25k/16-4	Turbid water	-ve	-ve
25k/16-7	Clean water	-ve	-ve
25k/16-13	Wet soil	-ve	-ve
25k/16-17	Mud	-ve	-ve

discharged effluents, yet all the sewage water samples did not show any parasitic contaminants. This could be attributed to the volume of water samples which was small and reduced the chances to detect these contaminants (Cysts and *Cryptosporidium* oocysts and others). The results agree with those of Schaefer III (1996) who reported that these parasites are usually present in low concentrations in contaminated water and must be concentrated from large volumes of water before detection. The study emphasized that the sensitivity of the detection techniques such as immunofluorescent staining (Schaefer III, 1996), PCR (Mahbubani *et al*., 1991, 1992; Webster *et al*., 1993) and ELISA (Da la Cruz and Sivaganesan, 1995) should be used to examine water samples for various contaminants. The results did not agree with those of Uneke and Uneke (2008) who found Oocysts of *Cryptosporidium* species in all the sampling sites with the highest mean oocyst concentration of 183.3 L^{-1} of water while the least mean oocyst concentration of 120.6 L^{-1} of water recorded. The absence of parasitic contaminants in sewage water of the present study are supported by the results of Enriquez *et al.* (1995), who investigated efficiency of tertiary sand and coal filtration and chlorination on removal of both *Giardia* cysts and *Cryptosporidium* oocysts.

CONCLUSION

There were no detectable contaminants determined by wet mount and floatation method in the sewage water of Al-Ahsa Oasis. This could be attributed to the chlorination of sewage effluent which might have removed both *Giardia* cysts and *Cryptosporidium* oocysts. Overall, the study provided an excellent opportunity for future investigations of various types of parasitic contaminants in the drainage water in Al-Ahsa Oasis by following different techniques. Because the drainage water is being used indiscriminately for irrigation and can cause health harzards among the farming community.

REFERENCES

Abbaszadegan, M., C. P. Gerba and J. B. Rose, 1991. Detection of *Giardia* cysts with a cDNA probe and applications to water samples. pplied Environ. Microbiol., 57: 927-31.

Campbell, A.T., R. Haggart, L.J. Robertson and H.V. Smith, 1992. Fluorescent imaging of *Cryptosporidium* using a cooled charge couple device (CCD). J. Microbiol. Methods, 16: 169-74.

Carrington, E.G. and P. Gray, 1993. The Influence of Cattle Waste and Sewage Effluent on the Levels of *Cryptosporidium* Oocysts in Surface Waters. Foundation for Water Research, Marlow, Bucks, UK, pp: 205–208.

Craun, G.F., 1979. Waterborne Outbreaks of Giardiasis. In: Waterborne Transmission of Giardiasis, Jakubowski, W. and J.C. Hoff (Eds.). US Environmental Protection Agency, USA.

D'Antonio, R.G., R.E. Winn, J.P. Taylor, T.L. Gustafson and W.L. Current *et al*., 1985. A waterborne outbreak of cryptosporidiosis in normal hosts. Ann. Int. Med., 103: 886-888.

Da la Cruz, A.A. and M. Sivaganesan, 1995. Detection of *Giardia* and *Cryptosporidium* sp., in source water samples by commercial enzyme-immunosorbent assay. Proceedings of the 1994 Water Technology Conference, Nov. 6-10, San Francisco, California, pp: 1303-1318.

Davies, C. and L. S. Ritchie, 1948. Clinical manifestations and treatment of epidemic amoebiasis occurring in occupants of the mantesta apartment building, Tokyo, Japan. Am. J. Tropical Med. Hygiene, 28: 817-822.

Enriquez, V., J.B. Rose, C.E. Enriquez and C. Gerba, 1995. Occurrence of *Cryptosporidium* and *Giardia* in Secondary and Tertiary Wastewater Effluents. In: Protozoan Parasites and Water, Betts, W.B., D. Casemore, C. Fricker, H. Smith and J. Watkins (Eds.). The Royal Society of Chemistry, Cambridge, UK, pp: 84-86.

Fayer, R., 2004. *Cryptosporidium*: a water-borne zoonotic parasite. Vet. Parasitol., 126: 37-56.

Gary, H.L., S.R. Johnson an D.S.L. Ponce, 1983. Cattle grazing impact on surface water quality in a Colorado front range stream. J. Soil Water Conserv., 38: 124-128.

Graczyk, T.K., B.M. Evans, C.J. Shiff, H.J. Karreman and J.A. Patz, 2000. Environmental and geographical factors contributing to watershed contamination with *Cryptosporidium* parvum oocysts. Environm. Res., 82: 263-271.

Henriksen, A. and J. F. L. Pholenz, 1981. Staining of Cryptosporidia by modified Ziehl-Neelsens technique. Acta Veterinary J. Scandanavian, 22: 594-596.

Jellison, K.L., H.F. Hemond and D.B. Schauer, 2002. Sources and species of *Cryptosporidium* oocysts in the wachusett reservoir watershed. Applied Environ. Microbiol., 68: 569-575.

Kaucner, C. and T. Stinear, 1998. Sensitive and rapid detection of viable *Giardia* cysts and *Cryptosporidium* parvum oocysts in large-volume water samples with wound fiberglass cartridge filters and reverse Transcription-PCR. Applied Environ. Microbiol., 64: 1743-1749.

Lechevallier, M. W., W. D. Norton and R. G. Lee, 1991. Occurrence of *Giardia* and *Cryptosporidium* sp., in surface water supplies. Applied Environ. Microbiol., 57: 2610-2616.

Madore, M. S., J. B. Rose, C. P. Gerba, M. J. Arrowood and C. R. Sterling, 1987. Occurrence of *Cryptosporidium* oocysts in sewage effluents and selected surface waters. J. Parasitol., 73: 702-705.

Mahbubani, M.H., A.K. Bej, M. Perlin , F.W. Schaefer, W. Jakubowski and R.M. Atlas, 1991. Detection of Giarda cysts by using polymerase chain reaction and distinguishing live from dead cysts. Applied Environ. Microbiol., 57: 3456-3461.

Mahbubani, M.H., A.K. Bej, M. Perlin , F.W. Schaefer, W. Jakubowski and R.M. Atlas, 1992. Differentiation of *Giardia* duodenalis and other *Giardia* spp. By using polymerase chain reaction and gene probes. J. Clin. Microbiol., 30: 74-78.

McHarry, M.J., 1984. Detection of *Giardia* in sewage effluent. J. Protozool., 31: 362-364.

Njoku, O.M., C.J. Uneke, S. Omale, P.U. Inyama, B.I. Jagun and D.D. Duhlinska, 2005. Occurrence of *Cryptosporidium* species in surface water in the Jos-plateau. J. Sci. Agric. Food Technol. Environ., 20: 59-63.

Nwachcuku, N. and C.P. Gerba, 2004. Emerging waterborne pathogens: can we kill them all?. Current Opinion Biotechnol., 15: 175-80.

Parker, J.F.W., H.V. Smith and R.W.A. Girdwood, 1993. Survey of Loch Lomond to assess the occurrence and prevalence of *Cryptosporidium* sp., oocysts and their likely impact on human health. Foundation for Water Research, Marlow, Bucks, SL7 2HD, UK. FWR Report No. FR0409.

Robertson, L.J., A.T. Campbell and H.V. Smith, 1992. Survival of oocysts of *Cryptosporidium parvum* under various environmental pressures. Applied Environ. Microbiol., 58: 3494-3500.

Robertson, L.J., A.T. Campbell and H.V. Smith, 1993a. *In vitro* excystation of *Cryptosporidium parvum*. Parasitology, 106: 13-19.

Robertson, L.J., A.T. Campbell and H.V. Smith, 1993b. A low cost, low technology container for studying the survival of transmission stages of parasites and other pathogens in water related environments. Water Res., 27: 723-725.

Robertson, L.J., H.V. Smith and C.A. Paton, 1995. Occurrence of *Giardia* cysts and *Cryptosporidium* Oocysts in Sewage Influent in Six Sewage Treatment Plants in Scotland and Prevalence of Cryptosporidiosis and Giardiasis Diagnosed in the Communities Served by those Plants. In: Protozoan Parasites and Water, Betts, W.B., D. Casemore, C. Fricker, H. Smith and J. Watkins (Eds.). Royal Society of Chemistry, Cambridge, UK., pp: 47-49.

Robertson, L.J., C.A. Paton, A.T. Campbell, P.G. Smith and M.H. Jackson *et al*., 2000. Gilmour, S.E. Black, D.A. Stevenson and H.V. Smith, 2000. *Giardia* cysts and *Cryptosporidium* oocysts at sewage treatment works in Scotland, UK. Water Res., 34: 2310-2322.

Rose, J.B., A. Cifrino, M.S. Madore, C.P. Gerba, C.R. Sterling and M.J. Arrowood, 1986. Detection of *Cryptosporidium* from wastewater and freshwater environments. Water Sci. Technol., 18: 233-239.

Schaefer III, F.W., 1996. Detection of Protozoan Parasites on Source and Finished Drinking Waters. In: Manual of Environmental Microbiology, Hurst, C.J., G.R. Knudsen and M.J. McInerney (Eds.). American Society for Microbiology, Washington, DC.

Simmons III, O.D., M.D. Sobsey, C.D. Heaney, F.W. III Schaefer and D.S. Francy, 2001. Concentration and detection of *Cryptosporidium* oocysts in surface water samples by method 1622 using ultrafiltration and capsule filtration. Applied Environ. Microbiol., 67: 1123-1127.

Smith, H.V., L.J. Robertson, D. Reay and C.J. Young, 1994. Occurrence of *Giardia* Cysts in Raw and Treated Sewage in Relation to the Prevalence of Giardiasis in a Scottish Community. In: *Giardia*: From Molecules to Disease, Thompson, R.C.A., J.A. Reynoldson and A.J. Lymberry (Eds.). CAB International, Wallingford, UK., pp: 128-129.

Sykora, J.L., C.A. Sorber, W. Jakubowski, L.W. Casson, P.D. Gavaghan, M.A. Shapiro and M.J. Schott, 1991. Distribution of *Giardia* cysts in wastewater. Water Sci. Technol., 24: 187-192.

Uneke, C.J. and B.I. Uneke, 2008. Occurrence of *Cryptosporidium* species in surface water in South-eastern Nigeria: The public health implication. Internet J. Health, 7: 35-42.

Webster, K.A., J.D.E. Pow, M. Giles, J. Catchpole, M.J. Woodward, 1993. Detection of *Cryptosporidium parvum* using a specific polymerase chain reaction. Vet. Parasitol., 50: 35-44.

Wohlsen, T., J. Bates, B. Gray and M. Katouli, 2004. Evaluation of five membrane filtration methods for recovery of *Cryptosporidium* and *Giardia* isolates from water samples. Applied Environ. Microbiol., 70: 2318-2322.

Xiao, L., A. Singh, J. Limor, T.K. Graczyk, S. Gradus and A. Lal, 2001. Molecular characterization of *Cryptosporidium oocysts* in samples of raw surface water and wastewater. Applied Environ. Microbiol., 67: 1097-1101.

Xiao, L., A.A. Lal and J. Jiang, 2004. Detection and differentiation of *Cryptosporidium* oocysts in water by PCR-RFLP. Methods Mol. Biol., 268: 163-176.