The Journal of Microbiology, June 2003, p.148-151 Vol. 41, No. 2
Copyright © 2003, The Microbiological Society of Korea

Investigation of Waterborne Parasites in Drinking Water Sources of Ankara, Turkey

Bilal Bakir[1,*], Mehmet Tanyuksel[2], Fatma Saylam[2], Sultan Tanriverdi[3,4], R. Engin Araz[1], Ali Kasim Hacim[1], and Metin Hasde[1]

[1]*Department of Public Health, Gulhane Military Medical Academy, School of Medicine 06018, Ankara, Turkey*
[2]*Division of Parasitology, Department of Microbiology and Clinical Microbiology Gulhane Military Medical Academy, School of Medicine, 06018, Ankara, Turkey*
[3]*Department of Parasitology, Mustafa Kemal University, School of Medicine, Antakya*
[4]*Department of Infectious Disease, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts, 01536, U.S.A.*

(Received January 22, 2003 / Accepted March 26, 2003)

Waterborne parasite infections are considered a re-emerging threat. Most studies on the epidemiology of human cryptosporidiosis, giardiasis, and amebiasis have been carried out in developed countries, and there is little data on the occurrence of these infections in other areas. The objective of this study was to investigate the presence of waterborne parasites such as *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica* in various water samples in Ankara, Turkey. A total of 85 samples were examined, 43 from the municipal water supply, 34 from wells, 6 from the Ankara River, and 2 from two untreated dams; by conventional microscopy, immunologically and by polymerase chain reaction (PCR). Oocysts of *C. parvum* and cysts of *G. lamblia* were detected by using an indirect fluorescence (antigen) assay, whereas an enzyme linked immunosorbent assay was used to detect the cysts of *E. histolytica* and *E. dispar*. In addition, PCR was used for *E. histolytica, E. dispar, C. parvum* and *G. lamblia* detection. *G. lamblia* was found in 2 of the 34 well water samples, and parasites were found in 3 of the 6 Ankara River samples. The 1st contained *E. histolytica* cysts and *Strongyloides stercoralis* larvae, the 2nd *E. histolytica* cysts, and *Trichuris trichiura* eggs, and the 3rd *C. parvum* oocysts only. No parasite was observed in the municipal water samples and untreated dam water samples. These results extend our knowledge on waterborne parasites, such occurrence information on waterborne pathogens assists the management and treatment of municipal water.

Key words: water, waterborne parasites, *Entamoeba, Cryptosporidium, Giardia.*

In developing countries, waterborne gastrointestinal parasite pathogens such as *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica* are frequently associated with morbidity, particularly in children. These parasites are the most common cause of infection worldwide (Pickering *et al.*, 1984; Curry and Smith, 1998; Tanyuksel *et al.*, 2001). In developed nations, outbreaks of *E. histolytica* infections have been caused by sewage contaminated water supplies (Barwick *et al.*, 1999). In Turkey, the prevalence of *E. histolytica* is lower than that of giardiosis (Doganci *et al.*, 1997), and the prevalence of cryptosporidiosis is low in humans (Doganci *et al.*, 2002), though it has been reported between 25.7% and 42.6% in calves (Arslan *et al.*, 2001; Tanriverdi *et al.*, unpublished data). The Ivedik Water Treatment Plant of Ankara was developed to meet the water demands of a growing city. The plant produces drinking water for 95% of the city's population water to WHO (WHO, 1996) and Turkish Standardization Institute (TSE, 1997) criteria. The Ankara River separates the city into two and is a repository of domestic and industrial discharge. Thus, it is reasonable to expect its contamination by parasites. This study was undertaken to investigate the presence of waterborne parasites in different water samples in Ankara.

* To whom correspondence should be addressed.
(Tel) 90-312-3044670; (Fax) 90-312-3044667
(E-mail) bakirbilal@hotmail.com

Materials and Methods

Water Sampling

Eighty-five 2-liter water samples were collected from different locations in Ankara, that is from; the municipal water supply, water wells, the Ankara River, and for untreated dammed surface water. Samples were collected between May and June 2002. Municipal sampling sites were selected to include all main arteries of the city water network. Well water samples represented more than 90%

of all military areas in Ankara. River water was sampled at every 3-4 kilometers in the metropolitan area, from the point where the river enters the metropolitan area; one sample was taken from each sampling site. All water samples were stored at 4°C and processed within 48 h of collection.

Sample Processing

Water samples were filtered through a 47 mm diameter, 0.45±0.02 μm pore size membrane filter.

Microscopy

Materials retained by filters were examined microscopically as a 0.9% saline smear for parasite cysts, trophozoites and helminth eggs. A portion of each sample was stained with Lugol iodine on a separate slide. Fresh preparations were examined visually at magnifications of 100X and 400X over approximately 100 fields and then a cold acid-fast and a trichrome staining technique were applied for the identification of *C. parvum, E. histolytica, E. dispar,* and *G. lamblia*. Microscopic identification was performed based on cold acid-fast, trichrome, and lugol stains.

Indirect Fluorescent Assay for C. Parvum and G. Lamblia

Cryptosporidium/Giardia direct immunofluorescent detection kit (Meridian Diagnostics, USA) was used according to the manufacturer's instructions. Slides were examined under an epi-florescent microscope (Zeiss Axiolab, Germany) equipped with a fluorescein isothiocyanate filter. *Cryptosporidium* oocysts and *Giardia* cysts stained with FITC labeled monoclonal antibody were identified based on a diameter of 4 to 6 mm and a length of 8-12 mm long.

Enzyme linked Immunosorbent assay (ELISA) for E. histolytica

ELISA was performed on filtered materials according to the manufacturer's instructions (*Entamoeba* CELISA-PATH, Cellabs Pty Ltd., Australia).

PCR

Cryptosporidium DNA was extracted using the phenol-chloroform extraction method as previously described (Carraway *et al.*, 1996). PCR identification of *C. parvum* was based on the amplification of a fragment of the β-tubulin exon 2 locus (Tanriverdi *et al.*, 2002). A 160 bp portion of the β-tubulin gene was amplified with the primers btub1 (ATGCTGTAATGGATGTAGTTAGACA); and btub2 (GTCTGCAAAATACGATCTGG) as previously described by Tanriverdi *et al.*, 2002). A total of 35 cycles were carried out, each consisting of 94°C for 50 sec, 52°C for 1 min, and 72°C for 50 sec, and a final extension at 72°C for 5 min.

G. lamblia DNA was extracted as previously described (Paintlia *et al.*, 1988). A set of specific PCR primers (5'-TGGACTGGCGAGACAAG-3' and 5'-TCCGGCTTGA GGGAAGC-3') were used to amplify a 540 bp product of *the G. lamblia* triose phosphate isomerase (*tpi*) gene, as described by Samuelson *et al.* (1989). The PCR reaction contained 1X PCR buffer, 6 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate, 100 nM of each primer, 2.5 U of *Taq* polymerase, and 1 μl of DNA template in a 50 μl PCR reaction. PCR consisted of 35 PCR cycles, each consisting of 95°C for 1 min, 45°C for 50 sec, and 72°C for 1 min, with an initial hot start at 95°C for 1 min and this was followed by a final extension at 72°C for 5 min.

E. histolytica and E. dispar As previously described (Clark and Diamond, 1991; Clark and Diamond, 1992), the presence of *E. histolytica* and/or *E. dispar* DNA was detected in filtered water sample by the PCR amplification of a portion of the ribosomal RNA (*ssu* rRNA) gene. Initial and nested amplification reactions were performed as previously described (Clark and Diamond, 1992). PCR products were run on 1% agarose gels, stained with ethidium bromide and photographed under ultraviolet illumination. ϕX174 RF DNA/*Hae*III fragments (Gibco BRL, USA) were used as markers.

Results and Discussion

G. lamblia cysts were identified in two (5.9%) of 34 well water samples by microscopy, IFA, and PCR. Three (50%) of 6 Ankara River samples contained parasites. In the 1st, *E. histolytica* cysts and *Strongyloides stercoralis* larvae, in the 2nd *E. histolytica* cysts and *Trichuris trichiura* eggs, and in the 3rd, only *C. parvum* oocysts were

Table 1. Waterborne parasites found in the samples obtained from different water sources

Samples	Total n (%)	Total positive samples n (%)	No. of positive samples n (%)	Parasites
Municipal Water	43 (100.0)	0	0	-
Well Water	34 (100.0)	2 (5.8)	2 (5.8)	*G. lamblia* cysts
Ankara Creek`s Water	6 (100.0)	3 (50.0)	1 (16.6)	*E. histolytica* cysts and *S. stercoralis* larvae
			1 (16.6)	*E.histolytica* cysts and *T. trichiura* eggs,
			1 (16.6)	*C. parvum* oocysts
Untreated Dam Water	2 (100.0)	0	0	-
Total	85 (100.0)	5 (5.8)	5 (5.8)	

identified. *E. histolytica and C. parvum* were found by microscopy, IFA, ELISA, and PCR, and *Strongyloides stercoralis* larvae and *Trichuris trichiura* were detected using the ova and parasite (O&P) method. No parasites were observed in the municipal water or untreated dam water samples (Table 1). The pH values of the water samples are shown and the pH values of all water samples except for the Ankara River samples, fell into the "drinkable" pH range, according to the WHO criteria (data not shown).

In contrast to other waterborne pathogens, such as *G. lamblia*, the occurence of cryptosporidiosis is unknown in many parts of the world, However, the mean prevalence rate of *Cryptosporidium* infection is between 1 and 3% in Europe and North America and 5% in Asia (Current, 1994). Moreover, some parasites such as *C. parvum*, *Giardia* and *Entamoeba* have been identified as significant waterborne pathogens and have been found responsible for several serious outbreaks worldwide over the past ten years (Marshall *et al.*, 1997, Morris *et al.*, 1998). This study was the first to investigate waterborne protozoan parasites in Turkey.

The development of sensitive and specific molecular detection methods, such as PCR has greatly increased our knowledge of the presence of waterborne protozoan parasites. Standardized methods such as US EPA method 1623 have been successfully used to recover and detect waterborne parasites in different water sources (LeChevallier *et al.*, 1995; Stinear *et al.*, 1996; Lowery *et al.*, 2000 Rimhanen-Finen *et al.*, 2002). In this study, we used IFA (*C. parvum* and *G. lamblia*), ELISA (*E. histolytica* and *E. dispar*) and PCR (*C. parvum, G. lamblia, E. histolytica* and *E. dispar*) to detect parasites in different water sources in Ankara. We improved the efficacy of testing by preparing DNA templates from membrane filtrates, which was easily performed, and cost-effective (ca. $10/test). The method preserved DNA reliability, avoided DNA loses, and eliminated other pathogens.

As seen in table 1, Ankara River was found to be the water sources most contaminated by parasites in this study. The percentage of all parasites detected in the River samples was as high as 50% while the percentage of *C. parvum* in the same samples was 16.6%. This water source was also found to be undrinkable due to its high pH; however, the number of samples prevents us claiming a relation between water pH and parasitic contamination. As compared to other surveys, the percentages and concentrations of waterborne parasites were low. In contrast to this study, Robertson *et al.* (2001) found that 16% and 11.5% of surface water samples in Norway were positive for *Cryptosporidium* and *Giardia*, respectively, although the concentrations of parasites were low. However, in a study of river water in western Japan (Ono *et al.*, 2001) 47% of the samples tested were positive for *Cryptosporidium*. Rose *et al.* (1991) performed a similar study in 17 American States and found *C. parvum* oocysts in 51% of the samples tested. The percent of samples in which *C. parvum* was detected seems to be lower in our study than in the above studies. The sample quantity used in our study was 2 liters, which might explain the low frequency of detection found in the present study. In several studies, seasonal associations between *Cryptosporidium* and *Giardia* occurrence have been found (Wallis *et al.*, 1996). Additional studies with sample numbers and quantities will be needed to identify any seasonal patterns in the water sources of Central Turkey. In the future, it is anticipated that underground water sources will become well utilized. Therefore, the inclusion of underground sources in surveys of waterborne pathogens is relevant to public health. In conclusion, the data presented here indicates that the prevalence of waterborne parasites in different water sources from the Ankara region is relatively low.

Acknowledgments

We thank to William A. Petri, Jr. (University of Virginia) and Giovanni Widmer (Tufts Univerity, N. Grofton, MA) for donating PCR reagents and for their helpful suggestions.

References

Arslan M.O., Y. Gicik, H.M. Erdogan, and B. Sari. 2001. Prevalence of *Cryptosporidium* spp. oocysts in diarrhoeic calves in Kars Province, Turkey. *Turk. J. Vet. Anim. Sci.* 25, 161-164.

Barwick, R.S., A. Uzicanin, S.M. Lareau, N. Malakmadze, P. Imnadze, M. Iosava, N. Ninasvili, M. Wilson, H. Bishop, A. Hightower, W.A. Petri, Jr., and D.D. Juranek. 1999. Outbreak of amebiasis in Tblisi, Republic of Georgia. p. 234. Abstr. 48th Annu. Meet. Am. Soc. Trop. Med. Hyg.

Carraway M., S. Tzipori, and G. Widmer. 1996. Identification of genetic heterogenicity in the *Cryptodporidium parvum* ribosomal repeat. *Appl. Environ. Microbiol.* 62, 712-716.

Clark, C.G. and L.S. Diamond. 1991. Ribosomal RNA genes of 'pathogenic' and 'nonpathogenic' *Entamoeba histolytica* are distinct. *Mol. Biochem. Parasitol.* 49, 297-302.

Clark, C.G. and L.S. Diamond. 1992. Differentiation of pathogenic *Entamoeba histolytica* from other intestinal protozoa by riboprinting. *Arch. Med. Res.* 23, 15-16.

Connell, K., C.C. Rodgers, H.L. Shank-Givens, J. Sheller, M.L. Pope, and K. Miller. 2000. Building a better protozoa data set. *J. Am. Water Works Assoc.* 92, 30-43.

Curry, A. and H.V. Smith. 1998. Emerging pathogens: *Isospora, Cyclospora,* and *Microsporidia*. *Parasitology* 117 (Suppl), S143-S159.

Current W.L. 1994. *Cryptosporidium parvum:* household transmission. *Ann. Intern. Med.* 120, 518-519.

Doganci, T., E. Araz, A. Ensari, M. Tanyuksel, and L. Doganci. 2002. Detection of *Cryptosporidium parvum* infection with different techniques in childhood. *Med. Science Monitor* 8(12): MT223-226, 2002.

Doganci, L., M. Tanyuksel, and H. Gun. 1997. Overdiagnosis of

intestinal amoebosis in Turkey. *Lancet* 350, 670.

Jacubowski, W., S. Boutros, W. Faber, R. Fayer, W. Ghiorse, M. LeChevalier, J.B. Rose, S. Sauch, A. Singh, and M.H. Stewart. 1996. Environmental methods for *Cryptosporidium*. *J. Am. Water Works Assoc.* 88, 107-121.

Kuhn, R.C., C.M. Rock, and K.H. Oshima. 2002. Effects of pH and magnetic material on immunomagnetic separation of *Cryptosporidium* oocysts from concentrated water samples. *Appl. Environ. Microbiol.* 68, 2066-2070.

LeChevallier, M.W., W.D. Norton, J.E. Siegel, and M. Abbaszadegan. 1995. Evaluation of the immunofluoresence procedure for detection of *Giardia* cysts and *Cryptosporidium* oocysts in water. *Appl. Environ. Microbiol.* 61, 690-697.

Lowery, C.J., J.E. Moore, B.C. Millar, D.P. Burke, K.A. McCorry, E. Crothers, and J.S.G. Dooley. 2000. Detection and speciation of *Cryptosporidium* spp. in environmental water samples by immunomagnetic separation, PCR and endonuclease restriction. *J. Med. Microbiol.* 49,779-785.

Marshall, M.M., D. Naumovitz, Y. Ortega, and C.R. Sterling. 1997. Waterborne protozoan pathogens. *Clin. Microbiol. Rev.* 10, 67-85.

Morris R.D., E.N. Naumova, and J.K. Griffiths. 1998. Did Milwaukee experience waterborne cryptosporidiosis before the large documented outbreak in 1993. *Epidemiology.* 9, 264-70.

Nieminski, E.C., F.W. Schaffer, and J.E. Ongerth. 1995. Comparison of two methods for detection of *Giardia* cysts and *Cryptosporidium* oocysts in water. *Appl. Environ. Microbiol.* 61, 1714-1719.

Ongerth, J.E. and H.H. Stibbs. 1987. Identification of *Cryptosporidium* oocysts in river water. *Appl. Environ. Microbiol.* 53, 672-676.

Ono, K., H. Tsuji, S.K. Rai, A. Yamamoto, K. Masuda, T. Endo, H. Hotta, T. Kawamura, and S. Uga. 2001. Contamination of river water by *Cryptosporidium parvum* oocysts in western Japan. *Appl. Environ. Microbiol.* 67, 3832-3836.

Paintlia, A.S., S. Descoteaux, B. Spencer, A. Chakraborti, N.K. Ganguly, R.C. Mahajan, and J. Samuelson. 1998. *Giardia lamblia* groups A and B among young adults in India. *Clin. Infect. Dis.* 26, 190-191.

Pickering, L.K., W.E. Woodward, H.l. DuPont, and P. Sullivan. 1984. Occurrence of *Giardia lamblia* in children in day care centers. *J. Pediatr.* 104, 522-526.

Rabold, J.G., C.W. Hoge, and D.R. Shim. 1994. *Cyclospora* outbreak associated with chlorinated drinking water. *Lancet* 344, 1360-1361.

Rimhanen-Finen, R., A. Hörman, P. Ronkainen, and M-L. Hänninen. 2002. An IC-PCR method for detection of *Cryptosporidium* and *Giardia* in natural surface waters in Finland. *J. Microbiol. Methods* 50, 299-303.

Rose, J.B., C.P. Gerba, and W. Jakubowski. 1991. Survey of potable water supplies for *Cryptosporidium* and *Giardia*. *Environ. Sci. Technol.* 25, 1393-1400.

Robertson L.J. and B. Gjerde. 2001. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw waters in Norway. *Scand. J. Public. Health* 29, 200-207.

Samuelson, J.S., R. Acuno-Sato, S. Reed, F. Biagi, and D. Wirth. 1989. DNA probe for clinical diagnosis of *Entamoeba histolytica*. *J. Clin. Microbiol.* 27, 671-676.

Stinear, T., A. Matusan, K. Hines, and M. Sandery. 1996. Detection of a single viable *Cryptosporidium parvum* oocyst in environmental water concentrates by reverse transcription-PCR. *Appl. Environ. Microbiol.* 62, 3385-3390.

Tanyuksel, M., H. Tachibana, and W.A. Petri, Jr. 2001. Amebiasis, an Emerging Disease, p. 197-212. *In* W.M. Scheld, W.A. Craig, and J.M. Hughes (eds.), Emerging Infections 5. American Society for Microbiology, Washington D.C.

Tanriverdi S, A. Tanyeli, F. Baslamisli, F. Koksal, Y. Kilinc, F. Xiaochuan, G. Batzer, S. Tzipori, and G. Widmer. 2002. Detection and genotyping of oocysts of *Cryptosporidium parvum* by Real-Time PCR and melting curve analysis. *J. Clin. Microbiol.* 40, 3237-3244.

Turkish Standardization Institute. 1997. TSE-TS 266. Waters-Drinking waters, Ankara.

Wallis P.M., S.L. Erlandsen, J.L. Isaac-Renton, M.E. Olson, W.J. Robertson, and H. van Keulen. 1996. Prevalence of *Giardia* cysts and *Cryptosporidium* oocysts and characterization of *Giardia* spp. Isolated from drinking water in Canada. *Appl. Environ. Microbiol.* 62, 2789-2797.

World Health Organization. 1996. Guidelines for Drinking-water quality, Vol. 2, 2nd ed., Geneva.