

PARASITIC CONTAMINATION OF WELLS DRINKING WATER IN MAZANDARAN PROVINCE

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Received 18 August 2008; revised 23 September 2009; accepted 12 October 2009

ABSTRACT

There is a direct relation between the prevalence of some parasitic diseases and the presence of those etiologic agents in water. The purpose of this research was to determine the contamination rate of wells drinking water to parasites in Mazandaran province in the north of Iran. 989 water samples were randomly taken based on the population of towns and number of health centers from 12 cities of Mazandaran province and transferred to the laboratory in sterile containers. Water samples were then filtered and analyzed according to the World Health Organization guidelines. Direct method and Gram staining procedure were used to identify the parasites. If cryptosporidium was seen, floatation (sheather's sugar) and modified Ziehl-Neelsen staining method were performed. Parasites count was undertaken using McMaster counting slide (0.3 mL). 197 out of 989 water samples were contaminated with different parasites. From 197 contaminated samples, 20 different types of parasites were separated of which 53 (26.9%) were pathogenic, 100 (50.8%) non pathogenic, and 44 non-infective stages of parasites. Distance between wells and sources of contamination, type of water distribution systems, city and chlorination status had significantly statistical relationship with contamination prevalence ($p < 0.001$). According to the results and considering the direct correlation between safe water and human health, proper implementation of providing hygienic drinking water should be enforced.

Keywords: Parasites, Drinking water, Wells, Contamination, Human health, Mazandaran

INTRODUCTION

There is a direct or indirect relationship between the prevalence of the parasitic diseases and the larva of water sanitation (Mohammadi, 1995; Rowhani, 1995). In 1989 it was estimated that the prevalence of diarrhea in children (under 5 years) was about 1.362 billion, of which 4.9 million died due to the diseases. Even if only 1/3 of these diarrhea cases were related to water, then more than 1.5 million of children under 5 died as a result of drinking contaminated water. Water related diseases are still prevalent and revolt. Morris et al. in 1995 estimated that every

year in United States, about 56/000 and 7/100/000 suffer from mild to severe and moderate infections respectively with about 1200 deaths.

Norton and Lee (1991) reported finding cysts in 81% of raw surface water samples from fourteen American states and one Canadian province, at counts ranging from 0.04 to 66 cysts/L (geometric mean 2.77). There was high correlation with both fecal and total coliform counts and turbidity on average; surface waters from urban areas contained ten times more giardial cysts than waters from protected watersheds, suggesting human sewage as the main source of environmental contamination. Authors were also

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able to demonstrate that 17% of filtered drinking water samples in these areas were also positive (range 0.29-69 cysts/100L) (Paul and Hunter, 2001). *Blastocystis hominis*, *Himnolypis Nana*, *Giardia*, *Entamoeba coli*, *Entamoeba histolytica* and *Cryptosporidium* are water transmitted protozoa (Athari, 1996).

Particular parasites like *Giardia*, *Entamoeba histolytica* and *Cryptosporidium* are not destroyed by routine doses of chlorine in drinking water; hence, epidemics may occur (Mohammadi, 1995; Athari, 1996; Markell *et al.*, 1999).

Surveys in different areas of the United State revealed that 67% - 100% of sewage water and 7% -26.8% of drinking water were contaminated by *Cryptosporidium oocysts*.

A research by Columbia University performed on the people of three regions with drinking water provided from deep wells, protected springs and surface water, showed that parasitic diseases especially *Giardiasis* had higher prevalence in the second and third communities compared to people who drank from deep wells (Isaac-Renton *et al.*, 1999).

46% parasitic contamination was reported by Hosseini doust (1997) from several hospitals in Tehran with urban water distribution system, including: *Acanthamoeba* 17%, *Naegleria* 35%, *Volcamfia* 27%, *Hartmatela* 9%, other ciliates 2.1% and other protozoa 2.5%.

In a survey, Ajaib (2001) worked on the molecular characterization of *Cryptosporidium oocysts* in samples of raw surface water and wastewater in 2000. Method used was a small subunit rRNA based PCR restriction fragment length polymorphism (RFLP); a Technique to detect and characterize *Cryptosporidium oocysts* in 56 samples of raw surface water collected from several areas in the United States and 49 samples of raw wastewater collected from Milwaukee. *Cryptosporidium* parasites were detected in 25 surface water samples and 12 raw wastewater samples. *C. parvum* human and bovine genotypes were the dominant *Cryptosporidium* parasites in the surface water samples. *C. andersoni* was the most common parasite in wastewater. There may be geographic differences in the distribution of *Cryptosporidium* genotypes in surface water. The PCR-RFLP technique can be a useful after

native method for detection and differentiation of *Cryptosporidium* parasites in water (Paul and Hunter, 2001).

In Mazandaran Province the groundwater level is generally high and the domestic wastewaters may eventually enter the drinking water. On the other hand, due to improper management of solid wastes as well as domestic and industrial wastewaters, the potential of groundwater contamination is high. Hence, due to high prevalence of parasitic and zoonotic diseases in the province, this survey was undertaken in accordance to the goal of the world health organization (WHO) on safe drinking water, to examine the rate of parasitic contamination in well water in Mazandaran province.

MATERIALS AND METHODS

Based on the contamination rate of 34.7% in a pilot study in the area, the number of samples was estimated 989 with ($\alpha=0.05$) and ($d=0.03$). Water samples were randomly taken considering the population of towns and number of health centers from 12 cities of Mazandaran province, and transferred to the laboratory in sterile containers. Water samples were then filtered and analyzed according to WHO guidelines. From each health house, three samples were randomly selected; also water samples were collected from all wells of these households. Sampling from tap water and piped well water were performed according to the WHO guidelines. Additional information including the point of sampling, consumer population, water distribution system, type of protection, well depth, disinfection, history of incidents of the water distribution system and the distance of water resource to cesspool, toilet, the waste disposal sites and farm lands were collected by observation of the sites as well as interview with the residents.

Membrane filter (0.8 micron cellulose acetate filter, Germany) under vacuum was for microscopic studies according to WHO guidelines. Direct method and Gram staining procedure were used to identify the parasites. If *cryptosporidium* was seen, floatation (Sheather's sugar) and modified Ziehl-Neelsen staining method were performed. Parasites count was undertaken using McMaster counting slide (0.3 mL).

RESULTS

From 989 water samples 792 (80.1%) were non-contaminated and 197 (19.9%) were parasitic. The contamination rate of water samples from different cities of Mazandaran Province is presented in Fig. 1.

The maximum and the minimum levels of contamination rate were observed in cities with code 6 and 9 respectively. Significant difference

was noticed between the contamination rates of water samples from different cities ($\chi^2=49.21$, d. f. = 11, $p<0.001$).

20 different types of parasites were separated by pathogenic or non-pathogenic of parasites are showed in Fig. 2. These data are summarized in Table 1.

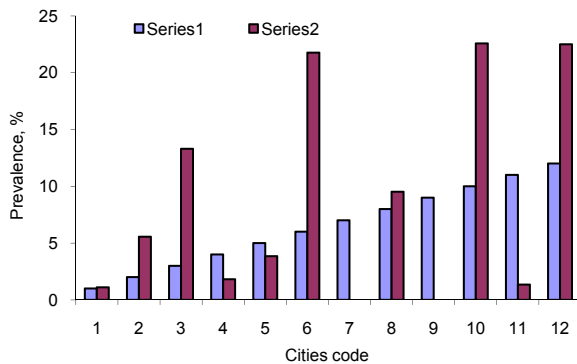


Fig1. Parasite contamination prevalence by cities code in Mazandaran Province

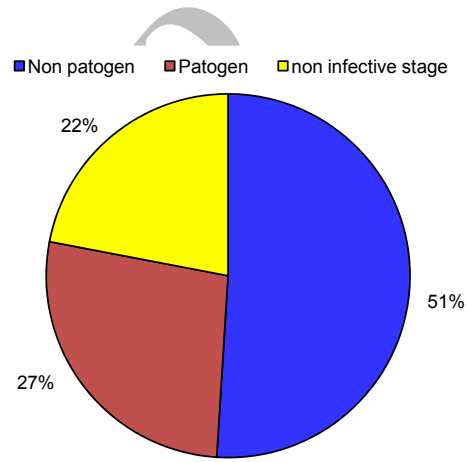


Fig2. Contamination rate by pathogenicity of parasite in drinking water of Mazandaran Province in Iran

Table 1: Diversity of well drinking water parasitic contamination dispersion in Mazaandaran Province according to type and parasites number.

Parasites contamination status	Contamination (One type)		Contamination (Two types)		Contamination (Three types)		Contamination (Four types)	
	Quantity	Percent	Quantity	Percent	Quantity	Percent	Quantity	Percent
Noninfective	792	80.1	---	---	---	---	---	---
Ciliate	13	1.3	8	0.8	2	0.2	---	---
Endolimax nana	18	1.8	---	---	---	---	---	---
Dicrocoelium dendricum	8	0.8	---	---	---	---	---	---
cryptosporidium oocysts	6	0.6	2	0.2	---	---	---	---
Ascaris ova	16	1.6	---	---	2	0.2	---	---
Trichocephal ova	4	0.4	2	0.2	---	---	---	---
Giardia	15	1.5	4	0.4	---	---	1	0.1
Iodamoeba butschlii	4	0.4	---	---	---	---	---	---
Free swimming mastigophora	53	5.4	12	1.2	1	0.1	---	---
Larvae of nematode	13	1.3	3	0.3	2	0.2	---	---
Blastocystis hominis	14	1.4	1	0.1	1	0.1	---	---
Fasiola hepatica ova	2	0.2	---	---	---	---	---	---
Entamoeba histolytica	16	1.6	6	0.6	---	---	1	0.1
Chilomastix mesnili	8	0.8	1	0.1	1	0.1	---	---
Naegleria	---	---	---	---	1	0.1	---	---
Entamoeba coli	4	0.4	2	0.2	---	---	1	0.1
Hymenolepis nana	---	---	---	---	---	---	1	0.1
Isospora belli	---	---	---	---	1	0.1	---	---
Taenia	2	0.2	2	0.2	---	---	---	---
Miracidia	1	0.1	---	---	---	---	---	---

From 197 cases of parasitic contaminated water; in 103 cases (53%) less than 10 parasites in 0.3 cc McMaster count slide and in 94 cases (47.7%) more than 100 parasites were counted.

From 989 samples, 750 of the samples (75.8%) were taken from individual wells and springs that were available with local piping for consumers. The contamination rate of these resources were 143 cases (19%) and 239 samples (24.2%) were taken from a network of wells or urban wells and the contamination was 83 cases (34.7%). Significant statistical difference was noticed between the types of water distribution systems

and the contamination ($p < 0.032$).

The contamination rate in 443 (44.8 %) samples with continuous chlorination was 69 (15.6 %). 508 (51.4%) samples of non chlorinated water and 38 (3.8%) samples of undetermined chlorination status were 122 (24%) and 6 (15.8%) respectively. Distance between wells and sources of contamination presented in table 2. Significant statistical difference were noticed ($\chi^2=16.82$, d.f.=4, $p < 0.001$) between infect and non-infect cases. Off-course significant statistical differences were not noticed between wells and distances from sources of contamination ($p > 0.05$).

Table 2: Dispersion of contamination with related to water distance (distance to cesspool, toilet, farmland, wastewater disposal site)

Variables	Under 18 meter				Upper 18 meter				Unknown			
	infect	%	Non infect	%	infect	%	Non infect	%	infect	%	Non infect	%
Distance to cesspool	88	22.9	296	77.1	101	17.5	476	82.5	8	28.6	20	71.4
Distance to farmland	39	15.7	209	84.3	136	22.1	478	77.9	22	17.3	105	82.7
Distance to landfill	17	0.14	104	0.86	165	20.5	640	79.5	15	23.8	48	76.2
Distance to toilet	80	21.2	299	78.9	109	18.7	475	81.3	8	30.8	18	69.2
Distance to wastes landfill	20	17.4	95	82.6	111	16.8	548	83.2	66	30.7	149	69.3

989 water samples were collected from drinking water wells in 12 cities of Mazandaran province and analyzed according to standard methods. 197(19.9%) out of 989 samples were contaminated with parasites. In Fig.1, city with code 9 has minimum and 6 has maximum percent of parasitic contamination rate. Significant statistical differences were observed between the contamination rates of water samples from different cities of Mazandaran province. The mean contamination rate is about 19.9 percent.

DISCUSSION

The contamination rate in drinking water in some areas in the USA was 7-26.8% (Lechevallier et al., 1991, Madore et al., 1987, Smith, 1998). On the other hand, the most researcher's attention to parasitic contamination in several parts of Mazandaran province is another reason for increase of parasitic prevalence and intestinal parasitic diversity specially protozoa

in Mazandaran populations (Soheila et al., 2001, Rezaeian, 2001, Ziaei, 2003, Mazandaran public health center, 1992, Shabankhani and Ziaei, 2002, Kianian, 2003, Tarane, 2002, Noroozian, 2002, Abedian, 2000, Sajjadi, 2000).

Therefore supplying safe drinking water which is emphasized by W.H.O. can reduce the prevalence of parasitic diseases (Lechevallier, et al., 1991, Madore, et al., 1987, Smith, 1998, Gholami and Mohammadi, 1999, Ghodratollah, 1999). In our study twenty different types of parasites were separated from the water samples.

From 197 parasitic contaminated samples, 53 cases (26.9%) were pathogenic parasites including cryptosporidium, oocysts, Giardia, cysts, Blastocystis humanus, Histolytica amoeba, Himnolipis nana' egg and etc. The contamination rate of Giardia parasite, was 2% and of Histolytica amoeba 2.3%.

The reports indicated that Giardia, E.histolytica and cryptosporidium cysts don't be destroyed

by chlorination, their presence in drinking water may result in the correspondent epidemics. From 443 water samples with continuous chlorination, 69 samples (15.6%) contaminated with different parasites is accord with the fact.

In this project 100 cases (50.8%) of non pathogenic parasites such as free ciliates and flagellates, *Aendulimax nana*, *Eyodamba boochli* and *Entamoeba coli* and etc were separated, with a prevalence of 0.1 -6.7%.(table2). Forty four cases (22.3%) of separated parasites were opportunist including: *Dicrosolium dandriticum*, *Fasciola hepatica*, *Negleria*, *Taenia saginata* and Mirasodium and etc .

Generally *Giardia* and *Cryptosporidium* are main water borne pathogens in developing countries. 750 (75.8%) of samples were from local wells and springs and 239 (24.2%) were from a local well water supply network (being was by several villages) with 19% and 34.7%. Parasite contamination respectively with significant statistical difference based on χ^2 test ($p < 0.032$). This contamination may recommend a possible contact, between drinking water and waste water, cyst's resistance against chlorination, lack of filtration and rottenness of water distributing pipes and irregular chlorination.

Several studies in different parts of the world indicated that water is a major source of Giardiasis epidemic (Shahnaz and Hamid, 2001, Mostafae, 2001, Addiss, 1992, Eugene, 1994, Theresa, 2000).

Considering the geography of Mazandaran with high ground water level and close distance between contamination sources (such as domestic wastewater, solid waste landfills, and etc) and water supply cause a uniformity of contamination is all water supplies which requires urgent sanitation measures. Significant statistical difference were noticed ($P_v < 0.001$) between infect and non-infect cases.

ACKNOWLEDGEMENTS

The authors are grateful to Mazandaran Management and Program Organization for financial support. Also thank to Personnel of Environmental Health Department and Water and Wastewater Laboratory for the practical work.

REFERENCES

- Abedian, H., (2000). Survey on parasitic contamination in east of Mazandaran province, proceeding of 3th National Congress on Iran parasitology-Sari.
- Addiss, D.G., Jeffrey, P. D., Jacquelin, M. R., Eric, E. M., (1992). Epidemiology of Giardiasis in Wisconsin: Increasing Incidence of Reported Cases and Unexplained Seasonal Trends, *Am J Trop Med Hyg*, **47**(1): 13-19.
- Ajaib, S.Y., (2001). Molecular characterization of cryptosporidium oocysts in samples of raw surface water and waste water-applied and environmental microbiology, **67** (3):1097-1101.
- Athari, A., (1996). Clinical parasitology, Tehran, 2nd ed, Daneshpajooch Pub.,pp. 209.
- Eugene, J., (1994). Sever giardiasis in the United States *J. clinical infect. Dis.* **18** (5): 160-3.
- Ghodratollah, S.h., (1999). Drinking water sanitation, Seasonal journal of Lorestan University of medical sciences, **1**(3): 43-47.
- Gholami, M., Mohammadi, H., (1999). Water and wastewater microbiology, Hayyan Pub.
- Hosseini doust, S. R., (1997). Water parasitic contamination in Tehran hospitals, 2nd National Congress of Iran parasitic diseases, Tehran, 19-22 Oct. Mohammadi R.P., (1995). Basic clinical parasitology, Jolodar Tabriz, Rezaee Pub.,pp. 46
- Isaac-Renton, J., Blatherwick, J., Bowie, W.R., Fyfe, M., Khan, M., Li, A., King, A., McLean, M., Medd, L., Moorehead, W., Ong, C.S., Robertson, W., (1999). Epidemic and endemic seroprevalence of antibodies to cryptosporidium and Giardia in residents of three communities with different drinking water supplies. *AM. J Trop. Medicine Hig.*, **60**(4):548-583.
- Kianian, A., (2003). Epidmiological survey on intestinal parasites in Sari and Joybar animal husbandmans in 2002-2003, MS thesis,pp. 569
- Lechevallier, M.W., Norton, W.D., lee, R.G., (1991). Giardia and cryptosporidium spp. Infiltered drinking water supplies. *Appl. Environ. Microbiol.* **57**:2617-26215.
- Madore, M.S., Rose, J.B., Gerba, C.P., Arrowood, M. J., sterling, C.R., (1987). Occurrence of cryptosporidium, oocysts in sewage of effluents and selected surface waters, *J. Parasitol.* **73**:702-705.
- Markell, E.K., John, D.T., krotoski, W., (1999). A medical parasitology, 8th ed w.B. Saunders Company. Philadelphia : 22-42.
- Rowhani, S., (1995). Principal of internal medicine, Harrison, 13th ed., Tehran, Chehre Pub.,pp. 13.
- Mazandaran public health center, (1992). A survey on prevalence of parasitic diseases in rurals of Mazandaran.
- Mostafae, G.h., Hosein, A., (2001). Relation between subterranean water consumption and parasitic contamination in referring peoples to medical diagnosis laboratory of Aboozaidabad Kashan in years 1997-8, Kashan University of medical sciences, health faculty, **13**: 55-60.
- Noroozian, M.B., (2002). Survey and properties of Intestinal worms in Siahrood river suburbs rurals (Ghaemshahr), Tarbiat Modarres University, medical faculty.
- Paul, R., Hunter, J., (2001). Waterborne Disease Epidemiology and Ecology (2nd ed) WILEY and sons: 1-93.
- Rezaeian, D., (2001).A survey on Iran parasitic diseases, Iran health journal, **22** (1-4): 155-164.
- Sajjadi, P., (2000). The prevalence Rate of parasitic contamination in health-cure centers of Shohaday Narivaran Babol, proceeding of 3th National Congress on Iran parasitology-Sari.
- Shabankhani, H., Ziaei, H., (2002). Sampling study in order to determination of parasite disease international conference the humanistic Renaissance in Mathematics education September 20-25 2002, palermo Italy.

- Shahnaz, S.h., Hamid, A., (2001). Giardia lamblia prevalence in children under 10 years deferring to 5 public health centers in Tehran, Baghiatollah University of medical sciences, medical faculty, microbiology group, kowsar medical journal, summer, **2**: 105-109.
- Smith, H.V., (1998). Water borne cryptosporidiosis current status parasitology today (**2th** ed), **14**:14-22.
- Soheila, R., Hormoz, K., Amid, A., (2001). Intestinal parasites prevalence in rural areas of Sari city 2000, Zanzan University of medical sciences scientific journal, **34**: 33-40.
- Tarane, R., Jafar, M., (2002). Intestinal parasitic contamination in urban and rural areas of Feridoonkenar City in Mazandaran, Tehran public health school Journal, **1**(1): 39-48
- Theresa, R. S., (2000). Emerging parasite zoonoses associated with water and food international journal for parasitology **30**: 1379-1393.
- Ziaei, H., (2003). Prevalence Rate of cryptosporidium in patients receiving immunosuppressive drugs in Amircola Iran 2002 **3_u** Bulkan conference Istanbul Turkey.

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