PARASITIC CONTAMINATION OF WELLS DRINKING WATER IN MAZANDARAN PROVINCE

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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 Cryptosporidum are water transmitted protozoa (Athari, 1996).

Particular parasites like Giardia, Entamoeba histolytica and Cryptosporidum are not destroyed by routine doses of chlorine in drinking water; hence, epidemics may occure (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 oocyts.

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 Milwoukee. 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 (a=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 ($\chi2=49.21$, 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.

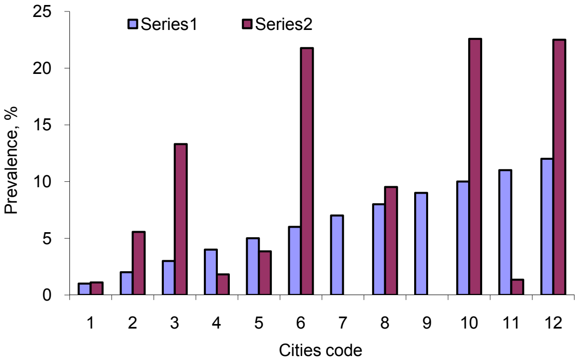

Fig1. Parasite contamination prevalence by cities code in Mazandaran Province

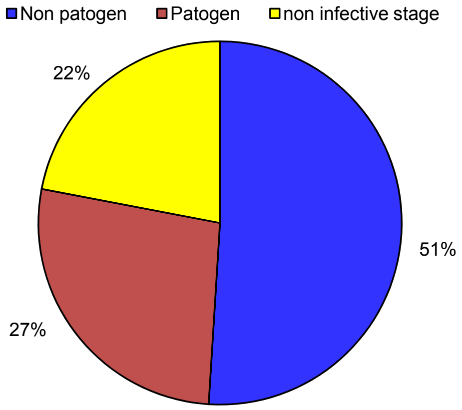

Fig2. Contamination rate by patoginicity of parasite in drinking water of Mazandaran Province in Iran

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	---	---	---	---	---	---
Dicrocelium 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	---	---	---	---	---	---
Entamoba 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 ($\chi2=16.82$, 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, oocyts, Giardia, cysts, Blastositis humanus, Histolytica ameba, 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 $\chi 2$ 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, Mostafaee, 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.

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