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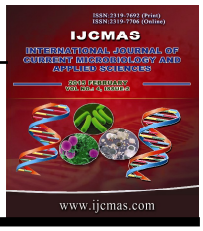


Original Research Article

Prevalence of *Entamoeba histolytica*/dispar in drinking water in the city of Shush, Khuzestan Province in 2011

Ezatollah Ghasemi[1]*, Mahmoud Rahdar[1] and Morad Rostami[2]

[1]Department of Parasitology, Faculty of Medicine, Ahvaz Jondishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Biochemistry, Faculty of Medicine, Ahvaz Jondishapur University of Medical Sciences, Ahvaz, Iran
**Corresponding author*

Keywords

Shush, *Entamoeba histolytica*/ Dispar, Water, Well, Rural, Urban.

A B S T R A C T

In most areas, water pollution is one of the most important primary sources of E. histolytica infection. Due to the high groundwater levels and Use of surface water of three rivers Karkheh, Dez and Shavour used to drinking in many areas of the city, this study was designed to examination the prevalence of *E. histolytica*/dispar in the city of Shush. This cross-sectional study was doe on 160 condensated water sample through a a filter with defined pore size and preparation of samples for microscopic examination. 500 ml of water was prepared and coded at standard dry plastic containers (Polyethylene) for carrying samples and transferred with the cold chain to the laboratory. The extracted samples were divided to 5 sterile tube each with a 10 ml volume and centrifuged for 5 minutes at 2000 rpm and studied through microscopic examination. From 160 samples with source of drinking water wells and surface water in rural and urban areas in the city of Shush, 64 samples (40%) were infected with at least one of the active stages of parasitic organisms; that of these, 46 samples (28.7%) the protozoa and 18 samples (11.2%) were infected with the worm process of living organisms. From 64 infected samples, 10 isolates (6.3%) were related to the parasite *Entamoeba histolytica*. Prevalence of 3.6% of drinking water in the city of Shush to *Entamoeba histolytica*, is remarkable, that need to health planninig to reduced it.

Introduction

Nearly 1.1 billion people still remain without access to improved sources of drinking water, and also about 2.4 billion have no access to improved sanitation services (1). drinking water contamination has devastating effects on the health of human society and implies the presence of microorganisms which could potentially created water-borne diseases threat in young and teenager people, particularly those with impaired immune systems (2). One of the most important parasitic pathogens in contaminated water supplies, are *Entamoeba histolytica* cysts (3). In most areas, water

pollution is one of the most important primary sources of *E.histolytica* infection (4). This parasitic disease, after malaria and Shistosomiasis, Is responsible for the highest mortality rate (5). In the United States, attributed the 6 water and food-borne outbreaks to this parasite during 1946- 1980 (6). About 68% of all amoebic dysentery, especially in non-industrialized countries, are teenagers (7) and 17% of all deaths due to this disease are children under five years of age (8). In patients infected with the human immunodeficiency virus (HIV) or with acquired immunodeficiency syndrome (AIDS), this fatal disease, Is long and life-threatening (9). 4 nuclei cyst of *Entamoeba histolytica*, can be transmitted through non-chlorinated drinking water sources contaminated with human feces (10). In this regard, often the surface waters, especially in tropical and subtropical developing countries are contaminated to this parasite (11-14). But to improve the microbiological quality of drinking water, the amount of pollution can be controlled (15). The disease is endemic in Iran and the prevalence of this parasite is variable between 40-60% (16).

Due to the high groundwater levels and Use of surface water of three rivers Karkheh, Dez and Shavour that used to drinking in many areas of the city of Shush and Lack of proper collection and disposal of standard sewage network that enables secondary pollution of water distribution network through sewage leaking into the wells, this study was designed to examination the prevalence of *E.histolytica*/dispar in the city of Shush and to designation of policies and appropriate strategies for the prevention and control of drinking water parasites in this city.

Material and Methods:

This study was done as a cross-sectional manner. The sample size of this study was determined 160 samples through 95% confidence and the contamination rate of d=10. Procedure was as follows and was according to condensation of water sample through a filter with a defined pore size and preparation of samples for microscopic examination.

A. The samples were collected randomly from each area, based on the list of health centers, dispersal and population covered. 500 ml of water was prepared and coded at standard dry plastic containers (Polyethylene) that special for carrying samples, then were transferred with the cold chain and within 6 hours to the laboratory (1, 3, 17).

B. Sample preparation for microscopic examination with using a vacuum pump and using membrane filters (Cellulose Acetate Filter Germany) with a size of 0.45µm (1, 18). Filters were immersed from the side of the Beakers wall in a volume of 50 ml of distilled water. For concentration of samples, the extracted samples were divided to 5 sterile tube each with a volume of 10 ml (3). The tubes were centrifuged for 5 minutes at 2000rpm (1 and 3). The sediment were studied and mixed with 2% formaldehyde solution in a ratio of 1: 1. If there was not possible tested due to time constraints, the sediment resuspended in 1 ml of PBS buffer and stored to -20 ° C (18 and 19).

C. Microscopic examination: At this stage, were used from 3 procedures, iodized and salt water wet (wet mount) and Trichrome staining to detect cysts and trophozoites of *Entamoeba histolytica*/Dyspar (3, 20 and 21). Parasites were counted with using of 10µL volume McMaster slide (22). To analyze the results, the SPSS version 18 and chi-square test was used (19).

Results and Discussion

From 160 samples with source of drinking water wells and surface water in rural and urban areas in the city of Shush, 64 samples (40%) were infected with at least one of the active stages of parasitic organisms such as protozoa and helminthes. Of these, 46 samples (28.7%) the protozoa and 18 samples (11.2%) were infected with the worm process of living organisms. Also, the 96 samples (60%) without contamination detected. From 64 samples contained a parasitic organism, 10 isolates (6.3%) were related to the parasite *Entamoeba histolytica* and the number of parasites was observed less than 10 number in 10 µl McMaster slide.

Drinking surface water in rural areas with 17.5% (7 samples from 160 studied samples), had the highest prevalence of *E.histolytica* cysts.

Between pollution of infected waters with this parasite by chi-square test (chi-square), significant differences were observed (P-value 0.006). None of the samples of drinkable water with Municipal water supply wells, were infected with this organism. Also, 2.5% (1/160 samples) of urban surface water and 5% (2/160 samples) of rural water wells contaminated with parasites were detected. This study has shown that in the four studied water source, rural surface water pollution has the highest frequency and organisms contamination.

Between contamination with *E.histolytica* in different areas (urban and rural) with type of drinking water, including drinking water wells and surface water, the difference was statistically significant (P-value = 0.000).

The city of Shush are in the Khuzestan province, northwest at 12 and 32 degrees north latitude and 17 and 48 degrees east longitude meridian of Greenwich. Its height is 112 meters above sea level and covers an area of over 3577 square kilometers. According to the census of 2012, the population of this city, was 210 thousands. In this area, three major rivers, including the Dez, Karkheh and Shavour were flowing. The total number of villages covered by this city, are 174 villages. Drinking water in rural areas of this city generally comes from two sources. About 152 villages have been Water supply facilities, of which, 59 rural drinking water supply from surface water (Karkheh and Dez) and 93 village source of water supply facilities are deep wells to a depth of approximately 80 to 110 meters. This despite the fact that about 22 villages have no drinking water supply facilities and their drinking water are provided from surface water sources (Karkheh and Shavour), shallow wells and hand trucks. But drinkable water in urban areas of this city, including the cities of Shush, Alwan, Hor Riahi, Fatholmobin, Shavour and the city region of Hafttapeh, is usually provided through deep wells.

From 80 specimens collected by source water wells in rural and urban areas of this city, 2 cases (2.5%) were infected with *Entamoeba histolytica*. Water wells height in rural areas of this city are differs from shallow wells to wells with 80 to 110 meters. Infection with parasitic organisms was observed in shallow wells. Sabiyeh and Farih villages that are around the Ubaid village, due to usage of these wells, had contamination with parasites; particularly an *E.histolytica* cysts were observed in Sabiyeh. Also, drinking water of Allahu Akbar Town that subset of Hafttapeh, were also infected with *E.histolytica* cysts. The high incidence of human amebiasis in this area in the last few years is a clear indication of this claim. Due to the high level of water wells, and some animal and human waste leak likely to become infected with parasitic

organisms, it is unavoidable. According to investigations, pollution of deep wells with 80-110 meter in urban areas of this city, confirms fracture in the water pipeline and It is expected that the parasitic organisms does not exist in deep wells.

Because of the high deep and low water levels of the wells, it seems unlikely the possibility of leakage of waste into the water of these wells. The infection may be justified due to the secondary contamination of water wells through leakage of wastewater and insufficiency of chlorine residual in the water distribution network.

Until the epidemiologic evidence not indicates to promoting the health of this region, there is no improvement in the quality of drinking water and the absence of parasitic organisms in them, remain unclear (23). From 80 specimens collected in urban and rural areas with surface water sources such as rivers Karkheh, Dez and Shavour, 8 cases (20%) was diagnosed infected with *E.histolytica* cyst. Contamination of 7 samples (17.5%) of all samples that infected with this parasite, is relate to use of surface water in different rural areas. Rural areas Ahu Dasht, Seyyed Rahimeh, Obeid and villages of Shavour were the most affected areas with this parasite. Contamination of drinking water wells in compared with surface water in this city, with certain parasitic organisms, especially *Entamoeba histolytica*, is much less.

The results of this study are somewhat in line with a study that was conducted at Columbia University. The results of a study at Columbia University on three populations that were used water from deep wells, protected springs and surface water, respectively, show that certain parasitic organisms *Entamoeba histolytica*, in the second and third population is a more common and It comes from the finding that the risk of exposure to parasitic diseases in the use of drinking water from deep wells, are more less (24). However, *Entamoeba histolytica* cysts are rarely found in sources of drinking water and open surface water (25). The contamination of drinking water in some areas of the United States to parasitic organisms have been reported between 7 and 26.8% (27-26).

In the study of Sultan A. et al. in 2011 in Pakistan, from 450 water samples that were prepared from the faucet, the pond (lake) and the wastewater, 295 samples (65.5%) were infected with the protozoan that of these, 65 samples (14.4%) was diagnosed with *Entamoeba histolytica* infection. This study demonstrated that public health care is essential for the prevention of diseases transmitted through water (19).

The present study suggests that the parasite *Entamoeba histolytica* infection rate in the city of Shush, is the rate of 3.6% that in comparison with study of Hezarjaribi et al. in the Mazandaran that reported the prevalence of *Entamoeba histolytica*, 2.3% of the positive samples in the water wells, is further (22).

Given the geography of the Shush city and the presence of three major rivers Dez Karkheh and Shavour and shallow wells, particularly in the rural areas, as well as vegetables cultivated lands that in some cases fertile by human manure, waste water and surface water, raises the possibility of infection with this parasite.

Since now the Shush city lacks wastewater collection system and the wastewater disposal are done by traditional methods as well as absorption takes place, therefore, there are the potential risk of contamination of groundwater supplies to these parasites.

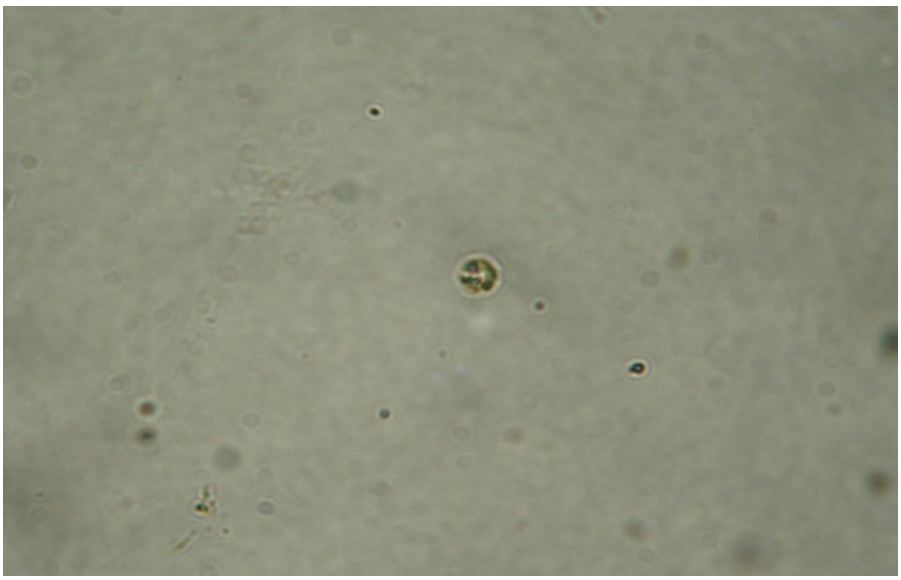
Therefore, the development wells, direct and continuous supervision over the operation of chlorination and filtration of water, as well as organizing wastewater collection systems and filtration of wastewater and.check and repair broken water pipes, can prevent this health problem; which is a complex health care in underserved countries in recent decades.

Prevalence of 3.6% of drinking water in the city of Shush to parasite *Entamoeba histolytica*, is remarkable. The importance of water-borne diarrheal diseases has led to the fact that contaminated drinking water in our society and the environment, it is unavoidable. In light of this study will be presented solutions to prevent this health problem.

Table.1 Distribution of water pollution in the studied parasite *Entamoeba histolytica*

Variable	The total sample (N=160)	Urban water wells (N=40)	Urban surface water (N=40)	Rural water wells (N=40)	Rural surface water (N=40)
Entamoeba histolytica cyst	10 (%6.3)	0 (%0)	1 (%2.5)	2 (%5)	7 (17.5%)

Figure.1 *Entamoeba histolytica* cysts in water samples



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