

Research Article

Waterborne Parasites: A Recent Status of Occurrence, Source and Human Intestinal Parasites in Sources and Tap Water; Dehloran, South West, Iran

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Abstract

Water is the most important element in the universe and preventing of water pollution is vital for the health of creature survival. According to Centers for Disease Control and Prevention (CDC), various microorganisms including parasitic agents can be transmitted by different sources of water. This study aimed to investigate existence of parasitic elements in piped water of Dehloran city, South West, Iran; before, after and in filters of household refinery system along with sources of water samples through Lugol's iodine and modified Ziehl-Neelsen stain methods. Based on population density, 50 filters were collected from 50 houses and 36 samples from pit by accumulated water as well as channel water and examined for parasitic agents. None parasitic agents were identified in samples from filters however, examined 36 collected samples from other sources were contaminated with 5 different strains of active stages of parasitic agents, of which 60% were potentially pathogenic and 40% nonpathogenic. Infective stage strains included *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Blastocystis hominis*, *Giardia* cyst and *Cryptosporidium* oocyst like. The study also confirmed that the distance between water sources and starting place of contamination, type of water samples and chlorination status had significantly statistical relationship with contamination prevalence ($p < 0.001$). Groundwater and surface water could directly connect with each other. If there is a healthy source in the vicinity of a contaminated source, potential for water pollution is very high, leading to the groundwater pollution specially in rainy seasons and floods. Therefore, in line with this issue stool samples of 11856 referrals to medical-health centers were also examined and detected 153 cases of *Giardia* cyst, 161 *Entamoeba histolytica/dispar* cyst, 72 *Blastocystis* spp., 332 *Entamoeba coli* and 1 case *Hymenolepis nana* ova. Consequently, in line with the results and in view of the direct association between safe water and human health, proper performance of providing hygienic drinking water should be available.

Introduction

The parasitic diseases are among the most important causes of morbidity and mortality in attrition of physical and intellectual in developing countries [1]. The prevalence of parasitic diseases and their transmission agents are dealt with different water sources [2]. *Giardia lamblia* (giardiasis cyst), *Cryptosporidium parvum* (human cryptosporidiosis oocystes), *Entamoeba histolytica* (amebic dysentery cyst) and *Balantidium* (balantidiasis cyst) are 4 important pathogen protozoan agents that water plays an important role in their distribution [1,3]. Based on conducted studies *Cryptosporidium* oocystes and *Giardia* cysts are present around the world in the aquatic environment and are often found in surface water sources which their concentrations are associated with fecal contamination or human use of water [4]. *Cryptosporidium*'s distribution accounted for 25% of cases of diarrhea worldwide and in children with malnutrition and HIV tend to be lasting for long time. In addition to sustainable and fatal infection in patients with immune deficiency it is one of common factors causing diarrhea in travelers and boarding centers residents [5]. Approximately, 30 percent of people living with *Entamoeba coli* which emissions globally [6]. Well protected ground waters which do not mix with surface water or other contaminants sources are free of pathogen and other enteric agents. However, ground waters which mix with surface water or other sources of pollutants (rain water) may contain low levels of *Cryptosporidium* and *Giardia* causing water-borne diseases [7]. *Microsporidia* in most cases in hosts is as gastrointestinal form and typically transmits from animal

to man, man to man and from water and food to man [7]. There are underground water resources with productive plains (9 wells with a depth of at least 100m up to 140m) in various sectors of Dehloran city supplying residents' drinking water. Chemical examination of the water, categorized it as hard and very hard water type with low quality during the years 2011 and 2012. Samples from Susa, vicinity of Dehloran showed the prevalence of *Entamoeba histolytica/dispar* for 16.6% [8]. In another study on association of drinking water and prevalence of intestinal parasites in Bandar Abbas, individuals were positive for 22 (2.2%) *Entamoeba histolytica*, 66 (6.6%) *Giardia*, 17 (1.7%) *Ascaris* and 15 (1.5%) for *Hymenolepis nana* [9]. Parasitic infection prevalence in cities of Tonekabon, Chalus and Ramsar in north of Iran was 2.38%, which included *Giardia* 1.43%, *Blastocystis* 0.71% and *Entamoeba coli* 0.24 % while in none was viewed *Cryptosporidium* spp. [9]. Results from Columbia University in three groups of population used water from deep wells, protected springs and surface water showed *Giardia* and *Entamoeba histolytica* were the most common in second and third groups while the risk of exposure to parasitic diseases was much less for people using deep wells [10].

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Received: October 29, 2016; **Accepted:** November 29, 2016; **Published:** December 01, 2016

Study Area

The study was carried out in rural area of Dehloran, one of largest cities located in South West of Ilam Province and of East lead to Abdanan, South West to Iraq, North to Mehran and South to Andimeshk and Susa cities. The population is about 31700 people which makes up 25% of total population of Ilam [11]. The city has three sections with six rural districts and mean ambient temperature of 28–48.9°C and 16.4–24.9°C in summer and winter, respectively [12]. Musian, Meymeh and Pahleh are three counties of Dehloran. Average annually precipitation rainfall is 226.8 mm [1] and the altitude is 180 meters above sea level [1]. In terms of geographic location Dehloran is between 47°, 15' East longitude and 32° 25' North latitude [1]. The area is in developing form hence adequate facilities and infrastructural amenities are still lacking. The economy is based on agriculture of wheat and barley as well as livestock farming and handicrafts. Sources of water for drinking include streams and wells and there are source of water for other domestic activities in the area include channel and pits collected by rain water.

Figure 1

Methods

To the best of our knowledge and according to reports from Dehloran health centers there is no study for detection of parasitic contamination sources through water reservoirs as a way for parasitic elements expansion and dispersion. Therefore, this study aimed to determine the prevalence of parasitic elements with current diagnostic methods including direct smear/ Lugol's iodine and modified Ziehl-Neelsen stain in household filter systems, pit collected by rain water and channel water samples.

Filters samples

Filter samples were randomly collected from metropolitan area of Dehloran city with a population about 31700. Given that, similarity population density and prevalence of intestinal parasites, an equal number of 10 first water filter device that is at least 3 months of use have been collected from 5 regions; North, South, East, West and center of the city and transferred to the laboratory in sterile containers. Filters along with water of filter tank were placed in a beaker, washed from top to bottom with water pressure, cut into pieces and washed twice. The solutions were mixed thoroughly, allowed to settle for two hours at room temperature, supernatant was discarded and sediments were centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and smears of sediments were made on grease-free slides. Two slides were prepared for each sample; one was stained with Lugol's iodine [5] while the other with acid fast stain using Ziehl-Neelsen technique as described by [13]. The stained slides were covered with cover slips and viewed under the light microscope using 10x and 40x objective lens. Furthermore, pulled a swab on each filter and immersed in a saline drop on a slide to examine microscopically through direct method.

Sample collection

This study was designed as a cross-sectional descriptive epidemiologic study and numbers of filter samples were calculated at 53 according to the contamination rate of $P=16.6\%$ in a pilot study in the vicinity area; Susa, ($\alpha=0.05$) and ($d=0.01$) and ($Z_{1-\alpha/2}=1.96$):

$$n = \frac{(z_{1-\alpha/2})^2 * p(1-p)}{d^2} \quad n = \frac{1.96 \times 16.6(1-16.6)}{0.01} = 53$$

Pit collected by rain water and channel water samples

Water samples were collected directly from the various locations into 2-litre sterile wide mouth screw-capped bottles from six sites

included 3 sites of pit by accumulated rain water and 3 sites of channel water from 6 different locations. In each journey, containers were collected from each sample site between 3pm and 5pm and containers labeled according to standard water sampling and tested within early 6 hours [4,14]. In total, 36 water samples were collected in 6 journeys.

Additional facts including the point of collected samples, user population, water distribution system, type of protection, applied possible disinfection, history of incidents of the water distribution diseases and the distance of water supply to possible contamination sources, the waste disposal sites and farm fields were also considered by observation of locations as well as interview with residents. To test water samples, membrane filter (0.2 and 0.4 μ cellulose acetate, Germany) under vacuum was used for microscopic studies according to WHO guidelines. All water samples were viewed with naked eyes after which fresh drops of samples were viewed under a light microscope to note the presence of visible particles/debris.

Statistical Analysis

Differences were analyzed through Chi-square and a *P value* of < 0.05 as significant.

Findings

Filter sample

Of 53 tested filter system samples using direct smear via Lugol's iodine and modified Ziehl-Neelsen stain methods found no parasitic agents such as protozoa, larvae, worms, eggs or oocytes.

Pit collected by rain water and channel water samples

Examining samples from Pit by accumulated rain water and channel water applying direct smear Lugol's iodine documented at least five type water-borne parasites include *Giardia spp* which accounted to 18% of all the parasites, *Entamoeba histolytica/disbar* 10%, *Entamoeba coli* 32% and *Blastocystis hominis* 26%. In addition, applying modified ziehl-Neelsen stained method for sediment samples indicated *Cryptosporidium* oocytes like at 14% (Table 1) (Figure 2, 3) Collected samples from different water sources showed that *E. coli* had the highest rate of contamination (32%) followed by, *Blastocystis hominis* (26%), *Giardia spp* (18%), *Crypto. oocytes like* (14%) while, the *Entamoeba histolytica/disbar* had the least (10%) (See Table 1). Analyzing obtained results proved that there was statistically significant differences in the level of parasitic contamination and the number of times each parasite was observed ($P < 0.05$). Table 1 demonstrates the percentage of recognized parasites in different sample water sources, various water sample sites and the number of times the parasites recognized in each site. The results revealed that water samples from all locations, incorporated 5 different parasite types and regarding number of times each parasite was detected; the *Entamoeba coli* cysts were the most widely distributed as it observed in the entire sites followed by *Blastocystis hominis*, however *Entamoeba histolytica/disbar* cysts were the least distributed parasitic agent (Figure 2). In terms of water sources the channel water samples were more contaminated (56%) than Pit by accumulated rain water (44%) (Figure 1) and significant statistical difference were noticed ($\chi^2=16.82$, d.f.=4, $p<0.05$). With respect of various water sample sites, Post-code area 4 from canal water showed the highest number of parasites [15] while Post-code area 3 and Post-code area 4 from Pit by accumulated rain water equally had the least parasites [7] and significant statistical difference was noticed for types of water sample sources, Post-code areas and the contamination ($p<0.05$).

Table 1: Summary of results of the various examined water sample sources and sites.

| Water source | Water sample site | Total NO. of Samples | Number of times each parasite was observed | | | | | Total No of parasites | Parasite Incidence (%) | Parasite Incidence (%) based on water source |
|---------------------------|-------------------|----------------------|--|-----------------------|----------------|-----------------------------|----------------------------|-----------------------|------------------------|--|
| | | | <i>Giardia</i> spp. | <i>Entamoeba</i> spp. | <i>E. coli</i> | <i>Blastocystis hominis</i> | <i>Crypto. Oocyst like</i> | | | |
| Pit by accumulated water, | Post-code 2 | 7 | 2 | 1 | 3 | 1 | 1 | 8 | 16 | 44% |
| | Post-code 3 | 5 | 2 | 1 | 2 | 2 | 0 | 7 | 14 | |
| | Post-code 4 | 6 | 1 | 0 | 2 | 3 | 1 | 7 | 14 | |
| Channel Water | Post-code 2 | 5 | 1 | 0 | 2 | 2 | 3 | 8 | 16 | 56% |
| | Post-code 3 | 7 | 0 | 1 | 3 | 2 | 2 | 8 | 16 | |
| | Post-code 4 | 6 | 3 | 2 | 4 | 3 | 0 | 12 | 24 | |
| Total | - | 36 | 9 | 5 | 16 | 13 | 7 | 50 | - | |
| Percentage (%) | - | - | 18 | 10 | 32 | 26 | 14 | 100 | - | 100 |



Figure 1: Map showing the study Area

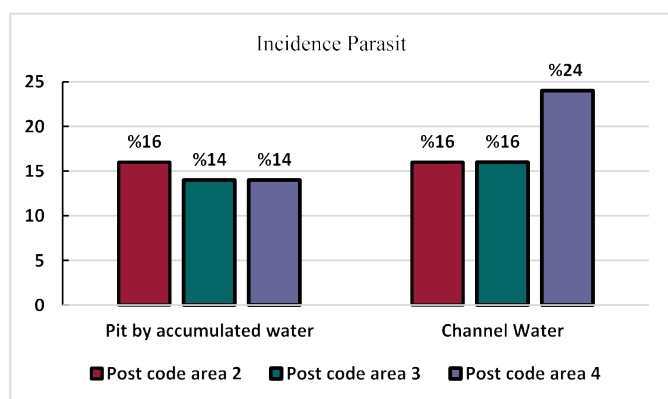


Figure 2: Percentage of parasite contamination based on post code area and water samples

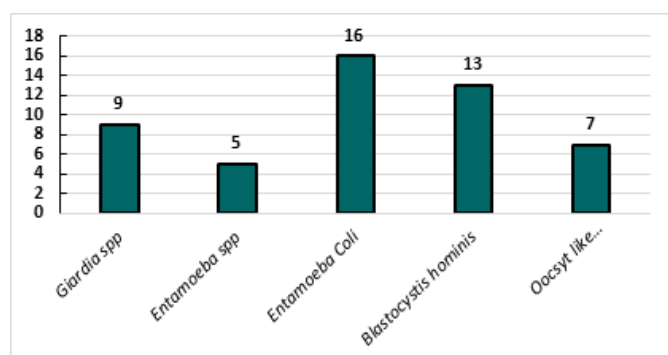


Figure 3: Number of times each parasite was detected in water samples

Discussion

Filter system samples

Outbreaks of parasitic waterborne elements in developed countries show that transmission of parasitic elements by water reservoirs is a significant risk. In the case of *Cryptosporidium*, the lack of an adequate treatment for immune-compromised patients raises the difficulty and outbreaks in developed countries receive most awareness however, epidemics and low-level spread of protozoa through drinking-water in both countries types are in a similar way [8,15]. Cysts and oocysts are regularly found in drinking-water [15-17] although only a small quantity may be viable and transferable to man. Disinfection with chlorine has always been an important means of preventing spread of waterborne parasites. Therefore, the chlorine of drinking water tanks is measured averagely 145 times monthly by Dehloran water laboratory and the limit is roughly 0.4 to 0.6 ppm. However, due to chlorine device downtime the rate of chlorine may report at zero point. In addition, the Dehloran water laboratory, solitary carrying out the test of water in term of Thermo-tolerant coliform bacteria. In addition, resistance to chlorine makes problematic and unsuccessful the disinfection procedure for parasite elements' inactivation in drinking-water reservoirs. That is why we often encountered with contamination among people especially those who lack of piped drinking water filters system. Therefore, in line with this issue in a retrospective study by the current authors on the prevalence of parasites in human stool samples, of 11856 referred to medical-health centers for the period of 2015, we detected 87 cases of *Giardia* cyst and 13 trophozoite, 161 cases of *Entamoeba histolytica/disbar* cyst and 11 trophozoite, 24 cases *Blastocystis* spp., 6 cases *E. coli* and 1 case *Hymnolepis nana* ova.

Pit collected by rain water and channel water samples and site of collection

Pathogenic parasites may cause serious hazard to health of dwellers such as farmers owing to their activity and children due to poor hygienic behaviors [6].

The study documented parasitic contamination for different water sample sources in Dehloran city and the incidence of contamination varies between the collected water samples spots and sources. Several studies at different parts of the country have also documented a number of incidence of water contamination with parasites [9,11,18]. In the current study channel water samples recorded for higher incidence of parasitic contamination (56%) compared to samples from pit by accumulated rainwater (44%). This could be due to the fact that during the shortage of water, channel water sources serve as reservoirs that collect run-off water from different routes. Therefore,

channel water stand a greater risk of contamination with parasites [6].

Subject to location and sanitation circumstances, flood water led to contaminate drinking water supplied from surface water, groundwater, and distribution systems. Groundwater wells can easily be contaminated from municipal sewage and septic seepage. The surface water sources are also affected in similar manners. Having said that, Dehloran region has a temperate climate that is sometimes subject to extreme weather changes especially for the time of rainy seasons. In support of this, examining water samples after the recent huge floods in the region indicated more contamination for different parasitic elements (Pictures). Figure 4

Five water-borne protozoan parasites; *Giardia* spp., *Entamoeba histolytica/dispar*, *Cryptosporidium* spp., *Blastocystis* spp. and *Entamoeba coli* were recognized in this study in their cystic and oocystic forms (Figures 5). Apart from parasitic agents, majority of water samples contained dirt, debris and colored particles making water unsafe for consumption. In addition to erosion and influx of surface water into the water sources, the activities that go on within the area of the water sources could also take into account for their contamination. Isolated of parasitic agents from various sample water sources in this study consent with the completed studies by [3,6,19] and of 36 water samples, *Entamoeba coli*, *Blastocystis* spp., *Giardia* spp., *Entamoeba histolytica/dispar*, and *Cryptosporidium* spp., were the five prevalent parasites observed 16, 13, 9, 5 and 7 times, respectively. *Entamoeba coli* was the most common prevalent (32%) while, *Entamoeba histolytica/dispar* was the least prevalent (10%). Therefore, the results showed that there is a potential tendency of parasite contamination for animals and human in the study area. The *Entamoeba coli* high incidence in the current work was consistent with study of [20] which could be associated with the fact that during rains seasons and floods high rate of contamination of water sources is possibly originated from human or/and animal faeces. *Cryptosporidium* spp., *Giardia* spp., *Blastocystis* and *E. histolytica/dispar* are others water-borne protozoan parasites that cause major human infections. Studies in different parts of the world indicated that water is main source of parasites transmission and epidemics [2,7,10,21-26]. Furthermore, water pollution strongly linked with unsanitary activities near water sources and actions of animal farms which included the parasites agents, connection between drinking water sources and waste water, cyst's resistance against chlorination, lack of filtration and rottenness of water distributing pipes and irregular chlorination.

Considering the geographical location of Dehloran city with many ground water sources and close distance between contamination sources such as locally wastewater, hard waste landfills, and water sources especially with recent floods in the area and possibility of causing uniformity of contamination of all water supplies require urgent hygiene procedures.



Figure 4: Photos for recent floods in Dehloran; 2016

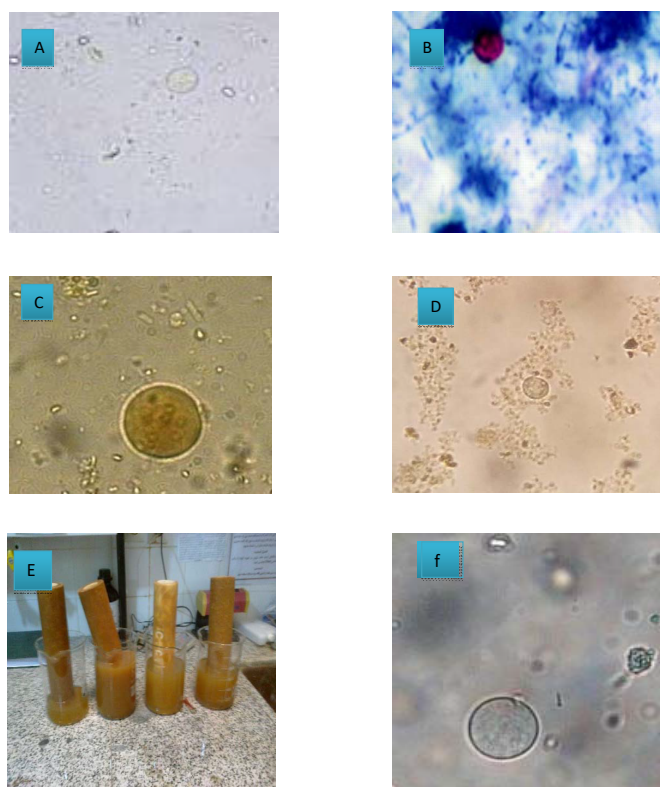


Figure 5: *Giardia* sps (A) Oocyst like *Cryptosporidium* spp (B) *Entamoeba coli* (C) *Blastocystis hominis* cyst (D) Household water treatment filters (E) *Entamoeba* spp (F)

Conclusion

The variety of protozoan parasite were presented in water sample sources and a significant statistical difference was noticed between the types of water sample sources and sites as well as the contamination ($p < 0.05$). Therefore, it is strongly suggested that all households use filter refinery system for piped water to remove parasites agents, dirt, debris as well as coloured particles to make the water safe for consumption. It is also recommended to encourage health-care providers to consider and test drinking water sources routinely for parasites in the workup for avoiding of gastrointestinal illness.

Acknowledgements

We would like to thank the staff at the Dehloran Shohada Hospital Laboratory, Dehloran Water Laboratory, and Dehloran Water and Sewage Organization for their assistance and collaboration with field sample collection. We would also like to thank the Health Center staff for their collaboration and assistance in data collection, and to all the individuals who kindly gave their time to participate in this study. This study was supported by the Vice Chancellor for Research and Technology Development of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, under Behsan Code 33009032 Project number. We thank the reviewers for their valuable input in this paper.

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