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Original Article

Detection of *Giardia lamblia* Cysts in Surface Waters of Rasht City, Iran

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Introduction: *Giardia lamblia* is a protozoan parasite with universal distribution in human populations. This infections transfer to human via contaminated foods and waters with *Giardia* cysts. The Knowledge on the incidence of this agent in the potential infection sources can provide valuable information for control and the spread of this parasite to human communities. This study was aimed to isolate and identify the *Giardia lamblia* cysts in the surface waters of Rasht city, Guilan province, north of Iran with microscopy and PCR assay. **Methods**: This cross-sectional study was performed on 45 samples of surface waters collected from rivers and wetlands in the vicinity of Rasht city. The samples were concentrated using nitrocellulose membrane filters, and the sediments were examined for *Giardia* cysts by microscopy with a magnification of 1000 X. Also, DNA was extracted from the sediments, and the heat shock protein gene of *G. lamblia* was amplified. **Results**: From 45 samples, 33.33% and 40% were positive by microscopy and PCR, respectively. **Conclusion:** In comparison with the standard microscopic method, PCR showed more sensitivity for detection of *G. lamblia* cysts in water samples. *J Med Microbiol Infec Dis, 2018, 6 (1): 8-12.*

Keywords: *Giardia lamblia* Cysts, Surface Waters, PCR, Rasht.

INTRODUCTION

Giardia lamblia, an intestinal flagellate protozoan, is the causative agent of one of the most common human parasitic infections worldwide known as giardiasis. It is estimated that 200 million people in Asia, Africa, and Latin America are infected with this parasite [1], and approximately, 500,000 new cases of the disease are reported in children annually [2].

Giardia lamblia cysts are highly pathogenic to humans; in a clinical study conducted on volunteers, ingestion of gelatin capsules containing 10 *Giardia* cysts caused giardiasis [3]. Symptoms of giardiasis are variable and range from no signs to chronic diarrhea, malnutrition, and weight loss [4]. In Iran, the diagnosis of *G. lamblia* is based on detection of *G. lamblia* cysts by microscopy in feces, which lacks the required sensitivity when the parasite is scanty [5]. Due to the intermittent shedding of cysts, microscopic examination of a single stool specimen has a low sensitivity of 46% [6]. Hence, at least three fecal samples should be taken and examined over a 3-5 day period to achieve 94% accuracy in diagnosing *Giardia* cases [7]. In 1952, Filice differentiated *Giardia duodenalis*, *Giardia morris,* and *Giardia argilis* by the mean body size and the measurement of the dimensions of *Giardia* trophozoites [8]. In comparative studies on staining methods of *Giardia* cysts, trichrome staining showed to be the most sensitive approach [9]. In developing countries, pathogenic water-borne parasites of the gastrointestinal tract, including *Entamoeba histolytica*, *G. lamblia*, and *Cryptosporidium parvum* are frequent causes of human death, especially among children [10]. These parasites are the most common infectious agents worldwide [11]. Water is not a suitable medium for the growth of *Giardia,* but a high potential environment for spread and transmission of this parasite. Despite the refinement of water resources, some cysts can remain in it and survive for a long time [12]. The presence of *G. lamblia* cysts in water resources can cause outbreaks, especially during catastrophes such as flood and earthquakes. Several studies are annually conducted worldwide to screen for waterborne infectious agents. The obtained data can play a crucial role in the control of these infectious agents. Regarding the importance of rapid detection of *G. lamblia* cysts, this study was conducted for comparison of microscopy and PCR methods to identify *G. lamblia* cysts in surface waters of Rasht city, Guilan province, Iran.

MATERIAL AND METHODS

The study area and sample collection. Rasht city is located in Guilan province of Iran with 37°16′51″N 49°34′59″E coordinates and a mean elevation of 5 m above the sea level with an annual rainfall of above 1000 mm (Fig. 1). Amounts of one liter of water were collected at a depth of ≈30 cm from 12 rivers and 8 wetlands near Rasht city

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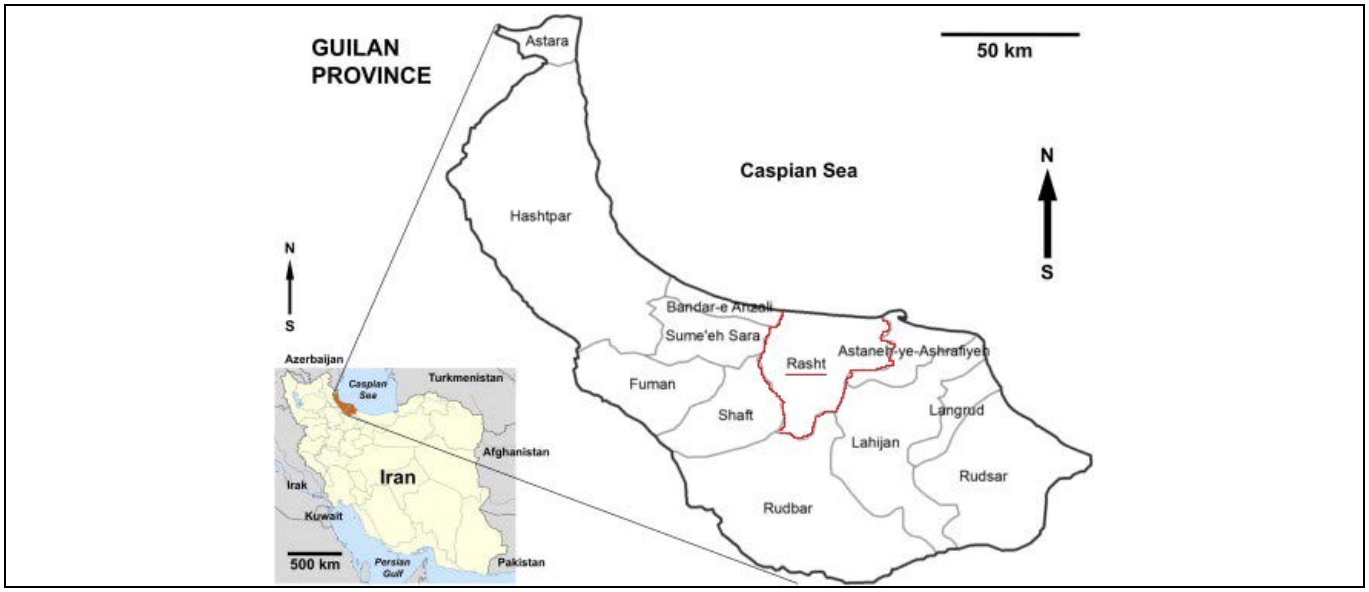

Fig. 1. The geographical location of Rasht city in Guilan province, Iran

during October to November 2014. All the samples were kept at 4°C and were analyzed within 48 h.

Sample processing and preparation. Water samples were filtered using 0.2 µm pore size qualitative Whatman filter papers (Sigma- Aldrich, Missouri, United States). The filter papers were washed with PBS buffer and centrifuged at 10000 rpm for 15 min. The sediments were stained with Lugol and examined for *Giardia* cysts with 1000 X magnification of microscopy

DNA extraction and PCR. A freeze and thaw method with 15 cycles of 3 min at -81°C and 100°C, alternatively was performed to rupture the wall of the cysts in sediments. The phenol-chloroform method was used to extract DNA from sediments as described by others [13]. For molecular diagnosis of *G. lamblia*, a 350 bp fragment of heat shock protein gene was amplified with the primers hspF 5'-GCATGTCGTCAGTATAGGCG-3' and hspR 5'-GCTTCACTGACTCGGCCTTA-3 designed in this study. The master mix for the PCR included 3 µl of 10x PCR buffer, 2.5 mM of $MgCl_2$, 3 µl of 10 mM dNTPs, 0.5 µl of *Taq* (5 u/µl) DNA Polymerase (CinnaGen, Tehran, Iran), 1 µl of the forward and reverse primers (10 pmol), 3 µl of DNA template, and 12.5 µl of double distilled water to make the final reactions 25 µl.

The PCR amplification consisted a single initial denaturation step at 94°C for 4 min followed by 35 cycles of 30 sec at 94°C, 30 sec at 47°C, and 30 sec at 72°C, with a final extension step at 72°C for 5 min. The amplified samples were run on a 1.5% agarose gel and visualized under a UV **t**ransilluminator. The DNA from *Giardia* cysts isolated from the stool samples of giardiasis patients by percoll gradient centrifugation protocol and distilled water were included as positive and negative controls, respectively.

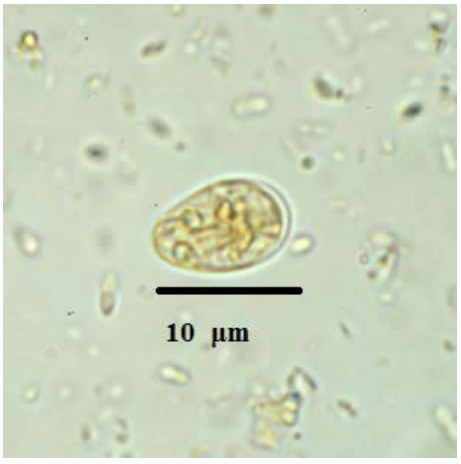

Fig. 2. A *Giardia* cysts recovered from a concentrated water sample

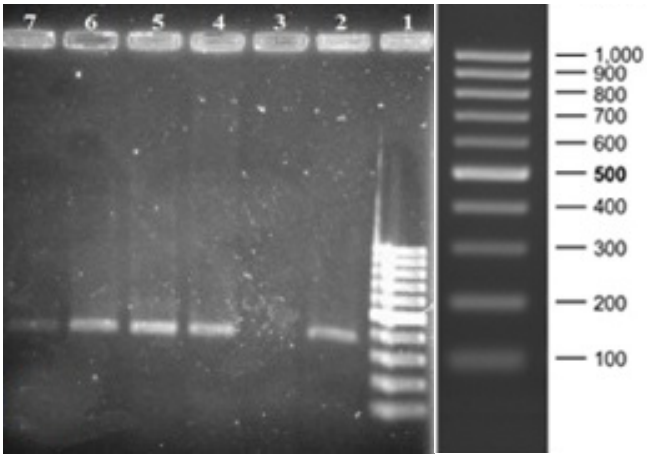

Fig 3. Detection of *Giardia* spp. in surface waters by PCR amplification of a 350 bp fragment of heat shock protein gene. Lane 1, 100 bp ladder (CinnaGen, Tehran, Iran); lane 2, positive control; lane 3, negative control; lanes 4 to 7, DNA from surface waters

Table 1. Positive concentrated water samples in different sampling sites

Sample number	Sampling site	Number of collected samples	Microscopy	PCR
1	Taleshan river bridge	1	-	+
2	Taleshan river bridge	1	+	+
3	Barban river	2	+	+
4	Khubak river	2	+	+
5	Khomamrud	1	-	+
6	Khomamrud	3	+	+
7	Pasikhan river	2	+	+
8	Sufian siah river	2	+	+
9	Jafar abad river	1	+	+
10	Jafar abad river	2	+	+
11	Nyshavandan river -Daroosazi	2	+	+
12	Nyshavandan river -Daroosazi	1	+	+
13	Eynak wetland	1	+	+
14	Eynak wetland	2	-	+
15	Ahmad Goorab	1	+	+
16	Ahmad Goorab	2	+	+
17	Eshkik-Anzali highway	1	+	+
18	Eshkik-Anzali highway	2	+	+

RESULTS

Out of a total of 45 sediments from surface waters, 15 (33.33%) were positive for *Giardia* spp. by microscopy with the cysts appearing in brown color in Lugol-stained smears (Fig. 2). The PCR yielded the expected band in 18 (40%) of the specimens (Fig. 3). The details of the water samples and microscopy and PCR assay are reflected in Table 1.

DISCUSSION

Giardia lamblia is a common parasitic cause of diarrhea, particularly in children leading to their malnutrition and delayed growth. Laboratory diagnosis is frequently based on detection of cysts and trophozoites of the parasite by direct wet mount microscopy [14]. Microscopical examination of three stool samples is considered as the "gold standard" for diagnosis of *G. lamblia* infection in humans, but the sensitivity of this method is low (46%) even after multiple examinations [15]. PCR assays for detection of *G. lamblia* have proven to be very useful with the advantages of reduced labor and time consumption required for diagnosis [16]. When compared with direct microscopy method, PCR with a sensitivity of 100% and specificity of 94% can be utilized as an alternative method for diagnosis of giardiasis [17].

Contamination of water with *Giardia* cysts is directly related to the health and economic status of the community, especially in areas in which the wastewaters are disposed in the environment without any treatment. This problem primarily is more evident in the rainy seasons. In such regions, runoff waters transfer the cysts to surface waters and increase the risk of disease transmission if they are not controlled [18]. Mostly, surface waters are used for domestic consumption after treatment with disinfecting agents. Chlorination is one of the most commonly used methods in water treatment [19]. However, due to the high resistance of protozoa (including *Giardia* cysts) to disinfection, they should be filtered out rather than just treated through tap water chlorination [20]. The use of sand or diatomaceous filters can remove the majority of protozoa from contaminated waters. There are reports of the isolation of *Giardia* cysts from drinking waters by filtration [21]. Epidemiological studies on water resources can be valuable during outbreaks of giardiasis. In 1974, *Giardia* cysts were detected following the incidence of giardiasis in water resources in New Jersey, USA [22]. Tracking and detection of *Giardia* cysts in water samples involves three steps; concentration, purification, and recovery phases. Today, filtration is used in the majority of studies to concentrate collected water samples. Microfilters of 0.1 µm or larger pore size are considered as the most appropriate filters in slow sand filtration and precoat filtration [23].

Floatation methods using zinc sulfate, sucrose, percoll/**s**ucrose, and potassium citrate solutions are used for the purification and recovery of *Giardia* cysts from concentrated water samples [24]. In this study, we used percoll/**s**ucrose method to recover the cysts [25]. Before the 1990s, most studies used microscopy and IFA for the detection of *Giardia* cysts in water resources [26]. In 1979, IFA method was introduced for the detection of *Giardia*. In IFA test, *Giardia* cysts are detected using fluorescent monoclonal antibodies following a concentration step [27]. Today, this method is rarely used as the efficacy of recovery is affected by water quality, especially turbidity of water. Moreover, IFA method is time-consuming, tedious and expensive and requires experienced and skilled staff.

With the advent of molecular methods, more useful tools were developed for detection of protozoa in contaminated waters. Abbaszadegan and colleagues (1991) detected *Giardia* in water samples by a cDNA probe [28]. In subsequent studies, the sensitivity level increased to detection of a DNA equivalent to a single *Giardia* cyst by a PCR assay [29]. The advantages of PCR technique included the distinction between live and dead cysts as well as identification of, and differentiation between *Giardia* species [30, 31]. In this study, with specific primers for the heat shock protein gene of *G. lambelia*, we reported a contamination rate of 40% in surface waters of Rasht city. Using IFA, LAMP, and PCR for the detection of *Giardia* cysts, Mahmoudi *et al.* (2013) reported 37.5% contamination in two rivers of Guilan province, which is almost similar to our results [32]. In a study in Brazil by Fernandes and colleagues (2011), PCR detected *Giardia*

duodenale in 41.6% of surface waters, agricultural waters, and sewage [33]. Monitoring of hygienic principles to prevent the infiltration of infectious agents into water resources can result in significant reduction of water supplies contamination. In developed countries, *e.g.*, Germany and Finland, the contamination rate of water resources with *Giardia* cysts was reported to be 4.2% and 13.7%, respectively [34, 35]. Guilan province is a significant location for agricultural activities in Iran and comprises a considerable proportion of fertile lands of the country. In this region, agriculture systems, mostly traditional, use surface waters sources for irrigation, which may contaminate many products leading to a variety of foodborne diseases among the consumers [36]. In Iran and Turkey, several studies reported contamination of fruits and vegetables with *Giardia* cysts [37, 38, 39]. The results of this study indicate a significant presence of *Giardia* cysts in surface waters in Rasht. Given the importance of this contamination as a hygienic problem for residents of Rasht city, we recommend treatment of human wastewater as the most crucial sanitary measure to avoid contamination of surface waters with parasitic cysts.

The staining method was efficient for the diagnosis of *Giardia* cysts in concentrated surface water samples. In the detection of *G. lamblia* cysts from water samples, PCR was more sensitive and more specific than microscopy method. Considering the widespread use of water resources for agricultural activities, monitoring of sanitary measures for pathogen control such as preventing human sewage from entering into water sources, is highly recommended.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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