The Occurrence of *Cryptosporidium* and *Giardia* Parasites in Drinking Water Resources of Alborz province, the Central Part of Iran in 2018

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Abstract

**Background:** *Giardia* and *Cryptosporidium* are common parasitic protozoa that cause acute intestinal infections in children. These two parasites are mostly found in aquatic environments, including raw water, wastewater, and even treated water.

**Objective:** The present study aimed to examine parasitic contamination of drinking water resources by cysts and the oocyst of *Giardia* and *Cryptosporidium* in Alborz province, Iran.

**Materials and Methods:** Water samples from three rivers and seven randomly-selected wells of Alborz province were examined using Sheather, formol-ether, and immuno-fluorescence assay (IFA) techniques. The prepared slides were examined with optical and fluorescence microscopes.

**Results:** IFA technique revealed that 28% of the wells were contaminated with both parasites. It was also shown that all rivers’ drainage basins were contaminated with *Cryptosporidium* parasite, while 66% of rivers’ drainage basins were contaminated with *Giardia* parasite.

**Conclusion:** The results showed that water resources of Alborz province contained *Giardia* cysts and *Cryptosporidium* oocysts, which required health care officials to pay serious attention to treating drinking water.

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**Background**

Numerous parasites can be transmitted to humans through water. *Giardia* and *Cryptosporidium* are two common protozoa parasites causing intestinal infections in children and adults throughout the world.1 The two parasites are found in aquatic environments, including raw water, wastewater, and even treated water.2,3 These parasites are usually transmitted by drinking/eating contaminated water and food. Water is the greatest source of large epidemics, which transmits parasites in the form of waterborne infections.4,5 Diarrhea is the second leading cause of death among children under 5 in the world, with about 2.55 million deaths each year. However, most diarrhea cases can be prevented by sanitation and providing healthy drinking water.5 *Cryptosporidium* parasites cause diarrhea in humans and animals.6 Most cysts and oocysts of *Giardia* and *Cryptosporidium* live in drinking water at 4-10°C for months. Surface water might not be cleared from cysts and oocysts of the two protozoa via conventional treatment methods, since they are resistant to common disinfectants such as chlorine in normal concentrations.7,8 As a result, *Cryptosporidium* contained in water resources – especially urban water resources, can be spread even if the water is treated.7,9,10 *Giardia* is a protozoan flagellate that is usually found in the human gastrointestinal tract.11,12 *Giardia* causes diarrhea, abdominal pain, nausea, lethargy, and weight loss, but is rarely fatal.11,12 This parasite is one of the most important reasons for water-borne diseases.12 The contamination of drinking water resources with these two parasites was reported in several studies conducted in Turkey, Nigeria, and Hamadan city in the west part of Iran.13-15 Taking into account the health risks of these two parasites and given the fact that no study had ever been conducted on the contamination of drinking water resources in Alborz province, the present study aimed to examine the contamination of these water resources by cysts and the oocyst of *Giardia* and *Cryptosporidium*.

**Materials and Methods**

Karaj (35°49’57”, 50°59’29”) is the capital of Alborz province, with a population of 1973000, and located...
30 km from Tehran, the capital city of Iran.16 The total volume of the collected samples was 250 L of water from Karaj water resources including the Amir Kabir, Taleghan, and Beylagan rivers, as well as seven wells located in Mohmmadshahr and Mahdasht. A total of 180 liters of water samples from Karaj and Taleghan dams and Beylagan River drainage basin were collected before entering the treatment plant in three different courses (20 L each). The drainage basin of the Beylaghan River originates from Amir Kabir Dam and the Karaj River. About 70 L of water was randomly collected from wells in Mohammad Shahr and Mahdasht in two different periods (5 L each). The samples were collected using wide-mouth containers at a depth of 20 cm under water surface.

**Filtration**

Each water sample was passed through nitrocellulose filters with a 140-mm diameter and 0.45 µm pore size, at the recommended standard discharge of 4-10 L per minute, and recommended tolerable pressure for the filter and storage compartment (max 21 PSI) using a pump. After separating the filter from the holder, the water sample was transferred to a stainless steel container and cut longitudinally. The layers cut intended to separate the sediment material from the filter were washed with a TWEE® 80 solution in different steps.

Finally, the obtained solution was concentrated by centrifugation at 3500 g for 10 minutes. The supernatant was discarded and its sediment was tested using an immuno-fluorescence assay (IFA) technique to observe *Cryptosporidium* oocysts and *Giardia* cysts by a fluorescence microscope.

**Immuno-Fluorescence Assay**

*Giardia* and *Cryptosporidium* immunofluorescent detection kit containing fluorescein isothiocyanate (FITC), a conjugate solution with anti-*Cryptosporidium* oocyst, and anti-*Giardia* monoclonal antibodies (Cellabs Diagnostic Brook Vale, Australia) was applied according to the manufacturer’s instructions. An aliquot of 50 µL of sediment was diluted and placed in a microscope slide. After a few steps, the conjugate was added. The slides were then incubated in a humid container for 30 minutes at 37°C. Finally, the slides were examined under a fluorescence microscope (Zeiss, Germany) at ×400 magnification.13,15 The sediment of the centrifuged samples was also used to prepare slides for studying intestinal parasites under a microscope using the Sheather and Formol-ether techniques with some modifications as described previously.18 In sum, for preparing Sheather solution, 500 g of sugar was dissolved in 330 mL of distilled water; then 6.5 g pure phenol was added to the solution. One gram of the sediment of the centrifuged sample was added to the Sheather solution in a centrifuge tube and centrifuged at 3500 rpm for 10 minutes. After centrifugation, the materials were removed from the surface with a loop and placed on the slides. Each slide was stained with an acid-fast procedure and examined under the optical microscope.

**Results**

The water samples in this study were collected in rainfall seasons. Contamination with *Cryptosporidium* and *Giardia* protozoa in the rivers and wells is shown in Table 1. The sensitivity, specificity, accuracy, and ease of working with the IFA microscopy technique compared to the simple microscopic techniques were 100%. The sensitivity of Sheather and Formol-ether techniques to identify *Cryptosporidium* parasite compared to IFA techniques were 80% and 60%, respectively. The sensitivity of Sheather and formol-ether techniques to identify *Giardia* cyst compared to the IFA technique were 0% and 10%, respectively. A water sample diagnosed with *Giardia* using the formol-ether technique was again identified with *Giardia* using the other technique, namely the IFA technique.

No fluorescence pattern was observed in any of the negative cases. No false (negative) cases were observed using the IFA technique. The duration required for detection by the IFA technique was approximately one minute, which was 5 minutes in the light microscopic techniques.

**Discussion**

Drinking water resources contaminated with parasites – *Cryptosporidium* and *Giardia* in particular, can be a threat to public health. In the present study, the parasitic contamination of drinking water resources of Alborz province was investigated using the Sheather, formol-ether, and IFA techniques; however, the IFA technique was adopted as the standard technique to determine *Cryptosporidium* oocysts and *Giardia* cysts in our study. It was found that the Sheather and formol-ether (light microscopic) techniques were easier and faster to use compared to IFA, but they had insufficient sensitivity.18 In several studies, using the IFA technique has been found to be more efficient and sensitive in detecting *Cryptosporidium* oocysts than Sheather and formol-ether techniques in fecal specimens. It can particularly

### Table 1. Karaj Drinking Water Resources Contaminated With *Cryptosporidium* and *Giardia* Parasites in 2017

| Methods     | Wells No. (%) | Rivers No. (%) | Wells No. (%) | Rivers No. (%) |
|-------------|---------------|----------------|---------------|---------------|
| Sheather    | 2/7(28)       | 2/3(66)        | 2/7(28)       | 2/3(66)       |
| Formol-Ether| -             | 3/3(100)       | 1/3(33%)      |               |
| IFA         | 2/7(28)       | 3/3(100)       | 2/7(28)       | 2/3(66)       |

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determine a low number of Cryptosporidium oocysts similar to Giardia cysts in various specimens.\textsuperscript{19-21} This finding was consistent with the result from a study by Ani et al investigating the parasitic contamination of drinking water in the Ebonyi State of Nigeria in five different sources (i.e., ponds, wells, rivers, boreholes, and runoff) using a simple optical microscope technique. They found that the performance of the simple microscopic technique was much lower than that of the IFA technique.\textsuperscript{14} Three parasites of Giardia (34.4%), Entamoeba histolytica (45.3%), and Cryptosporidium parvum (20.3%) were detected as the result of their study. Moreover, their study results demonstrated that each site had at least two parasites, and the level of contamination in given city was very high.\textsuperscript{14} The difference between our study results and those from their study was mostly due to the differences in cultural and geographical conditions between the countries. However, both studies confirmed the contamination of the environment with Giardia and Cryptosporidium parasites. As for the Amir Kabir River, the Formol-ether technique detected amoeba parasite and plant seeds, confirming the contamination of the river with other parasites.

The lack of conservation measures for surface water resources causes the transmission of any contamination to rivers.\textsuperscript{22} It seemed that the disposal of human wastes and discharge of domestic, industrial, and agricultural wastewater into the surface waters could have led to the transfer of these parasites into the rivers of Alborz province. Similar contaminants were found in Chinese rivers.\textsuperscript{23} The results of a study in Ahvaz, Iran showed that 27.27%, 9.09%, and 50% of water samples were contaminated with the Cryptosporidium, Giardia, and Entamoeba histolytica parasites, respectively.\textsuperscript{24} In the present study, the presence of the Cryptosporidium parasite in water resources was also detected to be strong. Cryptosporidium contamination in both regions may have been due to the contamination of rivers by human and animal feces or wastewater, leading to the transmission of the parasite. Giardia parasite was discovered to be more frequent in Ahvaz than in Karaj. This difference may have been due to the climatic and cultural differences existed between the two cities.

In addition to investigating the parasitic contamination of raw water sources of drinking water, the present study examined the effluent of the Karaj wastewater treatment plant. Cryptosporidium and Giardia parasites were observed in the samples from the effluent using three methods of Sheather, formol-ether, and IFA. Since Karaj effluent was excreted in the environment and may have been reused for irrigation of agricultural lands, it could have been considered as a possible cause of parasitic contamination of raw drinking water resources in the province.

Lim et al visited two wastewater treatment plants in Malaysia to find out if Cryptosporidium and Giardia parasites existed in raw and treated wastewater. The authors used the same IFA technique adopted in this study to detect parasites and found that the concentration of Giardia parasite was 100% in both raw and treated wastewater, and the concentrations of Cryptosporidium parasite were 50% and 25% in raw and treated wastewater, respectively.\textsuperscript{25} In sum, these two parasites were found in treated wastewater in two wastewater treatment plants in Malaysia and Alborz province of Iran. The level of parasitic infection in Alborz province was higher than that found in Malaysia. This difference could have been attributed to the treatment methods of wastewater in Malaysian plants or the diameter of the filter in the Alborz province plant since the larger the diameter of the filter, the higher the rate of parasite passage would be. The present study showed that the rivers were infected with Cryptosporidium (100%) and Giardia (about 66%) parasites according to the IFA technique. Rainfall caused the mixing of river water and the runoff washing the surrounding lands, and increased parasitic pollution in the water. It should be noted that our study's water samples were mostly collected during rainfall seasons.

Agricultural lands were located upstream of the Karaj rivers and their wastewater flowed into the rivers. On the other hand, the entry of wastewater from houses, factories, and recreational centers around the rivers contaminated surface waters.\textsuperscript{26} In Portugal, Lobo et al sampled groundwater and surface waters to detect Cryptosporidium and Giardia in raw and treated water resources using IFA and PCR techniques. According to the results from the IFA technique, 53.6% and 41.5% of Cryptosporidium were observed in raw and treated water, respectively; while 80% and 25.6% of Giardia were detected in raw and treated water, respectively.\textsuperscript{27} These findings were almost consistent with the results obtained in the present study. In both studies, the raw water resources were severely contaminated with the two above-mentioned parasites that could have posed a threat to public health. In Portugal, parasitic pollution was introduced into surface waters through urban and agricultural projects, industrial pollution, rainwater, and septic system leakage, which were almost similar to the contaminants found in water sources from Alborz province. The level of groundwater contamination was low in both studies.

This study faced few limitations one of which was the fact that the parasitic contamination of outlet water from drinking water treatment plants was not investigated. Therefore, it is recommended that this issue be examined in future studies.

**Conclusion**

According to our study results, a higher percentage of rivers compared to wells were contaminated with
Cryptosporidium and Giardia. The main source of drinking water in this province was well. Therefore, consuming drinking water from wells could have reduced the rate of parasitic contamination through drinking water.

It was also revealed that three main rivers of Alborz province including Amir Kabir, Taleghan, and Beyaghan rivers were contaminated with the Cryptosporidium and Giardia parasites, which may have played a highly significant role in the transmission of parasites to individuals. In conclusion, most of the water resources in Alborz province had been contaminated with Cryptosporidium and Giardia before their water entered the treatment plant in the region. Moreover, parasitic contamination was not eliminated during water treatment, and the possibility of parasitic transmission in the area was high. Therefore, it was necessary to decontaminate all drinking water sources from parasitic contamination. It was recommended that the rivers be protected against the entry of the effluent from the Karaj wastewater treatment plant, which contaminated the drinking water sources.

Drinking water should also be treated using techniques that remove the protozoans after water treatment so that the contamination caused by them could have been a potential risk to public health.

Authors’ Contributions
FK, AH, and MS carried out investigation, performed laboratory operations, and wrote the original draft. ED, AM, MNS, and AH performed the conceptualization and methodology, as well as wrote, reviewed and edited the drafts. All authors read and approved the final manuscript.

Ethics Approval
This study was approved by the Regional Scientific Ethics Committee of Alborz University of Medical Sciences (Project number: IR.ABZUMS.REC.1395.156).

Conflict of Interest Disclosures
The authors have no conflict of interests to declare.

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