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RESEARCH ARTICLE

The Frequency of the Intestinal Parasites *Giardia Lamblia* and *Entamoeba Histolytica* in Pediatric Diarrhea Specimens from Central Iran

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Abstract:

Introduction:

Intestinal parasitic infections, particularly those caused by *Giardia lamblia*, are among the major health problems that exist worldwide, especially in developing countries. This study aimed to investigate the prevalence of the intestinal parasites *Giardia lamblia* and *Entamoeba histolytica* that were isolated from samples of infectious diarrhea in pediatric patients from Central Iran.

Methods:

This descriptive cross-sectional study included 230 samples of infectious diarrhea that were collected from May 2015 to February 2016. Direct observation, the formalin-ether sedimentation method and the technique using the Polymerase Chain Reaction (PCR) of *β-giardin* and *EH* primers were used for the identification of *Giardia lamblia* and *Entamoeba histolytica.*

Results:

Out of 230 samples of infectious diarrhea, five cases (2.1%) of *Giardia lamblia* and no cases (0%) of *Entamoeba histolytica* were identified using the formalin-ether sedimentation method and the same result were obtained using PCR technique. Of the five patients who had *Giardia lamblia*, three (60%) were male and two (40%) were female. The most common clinical symptoms in these patients were stomach ache and diarrhea (100%) and mucus in the stool (80%).

Conclusion:

Giardia lamblia was introduced as a parasitic agent causing diarrhea from Central Iran. The results indicate that pediatricians and, even more importantly, experts in laboratories should pay special attention to the identification of this parasite to treat the patients as effectively and as quickly as possible.

Keywords: *Giardia Lamblia*, *Entamoeba histolytica*, Intestinal parasites, Pediatric, Infection, Diarrhea, Iran.

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1. INTRODUCTION

Being contaminated with parasitic infections is now believed to be inextricably intertwined with personal hygiene, individual conduct, and the social scales of people in terms of their sanitary awareness. Consequently, hygiene and health scale evaluation are considered to be among the yardsticks of measurement, specifically for those in developing countries [1]. In spite of the enormous efforts made by the World Health Organization (WHO), the exposure to parasitic infections is highly prevalent in developing countries and is particularly widespread in communities where there are poor sanitary conditions and economic and social disadvantages [2].

According to reports issued by the WHO over the past 2010 years, almost two-thirds of the world's population is contaminated by parasitic infections, of which the most prevalent is *Giardia lamblia* [3]. Among the parasitic infections of the digestive system, *Giardia lamblia* and *Entamoeba histolytica* are principal pathogenic protozoa. The number of those who are afflicted with *Giardia lamblia* has in fact reached epidemic proportions worldwide, and it is the most commonly found parasite in excrement samples collected in Iran [4]. The prevalence of this protozoan in developing

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countries fluctuates between 2% and 30%, whereas in industrialized countries the reported figures vary from 2% up to just 7% [5]. *Giardia lamblia* is capable of causing asymptomatic contamination or spectral diseases, and infections range from acute to chronic, with clinical symptoms such as acute watery diarrhea, abdominal cramps, maladaptation syndrome, and weight loss among both infants and children [6].

Entamoeba histolytica, which is mainly a cause of amoebiasis (amoebic bloody diarrhea), involves primary pathogenic protozoans in the human body [4]. Studies conducted between 1988 to 2009 found a prevalence of less than 1.3% in different parts of Iran [7]. However, identifying intestinal parasites in clinical detection labs is not carried out efficiently, and there is no specific information concerning the abundance scale of parasites, such as *Giardia lamblia* and *Entamoeba histolytica* in Iran's central region. Therefore, this study is being conducted to examine the in depth abundance scales of *Giardia lamblia* and *Entamoeba histolytica*, by examining samples of diarrhea from pediatric patients who were referred to the pediatric educational and therapeutic center at the Arak University of Medical Sciences (Amir Kabir Hospital).

2. MATERIALS AND METHODS

2.1. Collecting Samples

For this sectional descriptive study, 230 samples of diarrhea were collected from pediatric patients who were referred to the Amir Kabir Medical Center from the beginning of May (the second month of the year in accordance with the solar calendar) 2015 to the end of February (The eighth month of the year in accordance with the solar calendar), 2016. This study was approved by the Ethics Committee of the Arak University of Medical Sciences under No. 93-176-10.

2.2. Microscopic Observation

The procedure was carried out using direct and formalin-ether sedimentation methods and according to WHO protocol, using samples of diarrhea that were collected in less than 10 minutes. The formalin-ether sedimentation method was used as described elsewhere [8].

The clinical sample that was available through Valiasr Hospital's part of parasitology was used during this procedure as a positive control.

2.3. DNA Extraction and PCR Analysis

DNA extraction from samples of feces, using the QIAamp DNA Stool Mini Kit by Qiagen GmbH Hilden Germany, was conducted as per the protocol. The amount and purity of the extracted DNA were measured with a NanoDrop apparatus. The primers *B-giardin*and *EH* were deployed as genetic markers to validate the isolated *Giardia lamblia* and *Entamoeba histolytica* in the PCR (Table **1**) [9, 10] .

The eventual volume of PCR used in this study was 25 µl, which consisted of 12.5 µlof the master mix (1X), 0.5 µl of Taq DNA Polymerase (2.5 units), 10 µl of the forward and reverse primers (10 pm), 2 µl of the DNA sample (5 ng), and 8 µl of the twice distilled water. (All the substances were purchased from Yekta Tajhiz Corporation, Iran). From each positive gene, a sample was tested using the sequencing of isolated PCR products (Gene Fanavaran Corporation, Iran), which was verified using Blast analysis.

3. RESULTS

Of the 230 analyzed samples, five of the *Giardia lamblia* samples (2.1%) and none of the *Entamoeba histolytica* samples (0%) were found to be positive using direct observation and the Formalin-ether sedimentation technique; this finding was also confirmed by PCR. Four *Balantidium coli* samples (80%) were obtained from the five *Giardia lamblia* samples (2.1%) using the direct observation method, and one cyst (20%) was found using the Formalin-ether sedimentation technique.

The average age of the people afflicted with *Giardia lamblia* was 5 years and 8 months. The youngest diseased person was a 4-year-old boy; the oldest was an 8-year-old girl. Of the five patients (2.1%) afflicted with *Giardia lamblia*, three (60%) were male and two (40%) were female.

The clinical symptoms among the people suffering from giardiasis included stomachache and diarrhea (100%), mucus spotted in feces (80%), and nausea and anorexia (60%).

4. DISCUSSION

Intestinal parasitic infections are widespread across the world, and they often occur among people in poor social and economic circumstances, miserable living conditions, and densely populated regions deprived of proper sanitary protection, with inappropriate garbage disposal mechanisms, poor water quality, and unsanitary hygiene habits [11, 12].

In this study, five patients (2.1%) were infected with *Giardia lamblia*. In other studies carried out in Iran, the percentages of patients with *Giardia lamblia* were: 2.3% in Tehran, 4.1% in the northern regions of the country, and 10.9% in Hamedan; in Italy, 6.5% of the patients were infected with *Giardia lamblia*; in Jordan, 29.6% were infected with it [13 - 17]. The difference in the prevalence of *Giardia lamblia* infection reported in different studies is due to the differences

Table 1. Primers used in this study.

References	Annealing Temperature	Amplicon size (bp)	Sequence 5'→3'	Primer	Target Gene Description
[9]	60	171	F: 5- AAGTGCGTCAACGAGCAGCT-3 R: 5- TTAGTGCTTTGTGACCATCGA -3	*β-giardin* -F *β-giardin* -R	*Giardia lamblia*
[10]	56	170	F: 5- AAGCATTGTTTCTAGATCTGAG-3 R: 5- AAGAGGTCTAACCGAAATTAG-3	*EH* -F *EH* -R	*E. histolytica species*

in the time, geographical living areas, and the hygiene habits and lifestyles in which the samples were obtained [17].

Amoebiasis is still reported to be one of the most consequential health maladies in tropical and subtropical areas [18]. The outbreak from *Entamoeba histolytica* is yet to be recognized, whether in Iran or other major parts of the planet [19].

Many studies have scrutinized the prevalence of *Entamoeba histolytica*, and its impact on certain groups of people in Iran. There is no evidence of the prevalence of *Entamoeba histolytica* in our study; however, in other studies conducted in Iran, the prevalence of *Entamoeba histolytica* was found to be 0.02% in Karaj, 0.1% in the northern region of Iran, and 0.4% in Oromia; the prevalence is 4.7% in Saudi Arabia and 18.8% in South Africa [17, 20 - 23]. The samples of intestinal parasitic infections collected from people can vary due to differences in their hygiene habits and lifestyles, and differences in the time and geographical living areas in which the samples are obtained [24 - 26].

The initial technique is to detect *Balantidium coli*, *Giardia lamblia* and *Entamoeba histolytica* within feces using a microscope. A PCR test can also be used for the detection of feces [27, 28]. Of these two methods of microscopic observation and PCR, the sensitivity of the PCR method was higher [27]. PCR can accurately identify parasitic species, and it produces less laboratory waste than microscopic observation methods [27]. In spite of being ultrasensitive and exclusive for detecting *Giardia lamblia* and *Entamoeba histolytica*, the use of molecular techniques, such as PCR, is extensively restricted to laboratories [27]. The microscopic method is unable to differentiate *Entamoeba histolytica* from *Entamoeba dispar*. Thus, the WHO recommends the development and utilization of new techniques, such as PCR for a specific diagnosis of *Entamoeba histolytica* infection [28]. It is not possible to distinguish *Entamoeba histolytica* from commensal species, *Entamoeba dispar*, with morphological indices and only using specific monoclonal antibodies for each species and genomic testing by PCR is possible [8].

In the present study, the average age of the people contaminated with giardiasis was 5 years and 8 months. In Ghana, the average age infected person was less than a year. The highest prevalence was found in children, under the age of 10, in Karaj, Iran [29, 30]. In the present study, to some extent, men were more often inflicted than women, which was virtually identical to the findings reported in a study conducted in the northern region of Iran [17].

In the present study, the most prevalent clinical symptoms among the afflicted patients were stomachache and diarrhea (100%) and mucus in the feces (80%); in a study conducted in Pakistan, the principal clinical symptoms among the patients were abdominal muscle cramps, nausea, vomiting, flatulence, anorexia, and weight loss. The most common clinical symptom was nausea, which was found in 77.6% of the people; the least common clinical symptom was diarrhea, which was found in 19.7% of the people [30, 31].

CONCLUSION

To date, detailed investigations about intestinal parasites in various parts of the world have suggested that this type of infection is still a hygiene-related issue, due to the provision of sanitary services, notably in countries lacking basic amenities. In this study, *Giardia lamblia* was the parasite with the highest number of leading-to-diarrhea protozoans from pediatric patients in the central part of Iran; thus, pediatricians, and especially laboratory authorities, should be exceedingly vigilant in order to detect this protozoan to ensure proper and early medical treatment for affected patients. Due to the high sensitivity of the PCR method, there is a need to use this method in clinical diagnostic laboratories. As no case of *Entamoeba histolytica* has been observed in this geographical region, which is due to the its increased information and social awareness of hygiene practices, and decreased use of contaminated water supplies and unwashed vegetables.

LIST OF ABBREVIATIONS

WHO	=	World Health Organization,
DNA	=	Deoxyribonucleic Acid,
PCR	=	Polymerase Chain Reaction,
BLAST	=	Basic Local Alignment Search Tool.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of the Arak University of Medical Sciences under the approval no. 93-176-10.

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. All reported human were experimented in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the *Helsniki Declaration* of 1975, as revised in 2008 http://www.wma.net/en/20activities/10ethics/10helsinki/.

CONSENT FOR PUBLICATION

Informed consent was obtained from a parent and/or guardian for participants under 16 years old. The authors neither use nor access any information that enabled them to identify individual patients.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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