ORIGINAL ARTICLE

Cross-Sectional Study on the Prevalence of Intestinal Parasitic Infections in Primary School Students in Kish Island, Iran

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Abstract

Background: Intestinal parasitic infections pose a significant public health challenge in developing countries, with children being particularly susceptible. The prevalence of these infections varies across communities. This study aimed to determine the prevalence of intestinal parasitic infections among students in 12 primary schools on Kish Island, Iran, and to evaluate the infection status of family members of infected students.

Methods: A cross-sectional study was conducted on 443 students aged 7–12 years in Kish Island, southern Iran, from May 2016 to 2017. Stool samples from 179 boys (40.4%) and 264 girls (40.4%) across 12 primary schools were examined for evidence of parasitic infections using direct wet mount, formalin ethyl acetate, and trichrome stain methods. Modified Ziehl-Neelsen (ZN) staining was used to detect coccidian parasites. Conventional PCR was also employed to identify the genotype of *Giardia lamblia*. Data were analyzed using SPSS version 19.

Results: The prevalence of intestinal parasites was 5.2%. The highest incidence rate was found in *Entamoeba coli* (2.0%), followed by *Giardia lamblia* (1.6%). The prevalence rate of infection was significantly correlated with the type of drinking water ($P<0.05$). No significant difference was observed in the prevalence of intestinal parasitic infections between males and females ($P>0.05$). In this study, the genotypes of *Giardia lamblia* were molecularly characterized by studying the glutamate dehydrogenase (gdh) gene. This study represents the first molecular characterization of *G. lamblia* in children on Kish Island, with sequence analysis revealing assemblage B (BIII 100.0%).

Conclusion: This study indicates a low prevalence of parasitic infections in a sensitive population (children) on Kish Island. The prevalence of *Giardia lamblia*, a more pathogenic parasite, was quite low in our study. This cross-sectional study was conducted on all island residents; no significant difference was observed among them.

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Introduction

Intestinal parasitic infections are among the most significant health challenges in developing countries. The prevalence of these infections varies in different parts of a county, influenced by factors such as climate, economic situation, geography, and population density.[1] Young students are particularly susceptible to these infections

due to frequent contact with soil and contaminated materials, lack of basic sanitation, and weaker immune system.[2] Accurate data on the prevalence of intestinal parasites in students reflects personal hygiene levels and can also provide an estimate of a society's health status.[3] The prevalence of these infections was 33.3%, 47.7%, and 27.5% among students from Sari, Southern Khorasan Province, and Abu Musa Island, respectively.[4-6]

Giardia lamblia is one of the intestinal parasites that infect humans.[3] This parasite has been shown to have different genotypes. Type A (AI and AII) and B (BIII and BIV) can infect humans and other mammals, while genotypes C-H are observed only in domestic and wild animals.[7, 8]

Kish Island, Iran, presents unique conditions, including a tropical climate, challenges in supplying and transporting drinking water, concentrated populations in certain urban areas, immigrants from various socioeconomic classes, and a high percentage of non-native populations. This region is important due to its attractiveness as a tourist destination and its relevance to tourism health. However, specific information on the current health status and infectious diseases in primary schools in this region is lacking.

Therefore, this study aims to assess the prevalence of intestinal parasites among primary school students in Kish Island in 2014 and to determine its relationship with demographic factors. The research also intends to identify the genotypes of the extracted *Giardia lamblia* parasite and enhance the molecular-epidemiological information level in this region.

Methods

Sampling and Data Collection

This cross-sectional study was conducted among primary school students. Four hundred forty-three students were randomly selected from 12 primary schools in Kish Island (southern Iran, in the Persian Gulf) in May 2016. Subsequently, a second sample was taken from family members of students infected with pathogenic parasites. Seven out of 12 families agreed to cooperate and were examined. A demographic questionnaire was filled out for each student.

Sample Collection and Microscopic Analysis

Students and their parents were given instructions on how and when to collect samples. The samples were immediately transferred to the laboratory and examined using direct wet mount and formalin-ether sedimentation. An extension was also prepared from the sediment for dichromatic staining to confirm the positive samples. Another extension was prepared to modify the acid-fast staining to examine the sedimentation of the coccidian. Specimens infected by *Giardia lamblia* were studied to determine this parasite's genotype without adding preservatives.

DNA Extraction

Briefly, 2–3 g of stool was rinsed with saline thrice. Then, 200 µg of it was mixed with 200 µL of lysis buffer, and a freeze-thaw cycle (for 3 minutes in a freezer (liquid nitrogen tank) and 2 minutes in an 80 °C water bath) was repeated 7–8 times. Afterward, it was placed in an incubator at 72 °C for 5 hours, and DNA was extracted using the Yekta Tajhiz Azma Kit (Tehran, Iran) according to the manufacturer's instructions.

PCR Amplification

The *gdh* gene was amplified using a single PCR with the modified GDHF (5'– TCA ACG TCA ACC GCG GCT TCC T–3') and GDHR (5'– GTT GTC CTT GCA CAT CTC C–3') primers as previously described[9]. The reaction mixture for PCR amplification contained 5 µL of 10× buffer (CinnaGen, Iran), 1.5 mM of $MgCl_2$ (CinnaGen, Iran), 0.2 mM of each dNTPs, 1 U of *Taq* polymerase (CinnaGen, Iran), 20 pM of each primer, and 5 µL of extracted template DNA.

Amplification of the extracted DNA was performed using an Eppendorf (Germany) thermal cycler under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 45 s at 60.5°C, 1 min at 72°C, and a final extension at 72°C for 10 min. The DNA sample extracted from cultured trophozoites was used as the positive control, and distilled water was used as the negative control. Electrophoresis of PCR products was performed on 1.5% agarose gel stained with DNA Green Viewer (Yekta Tajhiz Azma, Iran).

DNA Sequencing

The PCR products were purified using a Vivantis® Nucleic Acid Purification Kit (Selangor, Malaysia). Nucleotide sequences were aligned with all homologous sequences available in GenBank using the Basic Local Alignment Search Tool (BLAST).

Statistical Analysis

The data were entered into SPSS Statistics version 24 (SPSS, Chicago, IL, USA) and analyzed using Fisher's exact and Student's T-tests. A P-value less than 0.05 was considered statistically significant.

Results

Prevalence of Intestinal Parasitic Infections in Students

Of the 443 students in this study, 179 were male, and 264 were female. The incidence rate was 3.9% for male students and 6.1% for female students; however, the difference was insignificant. Additionally, the

prevalence of intestinal parasitic infection was 5.2% among primary school students at Kish Island, of which 3.2% were infected with pathogenic parasites. Furthermore, 4.5% of the infections were identified as enteric protozoa and 0.7% as worms. No infections with coccidian parasites were found (Figure 1).

Association of Intestinal Infection with Socioeconomic and Sociodemographic Factors

The results showed that students with a university-educated father were less likely to be infected with intestinal parasites, and the difference was statistically significant (Table 1). Additionally, the average age of infected and uninfected students was 10.1±2.1 and 9.1±1.8 years, respectively, which was statistically significant (P=0.014). Students whose parents rinsed vegetables hygienically with detergents or sanitizers, such as chlorine powder, prior to use, were less likely to be infected, but the difference was not statistically significant (P=0.07).

Prevalence of Intestinal Parasitic Infections in Family Members of Infected Students

The results indicated that, of the seven family members, two from the same family were infected with the parasite *Giardia lamblia*. Given the shared infection of students and their family members with this parasite, a genomic test was conducted to identify the assemblages of *Giardia lamblia*. Out of the nine samples infected with the *Giardia lamblia* parasite (comprising seven students and two family members), DNA was successfully extracted from five samples.

Following the extraction of DNA, a one-step PCR was performed using the GDH-eF and GDH-IR primers on the GDH gene. However, only the sequences of four samples were accurately determined. Subsequently, based on the BLAST system and the GenBank database (with accession numbers DQ90533 and AF069059), the assemblage of all specimens was identified as BIII[9, 10] (Figure 2).

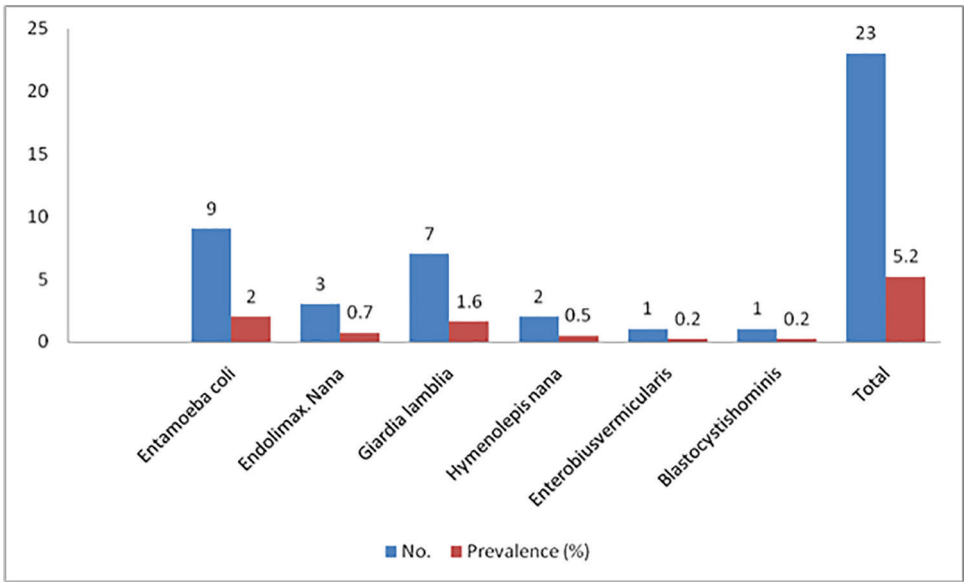

Figure 1: Prevalence of various intestinal parasites among elementary school students in Kish Island in 2016-2017 (N=443)

Table 1: Frequency distribution of intestinal parasite infection among elementary students in Kish Island in 2016-2017 based on the demographic data

Variables		School children (n %)		P value
		n=443 (%)	Infected (%)	
Sex	Male	179 (40.4)	7 (3.9)	0.31
	Female	264 (59.6)	16 (6.1)	
Place of residence	Native	286 (64.6)	19 (6.6)	0.60
	Un native	157 (35.4)	4 (2.5)	
Father's education	Primary	47 (10.6)	8 (17.0)	<0.0001
	Diploma	253 (53.0)	13 (5.5)	
	College Education	161 (36.4)	2 (1.2)	
Fathers job	Unemployed	19 (4.3)	3 (15.8)	0.092
	Employed	188 (42.4)	10 (5.3)	
	Self-employment	263 (53.3)	10 (4.2)	
Drinking water sources	Water piping	103 (23.3)	4 (3/9)	0.039
	Mineral water	294 (66.3)	13 (4.4)	
	Water storage tank	46 (10.4)	6 (13.0)	
Animal hold	Yes	3 (0.7)	0 (0.0)	1.0
	No	440 (99.3)	23 (5.2)	

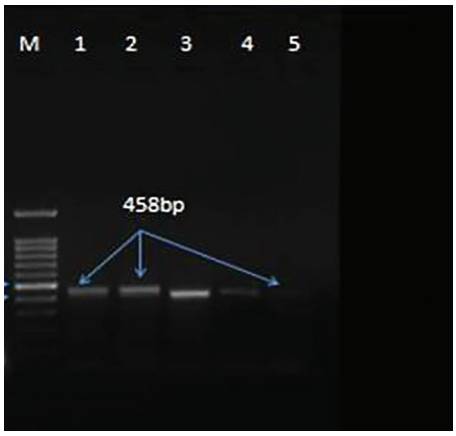

Figure 2: The results of the PCR-based amplification of the GDH gene of *Giardia* parasites isolated in the study. One to five: Positive samples indicating the presence of the *Giardia lambia* parasites (reproduction of fragment 458 base per)

Discussion

This study revealed that the prevalence of intestinal parasites among primary school students aged 7 to 12 on Kish Island was 5.2%. Comparable studies have been conducted in various regions of Iran and other countries, yielding different results. The infection rates reported for primary school students in Tabriz, Tehran, Bandar Abbas, and Abu Musa Island were 44%, 18.4%, 10.3%, and 27.5%, respectively.[1, 6, 11, 12] Furthermore, the infection rates reported in Thailand, Saudi Arabia, and Pemba Island were 48.7%, 27.2%, and 48.7%, respectively.[13-15] Our study found a lower prevalence rate of intestinal parasites compared to other studies, which could be attributed to the geographical location, improved sanitation, and economic conditions of Kish Island. In addition, vermicular parasites were found to be less prevalent than protozoan parasites. Similar studies have shown that protozoa are more common than worms due to their simpler life cycle and direct transmission from water and food sources.[3-10]

While the average prevalence rate of the *Giardia lamblia* parasite in various regions of Iran is estimated at 14.7%, this study found the prevalence of this parasite to be 1.6%.[6] Among parasitic worms, the prevalence of *Hymenolepis nana* and *Enterobius vermicularis* was 0.5% and 0.2%, respectively. Given that many children become infected by ingesting the eggs of these worms at a young age, primary and preschools are the most conducive environments for transmitting these infections.[11] It is worth noting that this study did not observe multiple parasitic infestations, and all students infected with pathogenic parasites received appropriate treatment.

In line with a study conducted in Nepal in 2013[12] our research demonstrated a significant correlation between the type of drinking water and the prevalence rate of parasitic infections on Kish Island. However, no significant relationship was found between family size and the prevalence of intestinal parasites (P=0.12).[10] The results showed no statistically significant correlation between the prevalence of parasitic infections and animal or religious maintenance. Furthermore, no coccidian parasites were observed in the students or their relatives.

Giardia lamblia infects a broad spectrum of animals, but only assemblages A and B have been identified in humans. Other assemblages are known to infect other mammals. For example, assemblages C and D have been found in dogs, cats, wolves, and coyotes, while assemblage E has been reported in cows, sheep, goats, pigs, and water buffalo. Assemblages G and F, on the other hand, have been reported in mice and rats.[16] Molecular genotyping of *Giardia lamblia* can effectively enhance epidemiological information and highlight the role of animals in transmitting this parasite.[16, 17]

A genomic study revealed that the *Giardia lamblia* genotype extracted from students and their families was 100% BIII assemblage. This value was 80% in Egypt, 93% in Argentina, and 74.4% in Belgium.[18-20] In Iran, a study conducted in Isfahan in 2014 showed that these values were 59.7% for assemblage A, 37.3% for assemblage B, and 2.2% for mixed assemblages.[21] In addition, another study conducted in Shiraz in 2012 revealed that 74.4% of the cases had all assemblages, 17.4% had BIII assemblage, 3.5% had BIV assemblage, and 4.7% had mixed assemblages.[9] In Ahvaz, 10%, 16%, and 74% of the cases showed A, B, and mixed assemblages, respectively.[22] In Sharjah, UAE, 17.1% and 18.9% of the cases showed assemblages B and A, respectively.[23] In Morocco, BIV, BIII, and other assemblages were reported in 72.2%, 9.08%, and 18.1% of the cases, respectively.[24]

In another study conducted in Lusaka, Zambia, in September 2017, *Giardia lamblia* was found in 10% of fecal samples from asymptomatic school children aged 3–16. Moreover, assemblages A and B were found in 27.3% and 72.7% of individuals, respectively. The analysis of the restriction enzymes revealed sub-assemblages AII (27.3%), BIII (12.1%), BIV (51.5%), and mixed assemblages of BIII and BIV (9.1%).[25]

Since the *Giardia lamblia* genotypes vary across different countries and even within different regions of a single country, this variation could suggest the role of animals and geographical location in transmitting this parasite. Given the studies demonstrating the presence of genotype B and subtypes BIII and BIV in humans and a wide range of animals, more attention should be paid to the zoonotic aspect of genotype B between humans and animals. The high prevalence rate of assemblage B in various regions may be due to human and animal waste contamination of the drinking water supply. However, since no molecular studies have been conducted in this area to determine the assemblages of *Giardia lamblia* in animals, this study

cannot definitively determine the role of animals in the transmission of *Giardia lamblia*. Therefore, further investigations are necessary to clarify Giardia lamblia's epidemiological and zoonotic status in this region.

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Conflict of Interest: None declared.

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