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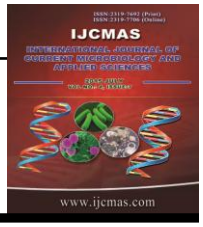

Original Research Article

Molecular detection of *Giardia lamblia* in different water sources of District Karak, Pakistan

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Keywords

G. lamblia, diarrheal disease, water, PCR, Takhte Nasrati

A B S T R A C T

G. lamblia (*G. lamblia*) is the major cause of diarrheal disease in humans, transmitted from person to person or animal to animal. The current study was designed to find out the prevalence of *G. lamblia* in different water sources of Tehsil Takhte Nasrati, District Karak (Pakistan) through molecular diagnostic tool, PCR. Three hundred samples were collected from different water sources (Pond Water, Dam Water, Spring Water, Well Water) in different areas including 75 samples from each source. All the samples were filtered and followed by DNA extraction and were subjected to PCR. In the present study the overall prevalence of *G. lamblia* in different water sources was observed 24.7% (74/300) including 25/75 (33.4 %) pond water, was in dam water 16/75 (21.4 %), similarly in well water sources, 14/75 (18.7 %), and in spring water 19/75 (25.4%) was recorded. The highest ratio was found in dam water. It was revealed from the current study that *G. lamblia* is present in water sources in some areas in Tehsil Takhte Nasrati, District Karak which may be, due to poor hygiene practices and improper management of water scheme.

Introduction

Water yields no energy, but it is one of the essential nutrients. The Cell structural composition is based on water. It is considered a primary component of diet [1]. According to the WHO report, more than 80 different diseases of humans are waterborne. Countries which are under developing have no entry to drink pure water [2]. Water systems which are mainly contaminated by the community are responsible to bring hazard affects globally [3].

The protozoan parasites G. *lamblia* are major causes of diarrheal disease in humans and many waterborne diseases worldwide are caused due to protozoan parasites [4]. In developed countries Giardiasis prevalence is about 2 to 5 percent and 20 to 30 percent in developing regions of Asia, Africa and Latin America [5]. Contaminated water transmits zoonotic infections and is serious threats to the financial system and global health [6]. Due to water-borne diseases about 3.5 million people, including 3 million children die throughout the world. About 98% deaths occur in the emerging republics where there water-born outbreaks are extensive. Only the diarrheal diseases cause greater than 1.5 million deaths per year [7].

The *Giardia* major transmission route is water, due to their vigorous form the cyst and oocyst of the parasite show highly resistat and ready for new infections [8, 9]. Transmission of *G. lamblia* may occur through person to person or animal to person [10]. This infection is also caused by oral-anal contact during sex [11, 12]. After the infection of *Giardia* symptoms start within 1 to 3 weeks [13].

Giardiasis outbreaks linked to drinking water [14], and food handlers are documented too [15]. Recreational water is mainly linked to Prolonged and large outbreaks of *Giardia* [16]. Diagnosis of Giardiasis is mainly based upon the microscopic examination, though the method is not reliable, consuming the time and mostly depends upon the skill of an experienced person [17]. Diagnosis of *Giardia*, through microscope has a low sensitivity and 50% of *Giardia* infections are not accurately diagnosed through this method [18, 19].

The current study is planned to find out the prevalence of G. *lamblia* in different water sources of Tehsil Takhte Nasrati, district Karak using molecular techniques and to make the people aware about the risk of *G. lamblia*.

Materials and Methods

Sample collection

A total of 300 water samples were collected A total of 300 water samples were collected for the detection of *G. lamblia* in different water sources of different villages/localities in Tehsil Takhte Nasrati, district Karak from 1st July 2013 to 31st October, 2013.

A total of 75 water from each water source (well, pond, spring and dam) containing one liter water for each sample in sterilized and labeled bottle with the collection date, area name and water type, and were transferred to Virology and Molecular Parasitology laboratory, Department of Zoology, Kohat University of Science and Technology Kohat for further process and evaluations after taking approval from the Ethical Committee of Kohat University of Science and Technology, Kohat

Samples processing

The samples of water were filtered through Whatman filter paper (Cat No 1442). A sample pellet was obtained and mixed with 1 ml buffer phosphate solution in an eppendorf tube and kept at -20ºC in refrigerator for further processing.

DNA extraction

The DNA was extracted from the above solution by using Genomic DNA purification kit (#k0512, Thermoscientific) following the method as previously described (20)

DNA Amplification (PCR)

PCR reaction was carried out in Amplitronyx thermal cycler (NyxTechnik Model No. A-6, Inc, San Diego, CA, USA) with Taq DNA polymerase (Fermentas USA). The amplification was performed with 5 µl of extracting DNA by using 10 pm of forward and reverse primers.

In 20 µl reaction mixture containing 1 µl 10 X PCR Buffer, 0.2 mM (1 µl) deoxynucleoside Triphosphate, 25 mM (2.4 µl) $MgCl_2$, 1 µM (1 µl) primers, target DNA 5 µl and 5 units of Taq DNA polymerase. DNA amplification at initial denaturation at 94°C for 5 min, 35 cycles of 96°C for 30 Sec annealing at 57°C at 72°C for 45 Sec. Primers used for *Giardia* were *ABB97F (AGGGCTCCGGCATAA CTTTCC)* and *ABB220R (GTATCTGTG ACCCGTCCGAG)* targeting HSP (Heat Shock Protein) gene [20,21].

Gel Electrophoresis

PCR product mixture of 10 µL was mixed with 2 µL loading dye and loaded in agarose gel. Gel was then examined under UV trans-illuminator and image was captured with gel documentation system (Clearver Scientific Model No. DI-HD, USA) (alam, ayaz, akbar, shahid niaz khan, ihsan)

The specific DNA amplified product of each sample was determined by identifying the 163-bp bands for *G. lamblia* (rubab, alam) which was compared with the 50bp ladder.

Prevalence rate

The prevalence rate of parasite was determined by using the following formula Prevalence Rate = (Positive samples/Total no. of water samples examined) ×100 . (20, 21)

Statistical analysis

The data was analyzed by using the Univariate ANOVA (Statistix 9) and $P \leq 0.05$ values were considered significant

Results and Discussion

In the current study *G. lamblia* was identified through PCR in different water sources of Tehsil Takhte Nasrati District Karak. In the present study the overall prevalence of *G. lamblia* in different sources of water was 24.7% (74/300). In pond water was 25/75 (33.4%), in dam water 16/75 (21.4 %), similarly in well water sources 14/75 (18.7 %), and in spring water 19/75 (25.4%) were observed (Table 1).

The prevalence of *G. lamblia* in Takhte Nasrati city was 10/25 (40%) and in Khadda Banda was 3/25 (12%), in Siraj Khail Gerang was 6/25 (24%), In Serki Nasrati was 16/75 (21.4%), In Alwar Banda 9/25 (36%), In Zeera banda 1/25 (4%), in Shnawa Gudi Khel 4/25 (16%), in Mianki Banda 11/25 (44%), in Jehangeri 8/25 (32%), in Inzer Banda 6/25 (24%).

In different sources of water the *G. lamblia* was observed, Pond water of village Mianki Banda was 11/25 (44%), in village Jehangeri 8/25 (32%), in Inzer Banda 6/25 (24%). The prevalence of *Giardia* in Dam water is 16/75 (21.4%) in Serki Nasrati. In Well water sources was 9/25 (36%) in Alwar Banda, 1/25 (4%) in Zeera Banda, in Shnawa Gudi Khel 4/25 (16%). Similarly *Giardia* prevalence in spring water was 10/25 (40%) in the Tehsil Takhte Nasrati city, 3/25 (12%) in Khadda Banda and 6/25 (24%) was observed in Siraj Khel (Table 2). *G. lamblia is a protozoan* parasite that affects humans and a wide range of domestic and wild animals. *G. lamblia* are the main cause of human Giardiasis [22].

Table.1 Prevalence of *G. lamblia* in different water sources

Serial No.	Water Source (n)	Positive	% Prevalence	P.Value
1	Dam water (75)	16	21.4	
2	Pond water (75)	25	33.4	
3	Well water (75)	14	18.7	0.0000
4	Spring water (75)	19	25.4	
Grand Total (300)		74	24.7	

n= total number, % for percent, p=0.00 <.5, significant

Table.2 Area wise prevalence of *G. lamblia* in different water sources of Tehsil Takhte Nasrati District karak

Location(n)	Pond Water Positive/total (%)	Dam Water Positive/total (%)	Well Water Positive/total (%)	Spring Water Positive/total (%)
TakhteNasrati City(25)	-	-	-	10/25 (40%)
Khadda Banda (25)	-	-	-	3/25 (12%)
SirajKhel (25)	-	-	-	6/25 (24%)
SerkiNasrati (75)	-	16/75 (21.4%)	-	-
Mianki Banda (25)	11/25 (44%)	-	-	-
Jehangeri Banda (25)	8/25 (32%)	-	-	-
Inzer Banda (25)	6/25 (24%)	-	-	-
Alwar Banda (25)	-	-	9/25 (36%)	-
Zeerabanda (25)	-	-	1/25 (4%)	-
ShnawaGudiKhel(25)	-	-	4/25 (16%)	-

- is denoted for not detected (%) = Percentage, n=total number, P <.05, significant

In the current study, *G. lamblia* was identified by PCR. In similar studies *Giardia* and other protozoan parasites were reported by different scholars in several parts of the world. For instance, different parasites were detected in different water sources of Lege Dini (Ethiopia) in which the overall prevalence was 47.5% in which the prevalence of *Giardia* was 35.3% [23]. , in Shanghai (China) was 50% in which *Giardia* was 18% [24], in Hungarian 40% and *Giardia* 26.7% [25].

In Mexico overall prevalence is 91% in which *Giardia* was 50% [26]. In Khyber Pakhunkhwa (Pakistan) overall prevalence was 33.6% and *Giardia* was 14.1% [20] and in Portugal overall prevalence was 18.6% in which *Giardia* prevalence was 8.2% [27], and in the current study overall prevalence in a given area for *Giardia* is 24.7%. A similar study was conducted in Russia and Bulgaria for the detection of *G. lamblia* and *Cryptosporidium parvum in* drinking water samples of different origin were collected from Rostov (southern Russia), Sofia and Varna (Bulgaria). 9.6% of the samples were positive for *G. lamblia.* The parasite was present in tap, river, well and waste water [28]. A similar study was also conducted in Kohat, Karak and Hangu districts of the Khyber Pakhtunkhwa province, Pakistan. Water samples were collected from tap, pond and drain water [20]. The contamination rates of *G. lamblia* and *C. parvum* in each water source samples were reported as follows: 65.5% of the samples, contained protozoa, among which *G. lamblia* was 18.5%. In the present study the prevalence of *Giardia* was 24.7% which was slightly different.

A similar study was also done by other world country like Iraq, showing 1.9% contamination in total of 22% by examining 100 water samples [29].

Statistical analysis

The data was analyzed by using the Univariate ANOVA (Statistix 9) and $P \leq 0.05$ values were considered significant.

It was concluded from the above study that Contamination of water with *G. lamblia* was found in water sources especially the drinking ones, which needs proper water treatment in water. It was recommended that Usage of Clean and boil water can prevent the users from the infection of this parasite.

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