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Prevalence and Molecular Detection of *Giardia* Spp in Different Drinking Water Sources in Karak

Lubna Hassan[1] and Fahim Ullah[2*]

[1]Department of Zoology, Kohat University of Science and Technology (KUST), 26000, Khyber, Pukhtunkhwa, Pakistan.
[2]Department of Environmental Engineering, College of Environment, Hohai University, 1 Xikang Road, Nanjing, 210098, P.R. China.

Authors' contributions

This work was carried out in collaboration between both authors. Author LH collected the samples and perform molecular detection. Author FU designed the study and performed analysis and wrote the first draft of the manuscript and also managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was conducted to know the prevalence of *Giardia* spp in different sources of water in District Karak Khyber Pukhtunkhwa, Pakistan.
Methods of Study: A total of 65 water samples was collected from different villages of District Karak was tested in the lab of the Department of Zoology in Kohat University of Science & Technology with the method of using PCR (Polymerase chain reaction) to detect the prevalence of G in tube well, bore, drainage and tap water.
Results: From the result the prevalence of G in tube well, drainage and bore water were found 10.76%, 1.53% and 1.53% respectively while in the sample of tap water there will be no prevalence of G was detected.
Conclusion: From the result it was concluded that a proper treatment of water for human consumptions is required.

**Corresponding author: E-mail: fahimullah320@yahoo.com;*

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1. INTRODUCTION

G. Campylobacter has been the most commonly identified zoonotic agents of waterborne disease outbreak from contaminated drinking water throughout the world and *Giardia* is a flagellated, binucleated protozoan, discovered by *Van Leeuwenhoek* in 1681 [1]. *Giardia* spp. was parasites of mammals and other animals, including reptiles and birds. It has a characteristic morphology; the vegetative trophozoite (15 mm in length) teardrop shaped two interiorly placed nuclei of equal size [2] and having four pairs of flagella (one anterior pair, two posterior pairs) and a caudal pair that emerges posterity from the disc [3]. The acquirement of *Giardia* occurs most commonly trough ingestion of the cyst in contaminated water, but person to person spread is common, particularly in settings of proof focal-oral hygiene [4].

The Evidence suggest that zoonotic waterborne pathogens will continue to be recognized as an increasing public health concern worldwide because of changing pattern in water use, climatic change severe weather events, increasingly concentrated livestock operation and international trade in animal product [5].

Giardia is an intestinal protozoan parasite and a chief cause of diarrheal disease in human's worldwide [6]. Possible symptoms of infection include malabsorption, loose or watery stools, dehydration and abdominal cramping. The symptoms of a *Giardia* infection are a suite of gastrointestinal unpleasantries, some of which have been described as "explosive" and "violent" [7]. *Giardia* is frequently found in water sources particularly those human and animal contamination is likely. Humans or animals become infected when they ingest cysts [8].

Giardiasis spread in communities where water supplies become contaminated with raw sewage or by the ingestion of foods contaminated with fecal material containing cyst and the infectivity dose may be as low as 10 cysts. It can be contracted by drinking water from lakes or streams where water-dwelling animals such as beavers and muskrats, or domestic animals such as sheep, have caused contamination [9]. The most common symptoms of Giardiasis are diarrhea, abdominal pain, bloating, flatulence and weight loss resulting from malabsorption [10]. Polymerase chain reaction (PCR), by allowing the rapid cloning analysis has revolutionized molecular genetics. PCR-based method used by technology *in vitro* mutagenesis, which can be accomplished. PCR is a readily accepted method and that there are numerous molecular methodologies (i.e. primers/genomic targets) [11].

Water born flagellated parasite *G. lambila* continuous to be most frequent protozoan agent of intestinal disease world-wide, causing an estimated 2.8 x 10^6 cases per annum [12]. *G. lambila* was the most commonly encountered parasite with a prevalence of 24.2% in Punjab. Prevalence of *G. lambila* is 11.8% in Muzaffarabad city. A total number of 3000 stool samples were collected from different laboratories of Sakkur, Sindh during the period of June 2005 to May 2007. Total 1050 (35%) cases were found with intestinal pathogenic parasite in their stools. The most common parasite was *G. lambila* found in 380 (36.19%) cases [13]. Prospective observational study of 239 children with recurrent abdominal pain was conducted at Department of Pediatrics, Postgraduate Medical Institute, Hyaytabad Medical Complex, Peshawar, from November 2004 to July 2006. Seventy-four (30.96%) children were positive for Giardiasis [14]. The present studies is designated with the objectives of the molecular detection of *Giardia* in different water sources of District Karak, Khyber Pukhtunkhwa, Pakistan and compare the water sources of contamination with drainage water.

1.1 Taxonomy and Classification of *G. lambila*

Kingdom: Protista	Subkingdom: Protozoa
Phylum: Sarcomastia	Subphylum: Mastigophora
Class: Zommastigophora	Order: Diplomonadida
Family: Hexamitidae	Genus: *Giardia*
Species: lambila [15]	

2. METHODS AND MATERIALS

2.1 Sample Selection

Karak is a district of the Khyber-Pukhtunkhwa, Pakistan. 65 samples of water were collected from different water sources of different villages. The quantity of water sample was 1.5 liters.

Karak is a district of the Khyber-Pukhtunkhwa, Pakistan. The 65 samples of water were collected from different water sources of different villages including Khojaki Kala, Ghulam Khel, Khaider Khel, Painda Khel and Atti Khel having samples of 17, 12, 27, 6 and 3 respectively as shown in Fig. 1. The different water sources include tap water, tube well (300ft depth), bore water tube well (150ft depth) and drainage water which is shown in Table 1.

2.2 Water Filtration and Processing

A total of 65 water samples were collected from different sources of District Karak like (tube well, bore, drainage and tap water) from 3/2012 to 3/2013. One liter samples were collected in sterilized bottles with the labeled date, site and nature. These samples were passed through filter paper (watt man grade 40) with the recommended flow rates. The high molecular weight settled down at the bottom and the low molecular weight remained above. The supernatant were discarded and pellet was poured into the appendrpph tube. Again those samples were run in micro centrifuge machine at 14000 rpm for 8 minutes.

Table 1. Prevalence of *Giardia* in different areas of Karak

Areas	Total	Positive	Percentage%
Painda Khel	6	1	16.66
Atti Khel	3	2	66.66
Khojaki Kala	17	3	17.64
Khaider Khel	27	2	7.40
Ghulam Khel	12	2	16.66

2.3 Extraction of DNA

The DNA (having unit Nucleotide) was extracted by DNA zole (Trizol USA) method with minor modification with the following steps. 124 µl from the sample was taken and added with 250 µl DNA zole. Then the mixture was mixed properly with vortex and incubated at room temperature for 5 minutes. For the precipitation of DNA 125 µl of iso-propanol was added to the mixture and centrifuged at 7000 rpm for 10 minutes. After centrifugation the supernatant was removed and add 125 DNA zole was added to the DNA pellet and centrifuged at 7000 rpm for 5 minutes. 200 µl of 70% ethanol was added to the pellet after discarding the supernatant and centrifuge at 7000 rpm for 5 minutes. Discard the supernatant. The DNA wash step was repeated and the tubes were stored vertically to dry for 10 minutes. 40 µl of distilled water was added to the pallet and incubated at 55ºC for 10 minutes in lowercase hotplates and were kept at -40ºC till use.

2.4 DNA Amplification (PCR)

PCR reaction was carried out in a thermal cycler (Nyx Technich USA) with *Taq* DNA polymerase (Ferments USA). The amplification was performed with 5 µl of extracted DNA by using 10 P mol of forward (5-AGGGCTCCGGCATAACTTTCC-3) and reverse (5-GTATCTGTGACCCGTCCGAG-3) primers. The reaction mixture for a single reaction was consisted of following terms:

a. Taq Buffer …..……..... 2.1 µl
b. MgCl2 (25 mM) …… 2.4 µl
c. dNTPs (500 µM) …… 1.0 µl
d. Forward Primer (10 Pm) …… 1.0 µl
e. Reverse Primer (10 Pm) …… 1.0 µl
f. dH2O ……7.2 µl
g. Taq, DNA Polymerase (5 U/ µl) … 5.0 µl
h. Extracted DNA ……5.0 µl

2.5 Gel Electrophoresis

The PCR product containing 0.5mg/ml of ethidium of 0.5×buffer of Tris-acetate EDTA at 120 volts, at room temperature for 15-20 minutes on a 2.0% agarose gel and containing 12µl per well and 2 micro-liters. The specific DNA amplified product of each sample was determined by identifying the 163-bp bands for *Giardia* comparing with 50-bp DNA ladder (Ferment's Germany) used as size marker.

The formula which is we used for the finding of prevalence is given below [16].

Prevalence Rate =

(No of parasite detected in water sample/Total no. of water samples examined) x100

3. RESULTS

Total of 65 drinking water samples were collected from different localities of Karak, Khyber Pukhtunkhwa, Pakistan from different sources like tap water, tube well (300 ft depth), bore (150 ft depth) and drainage water during the period of March, 2012 to March, 2013 and the prevalence of *Giardia* spp were examined through PCR. Over all prevalence of parasite was found 10/65 (15.38%). Prevalence in tube well, bore and drainage water was examined

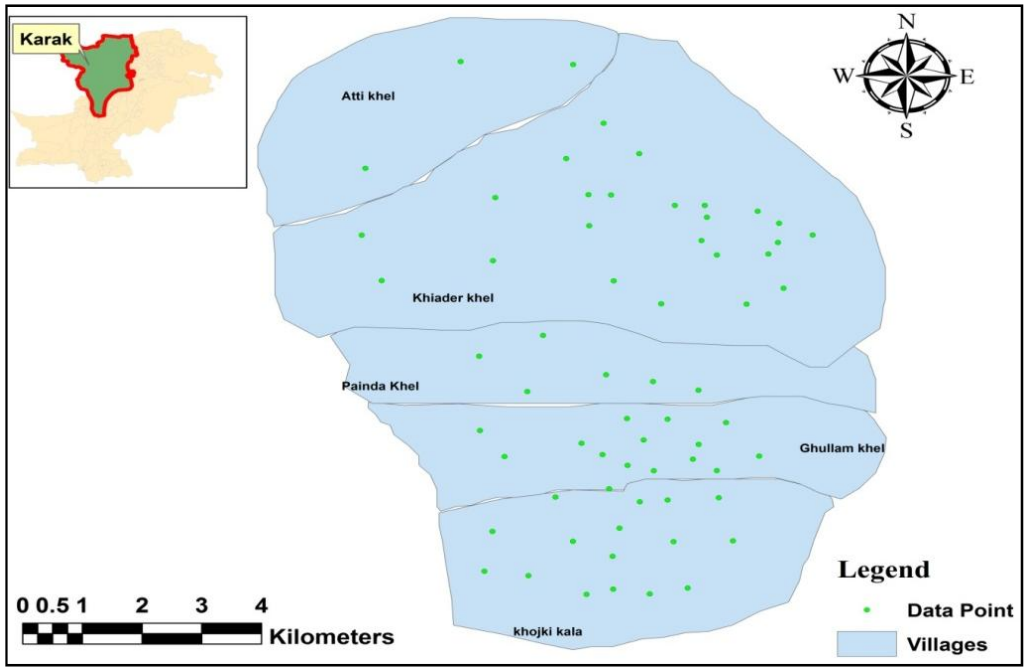

Fig. 1. Topographic view of study area where 65 samples were collected

7/65 (10.76%), 1/65 (1.53%) and 1/65 (1.53%) respectively which is given in Table 2. The result showed that tube well water was more contaminated with *Giardia* than other sources of water. The higher proportion of positive samples of *Giardia* was found in raw storage (72.6%) followed by raw (20.9%) and treated (18.2%) drinking water. water samples from 53 of the 72 municipalities sampled contained *Giardia* cysts at least once [16].

Table. 2. Prevalence of *Giardia* in different sources of water

Sources	Total	Positive	Percentage%
Tube well	29	7	10.76
Bore water	24	1	1.53
Drainage water	7	1	1.53

3.1 Locality Wise *Giardia* Prevalence

The sample were collected from different localities of District Karak, Khyber Pukhtunkhwa, Pakistan (Khojaki Kala, Ghulam Khel, Khaider Khel, Painda Khel and Atti Khel) from different sources (Tube well, bore, drainage and tap water) of Ghulam Khel were 12 in which 2 samples of tube well were positive for *Giardia.* 27 samples from Khaider Khel in which 1 of tube well and 1 of drainage were positive, 17 from Khojaki kala in which 1 of bore and 2 of tube well were positive, 3 from Atti Khel in which 2 positive of tube well and 6 from Painda Khel in which 1 positive of tube well which is given in the Table 3.

4. DISCUSSION

The present study revealed that *Giardia* Spp was widely disturbed in water sources in Karak, Khyber Pukhtunkhwa, Pakistan. A total of 65 samples were examined, among which 5 were of tap water, 29 of tube well (300 ft depth), 24 of bore water (150 ft depth) and 7 were of drainage water. The overall prevalence of parasite was 15.38% (10/65), in which prevalence in tube well water 10.76% (7/65), in drainage water 1.53% (1/65) and in bore water 1.53% (1/65). Result of this study revealed that prevalence of parasite was greater in tube well water than in other sources.

The prevalence of Giardia varies between 2% and 5% in industrialized countries and may exceed 30% in developing countries [17]. In 2001 the world Health Organization (WHO) estimated that around 280 million people are annually infected with *Giardia* spp in Asia, Africa and Latin America [18]. In contrast result of studies conducted in Karak have marked differences as

Table 3. No of water sources, positive for *Giardia* in Karak

Localities	Sources			
	Tube well	Bore water	Drainage water	Tap water
Ghulam Khel (12)	2	-	-	-
Khaider Khel (27)	1	-	1	-
Khojaki Kala (17)	2	1	-	-
Atti Khel (3)	2	-	-	-
Painda Khel (6)	1	-	-	-

out of 65 water samples only 10 were positive for *Giardia*. The presence of parasite more in tube well water was due to poor water supply system contamination of water supplies can also result agricultural runoff and leaking septic system [19]. The variation in the result was due to the different environmental condition of the area and the skilled man power.

5. CONCLUSION

From the study it was concluded that higher prevalence of *G.*spp in tube well water than other sources of water such as tube well, tap, bore and drainage water. It is suggested that a large scale study is required to explore the possibilities of zoonotic parasite in the water sources of Karak, Khyber Pukhtunkhwa and it is recommended that water should be treated before consumption.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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