Prevalence and Molecular Detection of *Giardia* in Different Sources of Water

1Lubna Hassan, 2Fahim Ullah and 3Mansoor Khan Khattak
1Department of Zoology, Kohat University of Science and Technology (KUST), 26000, Khyber Pukhtunkhwa, Pakistan
2Department of Agricultural Mechanization, The University of Agriculture Peshawar, 25000, Khyber Pukhtunkhwa, Pakistan

**Abstract:** The study was conducted to know the prevalence of *Giardia* in water in District Karak, Khyber Pukhtunkhwa, Pakistan. The collected water sample from different villages of District Karak was tested in the lab of the Department of Zoology in Kohat University of Science and Technology. *Giardia* is an intestinal protozoan parasite and a major cause of diarrheal disease in human worldwide. PCR (Polymerase Chain Reaction) method was used to detect the prevalence of *Giardia* in tube well, bore, drainage and taper water of District Karak, Khyber Pukhtunkhwa, Pakistan. The total of 65 water samples were collected amongst. From the result the prevalence of *Giardia* in tube well, drainage and bore water were found 10.76, 1.53 and 1.53%, respectively while in the sample of tape water there will be no prevalence of *Giardia* was detected. From the result it was concluded that a proper treatment of water for human consumptions is required.

**Key words:** PCR, *Giardia*, water source

**INTRODUCTION**

*Giardia camphylobacter*, *Cryptosporidium salmonella* and *Escherichia coli* have been the most commonly identified zoonotic agents of waterborne disease outbreak from contaminated drinking water throughout the world (Craun et al., 2004). *Giardia* is a flagellated, binucleated protozoan as shown in Fig. 1, discovered by Van leeuwenhoek in 1681. *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 (Yu et al., 2002) and having four pairs of flagella (one anterior pair, two posterior pairs) and a caudal pair that emerges posterity from the disc (Elmendorf et al., 2003). The acquirement of *Giardia* occurs most commonly through ingestion of the cyst (10-12 mm) in contaminated water, but person to person spread is common, particularly in settings of proof focal-oral hygiene. Filth flies can carry potentially viable *Giardia Lamblia* cyst on their exoskeleton (Graczyk et al., 2003).

The zoonotic transmissions are any infective disease that can be transmitted from animals, both wild and domestic to humans or from human to non-human animals of the 1415 pathogens known to affect humans 61% are zoonotic. Zoonotic pathogens comprise 75% of the emerging infection disease in humans (Bolin et al., 2004). Many of these emerging zoonotic are transmitted indirectly through food, water or environmental contamination. Evidence suggest that zoonotic water borne pathogens will continue to be recognized as an increasing public health concern world wide because of changing pattern in water use, climatic change, severe weather events, increasingly concentrated livestock operation and international trade in animal product (Taylor et al., 2001).

*Giardia* is an intestinal protozoan parasite and a chief cause of diarrheal disease in humans world wide (Berkman et al., 2002). Possible symptoms of infection include mal absorption, loose or watery stools, dehydration and abdominal cramping. The symptoms of a *Giardia* infection are a suite of gastrointestinal unpleasantries, same of which have been described as “explosive” and “violent” (Amar et al., 2002). *Giardia* is frequently found in water sources particularly those human and animal contamination is likely. Humans or animals become infected when they ingest cysts. Trophozoite, the feeding stages emerge from these cysts...
in the digestive tract and these stages can asexually divide (Appelbee et al., 2003).

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 (Huang and White, 2006). The most common symptoms of Giardiasis are diarrhea, abdominal pain, bloating, flatulence and weight loss resulting from mal absorption (Chaudhry et al., 2004).

The diagnosis of Giardiasis has been classically on detection of cyst or trophozoite in stool and duodenal aspirating specimens by direct microscopic examination and duodenal endoscopic biopsy specimen by histologically. Recently proven, Enzyme linked immune sorbent assay methods for the detection of Giardia (Ali and Hill, 2003).

Water born flagellated parasite Giardia lamblia continuous to be most frequent protozoan agent of intestinal disease world-wide, causing an estimated 2.8×10⁶ cases per annum (Ali and Hill, 2003). Giardia lamblia was the most commonly encountered parasite with a prevalence of 24.2% in Punjab. Prevalence of Giardia lamblia is 11.8% in Muzaffarabad city (Chaudhry et al., 2004). 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 Giardia lamblia found in 380 (36.19%) cases (Shaikh et al., 2009). 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 (Younas et al., 2008). 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.

**Taxonomy and classification of G. lamblia:**

- Kingdom: Protista Subkingdom: Protozoa
- Phylum: Sarcomastigia Subphylum: Mastigophora
- Class: Zommastigophora Order: Diplomonadida
- Family: Hexamitidae Genus: Giardia
- Species: Lamblia (Sulaiman et al., 2003)

**MATERIALS AND METHODS**

**Sample selection:** Karak is a district of the Khyber Pukhtunkhwa, Pakistan. Sixty five sample of water were collected from different water sources of different villages. The different water sources include tap water, tube well (300 ft depth), bore water tube well (150 ft depth) and drainage water. The quantity of water sample was 1.5 L. The predominant language is Pashto, which is spoken by all the population.

**Water filtration and processing:** A total of 65 water samples were randomly collected in a sterilized and clean bottle having capacity of 1 L directly from the water sources in Karak from different sources like (tube well, bore, drainage and tap water). The sampling was continued in summer, winter, autumn and spring March, 2012 to March, 2013. Samples were labeled with date of collection, site and nature and transported to laboratory of Zoology for further process. Samples were filtered through watt man filter paper and the material was collected from the filter paper according to the instruction. All samples were filtered at recommended flow rates and transferred into a centrifuge tube at 6000 rpm for 15 min. The purpose of centrifugation is to separate particles according to their density. Those having high molecular weight were settling down at the bottom and those having low molecular weight were settled above according to their density. Two layer from supernatant and pellet. The supernatant were discarded and pellet was poured into the eppendorf tube. Again those samples were run in micro centrifuge machine at 14000 rpm for 8 min.

**DNA extraction and lyses:** The DNA was extracted by DNA zole (Trizol USA) method with minor modification with the following steps. from 124 µL the sample was taken and added with 250 µL DNA zole. Then the mixture was mixed properly through vortex and incubated at room temperature for 5 min.

**DNA precipitation:** The 125 µL of iso-propanol was added to the mixture and centrifuged at 7000 rpm for 10 min. After centrifugation the supernatant was removed and add 125 DNA zole was added to the DNA pellet and centrifuged at 7000 rpm for 5 min.

**DNA wash and hydration:** Tow hundred microliter of 70% ethanol was added to the pellet after discarding the supernatant and centrifuge at 7000 rpm for 5 min. Discard the supernatant. The DNA wash step was repeated and the tubes were stored vertically to dry for 10 min. Forty microliter of distilled water was added to the pellet and incubated at 55°C for 10 min in hotplates and were kept at -40°C till use.

**DNA amplification (PCR):** Polymerase chain reaction reaction was carried out in a thermal cycler
Table 1: PCR cycle setup for *Giardia*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cycle</th>
<th>Step</th>
<th>Temperature (°C)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>94</td>
<td>5:00 min</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>1</td>
<td>94</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>57</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>72</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>72</td>
<td>7:00 min</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td>Hold</td>
</tr>
</tbody>
</table>

(Nyx Technich USA) with Taq DNA polymerase (Ferments USA). The conditions for PCR cycle were given in Table 1. The amplification was performed with.

Five microliter of extracted DNA by using 10 PM of forward (5'-AGGGCTCCGGCATAACTTTCC-3') and reverse (5'-GTATCTGTGACCCGTCCGAG-3') primers. The reaction mixture for a single reaction was consisted of following terms:

- Taq Buffer 2.1 µL
- MgCl2 (25 mM) 2.4 µL
- dNTPs (500 µM) 1.0 µL
- Forward Primer (10 Pm) 1.0 µL
- Reverse Primer (10 Pm) 1.0 µL
- Dh2o 7.2 µL
- Taq, DNA Polymerase (5 U µL⁻¹) 5.0 µL
- Extracted DNA 5.0 µL

**Gel electrophoresis:** Ten microliter of PCR product was mixed with 2 µL DNA loading dye and loaded in agarose gel which was prepared by dissolving 2 g of agarose in 100 mL of 0.5×TBE buffer in reaction bottle and boiled it for 2.5 min in microwave oven. The solution was cooled down to about 50°C at room temperature or water bath. Then 5 µL ethidium bromides (1 µg L⁻¹) was added per 100 mL gel solution and then poured into the gel rack. The comb was set at one side of the gel, about 5-10 mm from the end of the gel fixed. When the gel cooled down and become solid, the comb was remove carefully.

The holes that remain in the gel were the wells or the slots. Gel rack was placed in gel tank containing 1000 mL TBE buffer. Then 12 µL of each sample was loaded in the wells and 12 µL of DNA ladder (50 bp). The gel was run for 25 min at voltage of 120 volts and 500 ampere current. Gel was then examined by UV transilluminator and get documentation for picture. 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.

**Prevalence rate:** The prevalence rate was determined by the following formula (Ayaz *et al.*, 2011). Prevalence Rate = (No of parasite detected in water sample/Total no. of water samples examined) ×100.
examined, among which 5 were of tape 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 (tube well 150 ft depth) 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 varies between 2 and 5% in industrialized countries and may exceed 30% in developing countries (Eligio-Garcia et al., 2005). In 1988 the World Health Organization (WHO) estimated that around 280 million people are annually infected with Giardia spp in Asia, Africa and Latin America (Ponce-Macotela et al., 2005). 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 out of the 72 municipalities sampled contained Giardia cysts at least once (Wallis et al., 1996). In contrast result of studies conducted in Karak have marked differences as out of 65 water samples only 10 were positive for Giardia. The study reflected that the prevalence of parasite was greater in tube well water than in other sources of water. 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 (Widmer et al., 2002). The variation in the result was due to the different environmental condition of the area and the skilled man power.

**CONCLUSION**

From the study it was concluded that prevalence of Giardia parasite in tube well water was more than other sources of water (tube well, tape, bore and drainage water). There are possibilities that some of the pipes and connection systems may be damaged (cracks or leaked) and due to which contamination from surface may occur. 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.

**REFERENCES**


Yu, L.Z., C.W. Birky and R.D. Adam, 2002. The two nuclei of Giardia each have complete copies of the genome and are partitioned equationaly at cytokinesis. Eukaryote Cell, 1: 191-199.