

Prevalence of *Giardia lamblia* in Different Water Sources of District Newshehra, Khyber Pakhtunkhwa Pakistan.

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Abstract

Giardia lamblia is a cosmopolitan parasite that occurs worldwide and generally effects gastrointestinal tract. Water played a media for transmission of *Giardia* to different hosts. A total of 300 water samples were examined from different water sources, i.e. tap, open well, bore well and drain waters and DNA was extracted by trizol method through prescribed protocol. DNA was amplified through PCR. The overall prevalence of *G. lamblia* was 27.66% (83/300). Among these 2.5% (1/40) in bore well water, 29% (29/100) open well, 18.83% (11/60) tap water and 42% (42/100) drain water. It is concluded from the study that *Giardia* is frequently found in all water sources and is the main cause of ill health.

Key words: *Giardia lamblia*, Diarrhea, Prevalence and PCR.

Introduction

Waterborne parasites transmission is considered to be the major cause of various disease outbreaks in human population (Rose *et al.*, 2002). According to the survey of UNICEF, above 800 million individuals have no access to pure drinking water in Africa

and Asia. Due to this reason people suffer various infectious diseases (Tanwir *et al.*, 2003). *Giardia* is a genus of phylum Protozoa which are flagellated parasites of small intestine and causes infection in many vertebrates. *Giardia* has three different species including *G. lamblia*, *G. muris* and *G. angilis* (Garcia *et al.*, 2005). The *G. lamblia* is also known as *G. intestinalis* or *G. duodenalis*. Anton Van Leeuwenhoek observed this parasite in 1681 by his self-invented microscope. Later on it was named by two scientists Professor A. Giard in Paris and Doctor F. Lambl in Prague in 1915 (Thompson *et al.*, 2002). The name *Lamblia* has its origin from Vilem Lambl who described the trophozoite in humans in 1859, and the cyst form was discovered by Grassi twenty years later. However, Antony van Leeuwenhoek described the parasite in his own stool as early as in the 17th century (Hill *et al.*, 2006). It has worldwide distribution. In developed countries prevalence rate is 2-5 % and 21-31 % in the developing countries (Farting *et al.*, 1994). The protozoan parasite *G. lamblia* is mainly considered to be a major cause of diarrheal illness in human (Yaeger *et al.*, 1996). One of the main complications of Giardiasis is lipid malabsorption so that steatorrhoea (foul smelling, greasy stool) is a clinical sign of Giardiasis (Huang and White, 2006). Giardiasis is generally identified through microscope by the detection of trophozoites or cysts in water filtrate after staining methods or direct immune fluorescence (Janoff *et al.*, 1992). Fecal-oral transfer of *Giardia* cysts is thus the major route for Giardiasis transmission (Robertson *et al.*, 2009).

Keeping in mind the importance of zoonotic parasites the present study is designed to determine the prevalence of *Giardia* in different water sources in District Nowshera, Khyber Pakhtunkhwa Pakistan.

Materials and Methods

The study was carried out in four different areas of district Nowshehra including Nowshehra City, Pubbi, Aza Khel and Akhora Khattak of Khyber Pakhtunkhwa province for detection of *Giardia* in different water sources including bore wells, open wells, tap water and drain water. A total of 300 water samples were collected from the selected areas. One liter of each water sample was collected in sterilized bottle, labeled (date of collection, name of area and type of water). The water samples were filtered through Whatman filter papers in water filtration assembly and the water which have residual particles (containing parasites) were collected in 50 ml bottles. Then each sample was centrifuged at 800 rpm for 15 minutes in 10 ml tubes. From the tube supernatant was discarded and pellet was taken in eppendorf tubes. Tubes were centrifuged at 14000 rpm for 10 minute, again supernatant was removed and 250 ul pellets was taken as a sample for DNA extraction. The DNA was extracted by DNA zol (Trizol) method with minor modification. 200 µl from the sample was taken and added with 250 µl DNA zol. Then 150 µl of Iso-propanol was added to the mixture and centrifuged at 7000 rpm for 10 minutes. After centrifugation the supernatant was removed and 125 µl DNA zol was added to the DNA pellet and centrifuged at 7000 rpm for 5 minutes. 150 µl of 70% Ethanol was added to the pellet after discarding the supernatant and centrifuge at 7000 rpm for 5 minutes and discard the supernatant. The DNA wash step was repeated and the tubes were stored vertically to dry for 10 minutes. Then 40 µl of distilled water was added to the pallet and incubated at 55⁰C for 10 minutes in Hotplates, and were kept at -4⁰C till use.

PCR reaction was carried out with *Taq* DNA polymerase. The amplification was performed with 5 μ L of extracted DNA by using 10 Pico moles of forward and reverse primers.

Primer	Primer sequence	Prediction amplicon size
Forward (GDF)	AGGGCTCCGGCATAACTTTCC	163-bp
Reverse (GDR)	GTATCTGTGACCCGTCCGAG	

The reaction mixture for a single reaction was consisted of *Taq* Buffer 2.2 μ L, $MgCl_2$ 2.4 , dNTPs 1 μ L, Forward Primer 1 μ L, Reverse primer 1 μ L, Distal water 7 μ L, *Taq* DNA polymerase 0.5 μ L and extracted DNA 5 μ L. The denaturation temperature in PCR was set 94⁰C, Anelling temperature 57⁰C and Elongation temperature 72⁰C for *Giardia*.

Gel was prepared by dissolving 2 gram agarose in 100ml 0.5X TBE buffer. The 20 μ L of PCR product mixture was mixed with 2 μ L loading dye. Then 12 μ l of each sample was loaded in the wells and 12 μ l of DNA Ladder Marker (50bp). The gel was run for 25 min at voltage of 120 volts and 500 ampere current. Gel was then examined by UV Transilluminator. The specific DNA amplified product of each sample was determined by identifying the 163-bp bands for *Giardia*. The data were analyzed by using the univariate ANOVA and P<0.05 values were considered the significant.

Results

All the 300 water samples were examined through PCR, which indicated total 27.66% (83/300) prevalence of *G. lamblia*. Among these samples the prevalence of *G. lamblia* was highest (42%) in drain water, followed by open well water (29%), tap water (18.33%) and bore well water (2.5%).

The PCR result showed variation in different areas of Nowshehra. From Pubbi 28% samples were positive for *G. lamblia* in open well, 44% in drain water, 13.33% in tap water while bore well water showed no positive results. In Akora Khatak 36% sample from drain waters, 20 % from tape water, 24% in open well were positive for *G. lamblia*, while bore well sources were negative for *G. lamblia*.

Nowshehra City showed high prevalence rate as compared to other regions. 56% prevalence was detected in drain water, 26.66% in tap water, 44% in open well water and 10% bore well has positive results for *G. lamblia*.

Similarly in Aza Khel drain water have 28% prevalence, tap water 13.33%, open well 25% for *G. lamblia*, while bore well water has no positive results for *G. lamblia*.

Prevalence of *Giardia lamblia* in different areas of District Nowshehra

Area	Bore well %	Open Well %	Tap water %	Drain water %	Overall %
Pubbi	0	28	13.33	44	26.66
Akora khatak	0	24	20	36	24
Nowshehra city	10	44	26.66	56	40
Aza Khel	0	20	13.33	28	20
Total	2.5	29	18.33	42	27.66

ANOVA was applied and $P < 0.05$

Discussion

Different studies on *G. lamblia* revealed different results. In Ethiopia 26.6 % were found positive for *G. lamblia* infection. The prevalence of *G. lamblia* was related with the source of drinking water with more cases of Giardiasis detected in study participants using water from unprotected water sources than those using the “protected” water (Tigabu *et al.*, 2010). In our study the prevalence rate of *G. lamblia* is 27.33% in different water sources, which is closely similar to the earlier result.

In a current observation based at the Aga Khan University in Karachi, 334 adults aged from 16 to 83 years old 178 (patients with long-lasting diarrhoea and 156 persons diarrhoea-free volunteers who replaced as controls) were diagnosed for contamination with these parasites, stool were used as diagnose by microscope and/or PCR. The result showed that overall prevalence, were 6.3% and 8.7% of the samples were positive for *G. lamblia* by microscopic method and PCR respectively (Yakoob *et al.*, 2010). Younas found that 30.96% children were positive for Giardiasis (Younas *et al.*, 2008).

A microscopic study conducted on different sources (Tap water, ponds and drain water) in three different districts (Karak, Kohat and Hangu) of Khyber Pakhtunkhwa Province Pakistan. In all the three sources, water was contaminated with eggs, cysts or oocysts of the parasite. This result showed that the prevalence of *Giardia* spp. was 14.1%. This result is less than the current study due to different methodology PCR / Microscopy.

In present study 300 water samples were collected from different water sources, i.e. tap, open well, bore well and drain waters. The overall prevalence of *G. lamblia* was 27.66 %

(83/300). Among these samples the prevalence of *G. lamblia* was 2.5 % (1/40) in tube well water, 29% (29/100) in open well, 18.83% (11/60) in tap and 42% (42/100) in drain water.

REFERENCES

- Ayaz, S., Khan, S., Khan, S.N., Bibi, F., Shams, S. and Akhtar, M. (2011). Prevalence of Zoonotic parasites in drinking water of three districts of Khyber Pakhtoonkhwa Pakistan. *Pakistan Journal of life and Social Sciences*. **9**: 67-69.
- Farting, M.J.G., Thompson, R.C.A., Reynoldson, J.A. and Lymbery, A.J. (1994). *Giardiasis as a disease*. *CAB International*. **1**: 15-37.
- Hill, D.R. (2006). *Intestinal Flagellate and Ciliate Infections*. vol. 2, Second edition: Elsevier.
- Huang, D.B, White, A.C. (2006). "An updated review on Cryptosporidium and *Giardia*". *Gastroenterol. Clin. North Am.*, **35**: 291–314.
- Janoff, E. N. (1992): Diagnosis of *Giardia lamblia* Infections by Detection of Parasite Specific Antigens. *Journal of Clinical Microbiology*. **27**: 431-435.
- Robertson, L., Gjerde, B., Hansen, E.F., and Stachurska, H.T. (2009). A water contamination incident in Oslo, Norway during October 2007. A basis for discussion of boil-water notices and the potential for post-treatment contamination of drinking water supplies. *J. Water Health*. **7**:55–66.
- Rose, J.B., Huffman, D.E. and Gennaccaro, A. (2002). Risk and control of water borne Cryptosporidiosis. *FEMS Microbiology*. **26**: 113–123.

- Tanwir, F., Saboor. A and Shan, M.H. (2003). Water Contamination, health hazards and public awareness, a case of the urban Punjab, Pakistan. *International Journal of Agriculture and Biology*. **5**: 460–462.
- Thompson, R. C. A. (2002). Towards a Better Understanding of Host Specificity and the Transmission of *Giardia*. The Impact of Molecular Epidemiology.
- Thompson, R. C. A. (2004). The zoonotic significance and molecular epidemiology of *Giardia* and *Giardiasis*. *Veterinary Parasitology*. **126**: 15–35.
- Yaeger, G.R. (1996). Protozoa, structure, classification, growth and development. *Medical microbiology*, Galveston, Texas, University of Texas Medical Branch. 4th edition.