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Occurrence of Giardia in Different Water Sources in District Bannu

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SNK, IU and SU collected the samples, designed the study and performed Microscopic and Molecular analysis. Authors SA, SK, MAK, SA, JK and IA helped in literature search and wrote the manuscript. Author NUA arranged tables and performed statistically analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: After air, water is one of the greatest significant essentials for life, which is considered as one of the nutrients. *Giardia lamblia* (*G. lamblia*) is one of the most common waterborne protozoan parasites, causing diarrheal disease in human beings and animal diseases throughout the world.
Material and Methods: A total of 150 containing 1.5 L from each water samples were collected from different water sources of district Bannu from 1st May, 2012 to 30th April, 2013 and for further

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process the samples were brought to the Department of Zoology Kohat University of Science and Technology, Kohat within 24 hours. Water samples containing different water sources (Tap water, Bore water, Stream water and Pond water) in seven different areas of District Bannu (Pakistan). The water was filtered through Whattman filter paper No. 42 having 2.5µm pore size and the residue was subjected to Microscopy, DNA extraction and PCR was conducted for detection of *G. lamblia.* To increase the sensitivity of the test a small region (125-bp) of the SSU rRNA was targeted for the PCR amplification.

Results: The overall prevalence of *G. lamblia* in drinking water of district Bannu was 20% microscopically, including 28.33% in Stream water, 12.5% in Tap water, 20% in Tap water and was absent in Bore water. While that of PCR based study the overall prevalence of parasite (*G. lamblia*) was 24%, including Stream 28.33%, Tap water, 20%, Pond water 26.66% and Bore water 15 %. The highest prevalence of *G. lamblia* was 25% recorded in Tap water of Basia Khel through microscopic study & that of PCR based study, the highest prevalence was recorded in the Stream water of Bannu City which was 37.5% and P<.05 was considered significant.

Conclusion: It was revealed from the current study that *G. lamblia* is present in water sources in some areas in district Bannu, which may be due to flooding and improper management of water scheme. The study recommended that a proper treatment of water for human consumption is required, especially in Bannu City and Basia Khel in district Bannu.

Keywords: Giardia lamblia; protozoan; PCR; microscopy and water scheme.

1. INTRODUCTION

Water is considered as one of the nutrients, although it yields no calories, yet it enters into the structural composition of the cell and is an essential component of diet [1] and it is one of the greatest significant essentials for life after air [2]. It is estimated that due to different water sources, more than 100 human diseases occurred [3]. In developing countries, 60% of the total population has no access to pure drinking water [3]. Safe and healthy water have been defined as “water that is free from pathogenic agents, free from harmful chemical substances, pleasant to taste and smell [4]. Safe and fresh water supplies are at risk condition in many areas of Pakistan i.e, Pakistan is in “high water stress condition”, which occurs when the ratio of usable to availability exceeds 40 percent [4]. Due to waterborne 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 [2]. About 325 water related outbreaks of parasitic protozoan diseases including *G. lamblia* has been reported throughout the world [5]. As the methodology for the detection of *G. lamblia* in water is difficult economically because of taking much time and needs outclass microscope experts for identification [6].

Giardiasis is caused by *G. lamblia*, which is one of the most common intestinal parasite in the world and cause 2.8 x 10^8 estimated infections per year in humans [7]. It is more frequently found in surface water and it has been also associated with at least 132 waters borne outbreaks worldwide [7]. Giardiasis is a global disease which infects nearly 2% of adults and 6% to 8% of children in developed countries worldwide [8]. The prevalence rate in temperate climates is 2-10% in adults and 25% in children, whereas in tropical countries 50-80% of people are carriers [9]. In developed countries, infection occurs most frequently among children in day care centers, hikers, among members of the same family, between male homosexual partners and immune-compromised individuals. Drinking untreated water is a common source of infection and can result in community wide epidemics [10].

The medical features and symptoms of Giardiasis are epigastric pain and sudden onset of watery diarrhea [11]. Diarrhea is often explosive and presents a foul smell without the presence of blood, gas, bloating, mucus, or cellular exudates. Most infections resolve spontaneously within six weeks. Chronic infections can occur and diarrhea leads to dehydration, malabsorption and weight lost and impaired pancreatic function. Chronic infections can last from months to years. *G. lamblia* is usually found in the upper small intestine, but can be found in the gall bladder and in biliary drainage [12]. We are facing a water crisis due to increasing world population and growing contamination of usual resources. It is assessed by the World Health Organization (WHO) that

one billion people absence access to clean water for drinking [13]. *G. lamblia* spp was detected 18.5% through microscopy in different water sources of Khyber Pukhtunkhwa [13].

Different methods are used nowadays for the detection of *G. lamblia*, including microscopy, Immunofluorescent Assay (IFA), Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) etc. Although for the molecular detection of the parasites, PCR method is more sensitive than Microscopy, therefore the present study is designed to analyze the prevalence rate of *G. lamblia* in different water sources of district Bannu by using the approach of molecular techniques in comparison with microscopy.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in District Bannu, Khyber Pakhtunkhwa, which lies between 32º43′, and 33.6º15′ North latitude and 70º22′ to 70º57′ East longitudes. The total area of this district is 1,227 square km. The total population is 817182 bearing 500,000 Male population and 31782 female population. The occupation is mostly agriculture, but few people are merchants, businesspersons and professional like doctors, engineers and teachers, mostly the people of the current area have low economic condition ranges from about R.s 15000 to R.s 50000 per month, while their water sources are mainly composed of tube well and water supply systems connected with different dams, where there the water stored from rain water.

2.2 Study Area Sites

The sites from where the water samples were collected were Bazar Ahmed Khan, Garhi Sher Ahmed, Fatma Khel, Bannu City, Basia Khel, Sitty Kale and Fazal Haq Malwana. The samples were collected from 1st May, 2012 to 30th April, 2013.

2.3 Sample Collection

A total of 150 water samples was collected from six different areas for the detection of *G. lamblia* including 60, Stream water, 40 Tap water, 30 pond water and 20 samples were of Bore water. About 1.5 L of each water sample was collected in sterilized bottles, labeled (date of collection, name of the area and type of water) and was transported to the Molecular Parasitology and Virology Laboratory, Department of Zoology KUST, Kohat, for further experimental analysis through Microscopy for confirmation of some sample and Polymerase Chain Reaction (PCR).

2.4 Sample Processing

The water samples were filtered through Whatman filter paper No. 42 having 2.5µm pore size in water filtration assembly. The filtered residue was further centrifuged at 6000 rpm for 10 minutes the supernatant was discarded and the residue was obtained in Eppendorf tubes were centrifuged at 10000 rpm for 8 min. 10µl of the residue were placed on the slides and made a thin film on it through wooden stick and stained with a Lugol's iodine and immersion oil observed under a binocular microscope (Olympus CX -31) at 10X, 40X and 100X magnification power. The microscopically confirmed positive and negative samples were then subjected to PCR amplification for comparison.

2.5 DNA Extraction and Amplification (PCR)

DNA was extracted from the filtered residue containing 200µl by DNA zole (Trizol USA) with prescribed protocol. After DNA extraction, in a thermal cycler (NyxTechnix, USA) the PCR reaction performed along with Taq DNA polymerase (Fermentas, USA). The PCR product was amplified by mixing of 5µL of isolated DNA with Taq Buffer 2.2 µL, MgCl2, 2.4 µL, 1.0 µL dNTPs, followed by dH2O (Medicated) 7.1 µL, Taq DNA polymerase enzyme 0.3 µL and 1.0 µL of each 10 Pico moles of Forward Gdf (3'- AGGGCTCCGGCATAACTTTCC -5') and Reverse Gdr (5'-GTATCTGTGACCCGTCCGAG -3') primers, which are used to target specific heat shock protein gene, containing 163-bp length to detect *G. lamblia*. For each reaction 35 cycles were applied in PCR initiated by 94ºC for 10 minutes as denaturation. Each cycle was consisted of 3 steps denaturation for 30 seconds at 94ºC, annealing for 60 seconds at 51ºC followed by elongation at 72ºC for 40ºC. The final elongation was for 5 min at 72ºC [14] with some modification for standardization.

2.6 Gel Electrophoresis

About 12 µL sample containing 10 µL of PCR product mixture and 2 µL loading dye were

loaded in 2% agarose gel along with 12 µl of DNA Ladder (100 bp). The gel was run for 25 min at a voltage of 120 volts and 500 mA current. The gel was then examined by UV transilluminator. The specific DNA amplified product of each sample was determined by identifying 163-bp bands for *G. lamblia* comparing with 100-bp DNA Ladder (Fermantas Germany) marker [15].

2.7 pH Values Determining

The pH values of different water sources were determined by means of digital pH meter and pH 7 solutions on the spot during collection.

2.8 Data Analysis

Statistical analysis was performed by One-Way ANOVA test, using "STATISTIX", version 9.0, Korean made software. Variables included for evaluation were Tap water, Bore water, Stream water and Pond water and P<.05 values were considered significant.

2.9 Prevalence Rate

The prevalence rate was determined by the following formula [13]. Prevalence Rate = (No. of parasite detected in water sample/Total no. of water samples examined) ×100.

3. RESULTS

Water samples (n=150) were collected from 7 different areas for molecular detection of *G. lamblia* including 60, Stream water, 40, Tap water, 30, Pond water and 20, samples were collected from Bore water. After PCR examination, *G. lamblia* showed different results in different water sources of different areas.

The current study, observed by microscope showed dissimilar results as compared to PCR. The highest overall prevalence rate of *G. lamblia* was observed 25% in Fatma Khel followed by 21.05% in Bannu City and Sitty Kale. While the lowest prevalence rate was 16.66% observed in Bazaar Ahmed khan in out of 30 samples. Although the Stream water showed significantly high prevalence rate 37.5% followed by 33.3% of Pond water and 16.66% in Tap water and there was no *G. lamblia* observed in Bore water (Table 1).

In the current study it was find out that out of four water sources, the Stream water was much more contaminated with *G. lamblia* followed by Tap and Pond water, while the lowest rate was observed in Bore water source. Similarly out of seven different localities, Fatma Khel water samples were much more contaminated with *G. lamblia* showing 31.25% followed by Bannu City water sources showing 26.3%. While the lowest prevalence rate was observed in Sitty Kale, showed 21.05% in all water sources as shown in (Table 2).

The results showed that the Stream water was more contaminated with *G. lamblia* than other sources both through microscopy & PCR. The negative results may be due to problem in handling or it was also possible that these samples may not contain *G. lamblia*.

The PCR results were significantly high than Microscopy. The Stream and Pond water showed same results microscopically as well as by PCR. While three samples of each of the Tap and Bore water were false negative. There was no false positive sample in the current study. The highest overall prevalence of *G. lamblia* in different water sources was 31.25% in Fatma Khel while the lowest was 20% by means of PCR in Basaar Ahmed Khan. The microscopy results were slightly different than PCR, containing 21.05% which was highest value in Bannu City and Sitty Kale while the lowest value was 16.66% in Bazaar Ahmed Khan (Table 3).

Over all *p*H values were slightly same in different water sources of dissimilar areas, the Stream water of different areas of district Bannu showed 7.35 (±0.34), Tap water contained 7.65 (±0.19), Pond water consisted of 7.35 (±0.34), Bore well water showed 7.89 (±0.24) and the *p*H of Drain water was 8.17 (±0.34). While over all *p*H value was 7.68 (±0.28) determined by *p*H meter and *p*H 7 solutions on the spot during water collection. P was 0.0000 less than 0.05 which showed most significant results (Fig. 1).

4. DISCUSSION

The current study was compared with different studies, which were carried out by different people in Pakistan or in the District Bannu.

In the present study *G. lamblia* were identified by simple microscopy and PCR. In others similar studies *G. lamblia* was reported in several parts of the world. In Shangai (China) *G. lamblia* detected in drinking water which was 18% [16], in Lege Dini (Ethiopia) 35.3% [17], in Hungarian

26.7% [18], in Japan 12% [19], in Mexico 50% [20], in Portugal 8.2% [21] and in Khyber Pakhtunkhwa (Pakistan) 14.1% [13]. The present study is also quite similar in accordance with these in respect of the parasite prevalence 20% by Microscopy while 24% through PCR.

A study was conducted in Russia and Bulgaria for the detection of *G. lamblia* in drinking water resources. The water samples was of different origin (surface, tap, bottle, well, spring and waste water) were collected from Rostov (southern Russia), Sofia and Varna (Bulgaria) in which (9.6%) was positive for *G. lamblia.* In tap, river, well and in waste water *G. lamblia* were detected [22] similarly. A similar study was conducted at Kohat, Kark and Hugu (Pakistan). In which water samples were collected from Tap water, Pond water and Drain water. The prevalence (%) of *G. lamblia* in each category of water samples was determined. In that study, *G. lamblia* species were found in Tap, Pond and Drain water in Kohat, Kark and Hungu districts of Khyber Pakhtunkhwa province Pakistan. Of all the samples, 65.5% contained protozoa, including 18.5% *G. lamblia* [13].

Another same study was conducted in the different areas of the same district, showing 36% (25/75), containing Tap 17.64% (9/51) and Pond water 75% (6/8), bore well water 41.66% (5/12) and hand pump water 50% (2/4) [23] varying from our current results which may depend on the time of collecting samples, location and geological changes due to rain fall and water flood as well as methodological approach towards the handling of the water samples.

Table 1. Prevalence of *G. lamblia* in different areas of district Bannu (Microscopic based study)

Areas Location (n)	Data Analysis Positive/Total (%)				Total
	Stream water	Tap water	Pond water	Bore water	
Bannu City (19)	37.5	0	20	0	21.05
Bazaar Ahmed khan (30)	33.33	12.5	12.5	0	16.66
Garhi Sheer Ahmed (24)	28.57	12.5	28.57	0	20.83
Fatma Khel (16)	33.33	0	50	0	25
Basia Khel (21)	20	25	33.3	0	19.04
Sitty Kale (19)	25	16.66	33.3	0	21.05
Fazal Haq Malwana (21)	28.57	12.5	50	0	19.04

n= Total number of samples, P= 0.0000 <.05, Significant

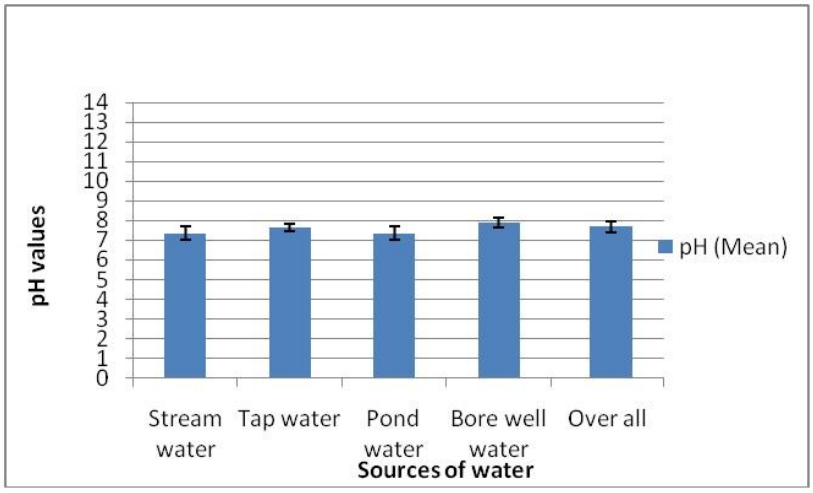

Fig. 1. Average pH values in different water sources collected from different areas in district Bannu

Table 2. Prevalence of G. lamblia in water samples of different areas of District Bannu (PCR based study)

Areas Location(n)	Data analysis positive/total (%)				Total
	Stream water	Tap water	Pond water	Bore water	
Bannu City (19)	37.5	25	20	0	26.31
Bazaar Ahmed khan (30)	33.3	12.5	12.5	25	20
Garhi Sheer Ahmed (24)	28.57	25	28.57	0	25
Fatma Khel (16)	33.3	50	50	0	31.25
Basia Khel (21)	20	25	33.3	25	23.8
Sitty Kale (19)	25	16.66	33.3	0	21.05
Fazal Haq Malwana (21)	28.57	12.5	50	25	23.80

n= Total number of samples, P= 0.0011 <.05, Significant

Table 3. Comparison of PCR and microscopy methods for the detection of *G. lamblia*

Areas location (n)	Microscopy	PCR
Bannu City (19)	21.05	26.31
Bazaar Ahmed khan (30)	16.66	20
Garhi Sheer Ahmed (24)	20.83	25
Fatma Khel (16)	25	31.25
Basia Khel (21)	19.04	23.8
Sitty kale (19)	21.05	21.05
Fazal Haq Malwana (21)	19.04	23.80

n= Total number, of samples P= 0.0340 <.05, Significant

As the pH values were nearly standard (6.5 to 8.5) according to WHO stated [24]. So no effect was observed in the prevalence of *G. lamblai* in the current study. In the present study, the positive samples mostly belonged to rural areas having low socioeconomic conditions. There was less awareness regarding cleanliness, sterilization and disinfection.

The PCR method was more sensitive [25], for detection of zoonotic parasite (*E. histolytica*) in water sources collected from different areas of district Peshawar. While our results showed too greater than microscope, which indicated that PCR is more accurate and sensitive than microscopic study. Microscopic study has also its own importance, because it is easily conducted and cheaper than PCR.

The present results may help the people for their health in prevention and supervision for giardiasis especially in children. It may also help the people in molecular detection of the parasites in the local laboratories.

5. CONCLUSION

It was concluded from the current study that high level of contamination of water was found both by microscopy and PCR in the Stream water of the District Bannu, that water was highly contaminated with *G. lamblia* as compare to the rest of water sources and among the drinking ones, the Pond water was more contaminated as compare to the Tap and Bore water of District Bannu.

It was recommended that PCR is more sensitive and accurate for the detection of the cysts or trophozoite form of *G. lamblia*. It was further recommended that filtered water should be used for drinking and cooking.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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