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Prevalence of Waterborne Parasites in Environmental Water Sources of Lahore and Faisalabad, Pakistan

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ABSTRACT

Water-borne parasitism has been an issue of concern in the recent past because of the pollution of different water sources. **Objectives:** To evaluate the prevalence and diversity of waterborne parasites in water samples of Lahore and Faisalabad. **Methods:** A total of 420 samples were collected from different sources (river, canal, borehole, and filter). A descriptive cross sectional study was conducted from April 2024 to July 2024. Different developmental stages of both protozoan and helminth have been isolated and examined from all the sampled water sources by filtration and centrifugation then the parasites were observed under a microscope. **Results:** Helminths isolated from water samples include *Ancylostoma duodenale* (1.19%), *Ascaris lumbricoides* (2.38%), *Clonorchis sinensis* (0.48%), *Dracunculus medinensis* (0.24%), *Enterobius vermicularis* (1.43%), *Hymenolepsis nana* (0.95%), and *Schistosoma* spp. (0.48%), *Strongyloides stercoralis* (3.33%), *Taenia saginata* (2.14%) and *Trichostrongylus* spp. (0.24%). Protozoans isolated include *Balantidium coli* (1.67%), *Blastocystis hominis* (1.19%), *Cryptosporidium parvum* (2.86%), Cystoisospora belli (1.9%), Entamoeba histolytica (2.38%), Entamoeba coli (1.43%), *Giardia duodenale* (0.48%), *Giardia lamblia* (1.67%), and *Iodoameoba butschii* (0.95%). Recreational water has the highest prevalence of 45.71%, followed by borehole 30% and filtered water 6.42%. Statistically significant difference ($P<0.05$) has been observed between borehole and recreational water. The highest prevalence of waterborne parasites has been observed in the month of April, 47.9%, followed by May, 37%, June, 14.7%, and July, 1.2%. **Conclusions:** Parasitic prevalence in water sources shows that water should be treated before use. High parasitic contamination in recreational water shows that it should not be used for human and animal activities.

INTRODUCTION

The pollution of multiple water sources in recent decades has raised concerns about water-borne parasite illnesses [1]. The WHO estimates that over 80 human diseases are waterborne. Approximately 30% of infections and 40% of deaths were waterborne in Pakistan [2]. One of the main signs of water-borne illnesses is diarrhea, which ranks as the ninth greatest cause of death globally. Unsafe water and poor sanitation were two of the main risk factors for diarrhea [3]. Unsafe drinking water, inadequate sanitation, and poor hygiene were responsible for around 88% of the burden. Parasitic diseases continue to be a hazard for public health in many regions of the world, despite recent efforts to enhance lifestyle choices and promote public health [4]. In Pakistan, exposure to dirty water was the leading cause of reported health issues. Diarrhea was responsible for 45% of child mortality, whereas waterborne illnesses account for 60%. The feces of infected humans, cattle, zoo animals, companion animals, and wild animals expelled the parasites from their bodies. The parasites in water supplies were spread by wild animals, agricultural

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operations, and recreational water activities [5]. According to estimates from the WHO, 2.4 billion people, or 40% of the world's population, live in unsanitary places; 1.1 billion people lack access to potable water; and up to 2.2 million children die from diarrhea every year [6]. Particularly, humans were susceptible to contracting WBPP infections via a variety of pathways (such as zoonotic, foodborne, and waterborne), and exposure to contaminated water sources could result in quite serious clinical problems [7]. According to the World Health Organization, at least 1.7 billion people globally use a drinking water source contaminated with feces, which poses the greatest microbial risk to drinking water safety [8]. This study was the first investigation and identification of waterborne parasites in Pakistani water sources in Lahore and Faisalabad. These cities lacked previous focused studies addressing the prevalence and varieties of waterborne parasites.

Despite the known risks of waterborne parasitic infections, there is limited data on the prevalence and diversity of such parasites in major urban centers of Pakistan, particularly in Lahore and Faisalabad. Previous studies have focused on microbial contamination or sporadic parasite reports, leaving a critical gap in understanding the seasonal variations, source-specific contamination, and public health implications of waterborne parasites in these cities. This study aims to address this knowledge gap by systematically evaluating the prevalence and diversity of waterborne parasites in different environmental water sources. The present study aimed to highlight the existence and diversity of harmful parasites in different water sources. The results helped shape public health policies while also addressing a major research gap.

METHODS

The study was carried out in Lahore and Faisalabad; both are districts of Punjab, Pakistan. Lahore is located in the north-eastern region of Punjab, along the River Ravi [9]. Faisalabad is located in the north-east of Punjab, lying between the plains of the Ravi and Chenab rivers (Figure 1) [10].

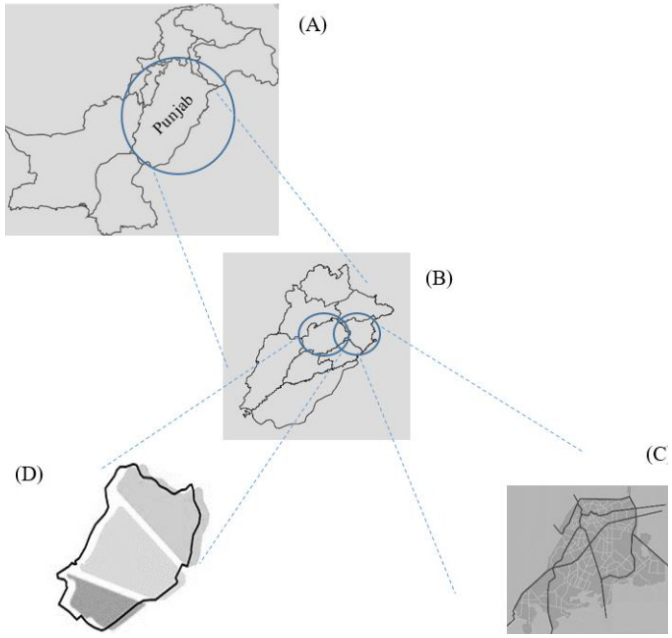

Figure 1: (A) Map of Pakistan, (B) Map of Punjab Province, (C) Map of Lahore, Where the Study Has Been Conducted. (D) Map of Faisalabad, Where the Study Has Been Conducted

A descriptive cross- sectional study was conducted to evaluate parasitic contamination in various water sources from Lahore and Faisalabad. Water sources were randomly selected in different areas of two districts. Water sources include borehole, filtered, river, lakes, canal, etc. Sampling was done in four consecutive months: April, May, June, and July. Samples of tap water, filtered water, streams, boreholes, and river water were gathered in clean plastic bottles. The samples were taken to the Parasitology lab in the Department of Zoology at Lahore College for Women University after being labelled with the dates of collection, the kind or source of the water, and the location of the collection. After being cleaned and rinsed with fresh water, 30ml sample vials with caps were brought to the sampling location. A total of 210 samples were gathered from each region. Collected samples were examined both macroscopically for color and the presence of adult parasites. After treating thirty milliliters of water samples with 0.35 grams of calcium carbonate, the samples were allowed to settle at the bottom of the bottle for one hour. Calcium carbonate treatment of water samples will guarantee effective parasite extraction in a state that permits accurate identification. After adding 0.5 ml of hydrogen sulphate (iv) acid to dissolve the debris, the supernatant was carefully decanted and left to stand for an additional hour. After that, the dissolved sediments were centrifuged for 15 minutes at 3000 rpm. While the sediments would be smeared on slides free of oil, the supernatant was disposed of. Using a Pasteur pipette, the deposit was placed on a clean, grease-free glass slide and was covered with a cover slip to avoid air bubbles and

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overflooding. A drop of Lugol's iodine was placed at the edge of the slide. Grease-free glass slides were smeared with the sediment and left to air-dry for a few minutes. The smeared slide was then fixed with methanol for three minutes to keep the parasite on it. The slides were then stained for fifteen minutes with carbol fuchsin and rinsed under tap water. The slide was decolored for 15 seconds with 1% acid alcohol, and any surplus acid alcohol was then rinsed off with tap water. After 60 seconds of counterstaining with 0.4% methylene blue, the slides were rinsed under tap water and allowed to air dry. The prepared slides were inspected with a binocular light microscope with objective lenses at 10x and 40x magnification. Parasites were identified by the morphological structures of their cysts, ova, or larvae when viewed under the microscope, as documented. SPSS version 27.0 was used to analyze data and determine the incidence and occurrence of parasites from water sources using basic descriptive statistics, chi-square, and t-test. Statistical significance was defined as p-values below 0.05.

RESULTS

A total of 420 water samples were collected from different areas of Lahore and Faisalabad. 210 samples were collected from each of the districts of Punjab. Recreational water has the highest prevalence of parasites in both districts, Lahore, 44.28%, and Faisalabad, 47.14%, and overall prevalence of 45.71% of waterborne parasites. Borehole water has an overall prevalence of 30%, and the lowest prevalence, 6.42%, has been observed in overall Filtered water samples. There is a statistically significant difference between the presence of waterborne parasites in water samples of the two study areas ($p<0.05$). (Table 1).

Table 1: Prevalence of Waterborne parasites in Water samples of Lahore and Faisalabad

Sample Source	Location Lahore			Location Faisalabad			Total		
	Sample Examined	No. of Positive	Prevalence (%)	Sample Examined	No. of Positive	Prevalence (%)	Sample Examined	No. of Positive	Prevalence (%)
Borehole Water	70	16	22.86%	70	26	37.14%	140	42	30%
Filtered Water	70	2	2.86%	70	7	10%	140	9	6.42%
Recreational Water	70	31	44.28%	70	33	47.14%	140	64	45.71%
Total	210	49	23.33%	210	66	31.42%	420	115	27.37%

Lahore (t-test=0.001; $p<0.05$), Faisalabad (t-test=0.001; $p<0.05$) Total (t-test=0.001; $p<0.05$)

There was a higher number of protozoans as compared to helminths in the sampled water of Lahore and Faisalabad. A total of 19 different parasites were extracted from water samples of both districts. *Ancylostoma duoedanale, Ascaris lumbricoides, Balantidium coli, Blastocytosis hominis, Clonorchis sinensis, Cryptosporodium parvum, Cystoisopora belli, Entameoba coli, Entameoba histolytica, Enterobius vermicularis, Giardia duodenale, Giardia lamblia, Hymenolepsis nana, Iodameoba butschii, Isospora belli, Strongyloides stercoralis, Taenia sanginata* while *schistosoma* spp. was only present in sampled water from Lahore, and *Dracunculus medinensis* and *Trichostrongylus* spp were present in sampled water of Faisalabad. These parasites, while extracted, were in their different developmental stages, like eggs, cysts, oocysts, and larvae (Table 2).

Table 2: Stages of Different Parasites from Two Study Areas

Sample Source	Location	
	Stages of Parasite (Lahore)	Stages of Parasite (Faisalabad)
Ancylostoma duodenale	Egg	Egg
Ascaris lumbricoides	Egg	Egg
Balantidium coli	Oocyst	Oocyst
Blastocytosis hominis	Oocyst	Oocyst
Clonorchis sinensis	Egg	Egg
Cryptosporidium parvum	Oocyst	Oocyst
Cystoisospora belli	Cyst	Cyst
Dracunculus medinensis	–	Larvae
Entamoeba coli	Oocyst	Oocyst
Entamoeba histolytica	Oocyst	Oocyst
Enterobius vermicularis	Larvae	Larvae
Giardia duodenale	Cyst + Trophozoite	Cyst
Giardia lamblia	Cyst	Trophozoite
Hymenolepsis nana	Egg	Egg
Iodamoeba butschlii	Cyst	Cyst
Schistosoma spp	Cercaria	–
Strongyloides stercoralis	Larvae	Larvae
Taenia saginata	Egg	Egg
Trichostrongylus spp	–	Egg

The presence of Waterborne parasites has been the highest in the month of April, 47.9%, while samples collected in the month of July have a 1.2% prevalence. Statistical analysis showed a significant difference in the presence of waterborne parasites in sampled water collected in the month of May ($p<0.05$). There is no statistical difference in the presence of waterborne parasites in sampled water collected in April, July, and June ($p>0.05$) (Table 3).

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Table 3: Month-Wise Prevalence of Waterborne Parasites in Sampled Water

Month of Sample Collection	Sample Examined	No. of Positive	Prevalence
April	121	58	47.9%
May	108	40	37%
June	109	16	14.7%
July	82	1	1.2%
Total	420	115	27.4%

The highest prevalence of *Strongyloides stercoralis*, 3.33%, has been observed. While the prevalence of *Cryptosporidium parvum* is 2.86%, *Ascaris lumbricoides* and *Entamoeba histolytica* have a prevalence of 2.38%. Statistical analysis showed a significant difference in the presence of parasite of parasites extracted from the borehole and recreational water (p<0.05). While results were non-significant for filtered water (p>0.05) (Table 4).

Table 4: Prevalence of Waterborne Parasites from different areas of Lahore and Faisalabad

Parasites	Lahore				Faisalabad				Total
	Borehole Water (n=70)	Filtered Water (n=70)	Recreational Water (n=70)	Total (n=210)	Borehole Water (n=70)	Filtered Water (n=70)	Recreational Water (n=70)	Total (n=210)	Total (n=420)
Ancylostoma duoedanale	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	2 (2.86%)	0 (0%)	1 (1.43%)	3 (1.43%)	5 (1.19%)
Ascaris lumbricoides	3 (4.28%)	0 (0%)	2 (2.86%)	5 (2.38%)	3 (4.28%)	0 (0%)	2 (2.86%)	5 (2.38%)	10 (2.38%)
Balantidium coli	2 (2.86%)	0 (0%)	2 (2.86%)	4 (1.9%)	0 (0%)	1 (1.43%)	2 (2.86%)	3 (1.43%)	7 (1.67%)
Blastocytosis hominis	0 (0%)	0 (0%)	3 (4.28%)	3 (1.43%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	5 (1.19%)
Clonorchis sinensis	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	2 (0.48%)
Cryptosporodium parvum	1 (1.43%)	0 (0%)	3 (4.28%)	4 (1.9%)	1 (1.43%)	2 (2.86%)	5 (7.14%)	8 (3.81%)	12 (2.86%)
Cystoisopora belli	1 (1.43%)	0 (0%)	2 (1.43%)	3 (1.43%)	1 (1.43%)	2 (2.86%)	2 (2.86%)	5 (3.81%)	8 (1.90%)
Dracunculus medinensis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	1 (0.24%)
Entameoba coli	2 (2.86%)	0 (0%)	2 (2.86%)	4 (1.9%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	6 (1.43%)
Entameoba histolytica	0 (0%)	2 (2.86%)	1 (1.43%)	3 (1.43%)	5 (7.14%)	0 (0%)	2 (2.86%)	7 (3.33%)	10 (2.38%)
Enterobius vermicularis	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	6 (1.43%)
Giardia duodenale	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	1 (1.43%)	0 (0%)	0 (0%)	1 (0.48%)	2 (0.48%)
Giardia lamblia	2 (2.86%)	0 (0%)	1 (1.43%)	3 (1.43%)	3 (4.28%)	0 (0%)	1 (1.43%)	4 (1.90%)	7 (1.67%)
Hymenolepsis nana	0 (0%)	0 (0%)	2 (2.86%)	2 (0.95%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	4 (0.95%)
Iodameoba butschii	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	0 (0%)	1 (1.43%)	2 (2.86%)	3 (1.43%)	4 (0.95%)
Schistosoma spp	0 (0%)	0 (0%)	2 (2.86%)	2 (0.95%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.48%)
Strongyloides stercoralis	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	5 (7.14%)	0 (0%)	6 (8.57%)	11 (5.24%)	14 (3.33%)
Taenia saginata	2 (2.86%)	0 (0%)	3 (4.28%)	5 (2.38%)	0 (0%)	1 (1.43%)	3 (4.28%)	4 (1.90%)	9 (2.14%)
Trichostrongylus spp	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.43%)	0 (0%)	0 (0%)	1 (0.48%)	1 (0.24%)

Borehole water (p=0.001; p<0.05), Filtered water (p=0.523; p>0.05), Recreational water (p=0.001; p<0.05)

DISCUSSION

Prevalence of parasitic contamination in water samples of Lahore and Faisalabad from the Punjab province of Pakistan is 27.38%, which is higher than the observations of (17.64%) and (12) (3.5%) from Khyber Pakhtunkhwa province of Pakistan and 23.4% from Ondo state of Nigeria [11]. A variety of helminths and protozoans have been observed during this study. Among protozoans, *Cryptosporidium parvum* has the highest prevalence of 2.86%. *Strongyloides stercoralis* has the highest prevalence of 3.33% among the helminths. Helminth eggs can either directly or indirectly have a major negative impact on human health, leading to gastrointestinal helminthiasis in both adults and children. It is well recognized that *Strongyloides* can damage the pulmonary, dermatological, and gastrointestinal systems [12]. In this study, *Ascaris lumbricoides* has the second-highest prevalence, 2.38%, followed by *Taenia sanginata*, 2.14%. while it was observed that the Ascaris species was the most common helminth, with a frequency of 33.9%. Ascaris lumbricoides has a prevalence of 4.86% in borehole and 2.83% in recreational water samples in each of the study areas separately [13]. This observation coincides with findings from other Great Lakes regions, where hookworm is frequently the predominant soil-transmitted helminth, followed by Ascaris and Schistosoma species [14]. Filtered water samples of Lahore have a prevalence of 2.86% with the presence of only one parasite, *Entamoeba histolytica*, while all other filtered water samples were clear of parasitic contamination. In Faisalabad, filtered water samples have a higher prevalence of 10% of parasitic contamination with the presence of 5 different parasites: *Balantidium coli*, *Cryptosporidium parvum*, *Cystoisospora belli*, *Iodoameoba butschii*, and *Taenia sanginata*. The reason for parasitic contamination in filtered water was that the presence of these parasites could be due to a fault in the treatment operations or post-treatment contamination [15]. As recreational waters include rivers, ponds, lakes, and canals, and have the highest prevalence

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of waterborne parasites, 45.71%. The presence of domestic animals near these water sites leads to high parasitic contamination in recreational water. Another study revealed that the prevalence of waterborne parasites in the river may be due to unsanitary practices by people who defecate near it, as well as the activities of farm animals like goats and cattle that harbor the parasites [16]. Borehole water samples have a 30% prevalence of parasitic contamination. This observation disagrees with other studies that similarly found no evidence of parasites in the borehole's source because of how it was built, and activities near that borehole, as ground activities could also influence the quality of borehole water [17]. According to our study, the high parasite prevalence was observed in the month of April, 47.9%, followed by May 37%, June 14.7%, and the lowest prevalence observed in the month of July, 1.2%. This observation disagrees with the study, which revealed that the parasitic contamination increases in the rainy season [18]. According to research, widespread waterborne epidemics were unlikely to happen in hot places, and parasites in the environment die quickly [19]. Aside from environmental toxins, a lack of water treatment endangers the health of unsuspecting populations. While no single method of filtration could eliminate toxins from drinking water, it can and should be safe to consume within accepted limits [20].
This study was limited by the sampling period of only four months and the focus on morphological identification, which may overlook low-density or molecularly distinct parasites. Future studies should incorporate year-round sampling and molecular techniques to detect a wider spectrum of parasites and assess their genetic diversity. Additionally, interventions such as water treatment efficacy and public awareness campaigns could be evaluated to reduce the risk of waterborne parasitic infections in urban populations.

CONCLUSION

It has been concluded that water sampled from various areas of Lahore and Faisalabad was contaminated with waterborne parasites, which means citizens were at high risk of getting parasitic infections. Therefore, it was very crucial to treat water before drinking or using it for other purposes. Even parasitic contamination in filtered water shows that there was a need for high-performance filtration to have parasite-free water. The highest prevalence of waterborne parasites in recreational water showed government should limit human and animal activities in recreational waters. There should be public education campaigns to raise awareness of the presence of waterborne parasites in water sources.

Authors' Contribution

Conceptualization: ZW, AW, MS
Methodology: ZW, AW, MS
Formal analysis: AI, TS, AAL, SH, AR, AMA
Writing and Drafting: ZW, AW, MS, AAL, SH, AR, AMA
Review and Editing: ZW, AW, MS, AI, TS, AAL, SH, AR, AMA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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