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Research Article

Investigation on Water Borne Protozoan Parasites in Drinking Water of Quetta and Sohbat Pur Districts of Balochistan

Tehmina Sajid[1], Abdul Razzaq[2], Asim Shamim[3], Sadaf Shahid[1], Dad Muhammad Anwar[4], Zahida Fatima[5], Munib Hussain[6], Muhammad Essa Kakar[7], Muhammad Bilal[7], Shakeel Ahmed Bugti[7], Muhammad Ramzan[7], Sajid Ali Khosa[4]

[1]*Department of Zoology Sardar Bahadur Khan Women's University Baluchistan Quetta, Pakistan.*
[2]*Animal Science Division, Pakistan Agriculture Research Council Islamabad, Pakistan.*
[3]*Department of Pathobiology, Faculty of Veterinary and Animal Sciences University of Poonch Rawalakot, Azad Kashmir, Pakistan*
[4]*Veterinary Research Institute, Quetta, Balochistan, Pakistan.*
[5]*National Reference Laboratory for Poultry Disease, NARC Islamabad, Pakistan.*
[6]*Animal Health Program, ASI, NARC Islamabad, Pakistan.*
[7]*Disease Investigation Laboratory, Livestock and Dairy Development Department, Balochistan, Pakistan.*

*Correspondence: asimshamim@upr.edu.pk

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Abstract

Current study was aimed to investigate the prevalence of water-borne protozoa in drinking water and stool samples from suspected intestinal effected patients in districts Quetta and Sohbat Pur, Balochistan. For this purpose, 200 water samples were collected from different water sources of the selected study areas. Collected samples were transported to the Sardar Bahadur Khan Woman University for further processing. Similarly, 200 human stool samples were also collected and transported to the Bolan Medical complex microbiology laboratory for further processing. Staining of stool samples was done by iodine and modified acid fast stain and examined under microscope. Culturing of positive stool samples weas carried out using lock egg media to culture *Entamoeba histolytica*. Overall, 15% protozoan prevalence was recorded from the water samples collected among the selected study areas where higher prevalence was observed in Sohbat Pur (20%) followed by Quetta (10%). Among water bodies higher prevalence was observed in canal system (40%). Protozoan distribution was recorded higher in May and August (17.5%) in water samples. Overall, 22% protozoan prevalence was recorded from the stool samples and higher prevalence was recorded in Sohbat Pur (27%) followed by Quetta (18%). Children from age group 11-20 years were found more susceptible as compared to adults. Based on month wise prevalence, higher prevalence was recorded in August (27.5%) and lowest in July (17.5%). *Entamoeba histolytica* was found more prevalent (17%) as compared to *Giardia lamblia* (5.5%). Statistical analysis shows there is no significant difference (P>0.05) among water and stool samples of Quetta and Sohabat Pur district of Balochistan. Further importance of the current study is discussed.

Keywords: Baluchistan; Drinking water; Protozoa; Stool samples

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Introduction

The main necessity of life is water and about one billion people in world are deprived from the access to the safe drinking water (WHO, 2017). Drinking water should be free from any noxious elements i.e., organic substances and infection causing microorganisms. Large portion of world population is affected either due to shortage of drinking water or with the contamination of drinking water with microorganisms (Ramírez-Castillo *et al.*, 2015). Water-borne infections are considered as threat for world and about 4% disease burden is concerned with water sanitation and hygiene and about 2.2 million people lost their life annually due to diarrheal diseases (Siddiqui *et al.*, 2012). Waterborne diseases are reported to cause 829,000 death each year from diarrheal cases annually and about 297,000 deaths among children of less than five years of age (WHO, 2017).

Water borne parasite can infect the individuals through various ways viz; water-washed diseases, water borne diseases, water related vector borne diseases, water-based diseases and water dispersed infections. Water-borne gastro-intestinal infections are the most emerging and re-emerging infections around the world and their impact on developing countries is an important aspect (Sente *et al.*, 2016; Siwila *et al.*, 2020).

Intestinal parasitic diseases are spreading due to lack of awareness regarding hygiene (personal and environmental) and low socio-economic conditions. Environmental conditions play a vital role in prevalence of intestinal parasitic diseases e.g. hot and humid conditions are causing rise in parasitic infections (Bethony *et al.*, 2006). Among intestinal parasites *Entamoeba (E.) histolytica, Giardia (G.) lamblia* and *Cryptosporidium (C.) parvum* are most important. Among these *E. histolytica* is causing annually 500 million cases and 40,000–110,000 deaths annually (Pham Duc *et al.*, 2011).

In developing countries raw water is mostly used for drinking, cooking and recreational activities and this raw water is contaminated with protozoa during collection, processing and storage (Young *et al.*, 2012; Siwila *et al.*, 2020). Low concentration of protozoan oocysts can cause infection even if 10 *Giardia* or *Cryptosporidium* oocyst ingested it may cause infection (Masangkay *et al.*, 2020).

Pakistan is blessed by nature with adequate surface and underground water sources but increased industrialization, population and urbanization has caused a detrimental effect on the water resources. Pakistan is ranked at 6th position based on population and its demand of water also increased with increase in population rate. In Pakistan approximately 38.5 million peoples are facing unavailability of portable water and 50.7 million are facing the sanitation problems (Khan and Javed, 2007). In urban and rural areas portable water is contaminated with micro-organisms. Per capita reduction in water accessibility is also a major issue in Pakistan due to increase in population (Soomro *et al.*, 2011).

Largest province of Pakistan is Balochistan having area of 2653 km^2 and Quetta is its capital city. Drought environmental conditions are present in Quetta and main water source is groundwater used for domestic as well as cultivation (Khan *et al.*, 2013). Untreated waste-water contaminates the underground water sources (Aina *et al.*, 2005). Main factors involved in water pollution are sewerage leakages, human activities, biofilm formation on water surface, leakages of pipes and irregular water supply (Uddin and Kurosawa, 2011). Based on the literature present study was designed to evaluate water-

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borne protozoa prevalence from drinking water sources of districts Quetta and Shbat Pur, Balochistan, Pakistan.

Methodology

Collection of samples

Collection of water samples

Different water sources (ponds, canal, tube wells, ground water sources, metropolitan water supply) from both study districts were visited and total of 200 water samples (2 liter each) were collected. Collected samples were further processed for examination.

Sample processing

Different parameters including pH and at room temperature the samples were further processed for filtration through Whatman filter papers having 47mm diameter and 0.45±0.02 mm pore size (Bakir *et al.*, 2003). Normal saline solution was used for rinsing of filtrate material. About 15 ml normal saline solution was used for this purpose and samples were centrifuged at 2000 rpm for 5 minutes. Sediments were collected after discard of supernatant and again centrifuged at the same conditions and subjected further for glass slide examination (Kwakye-Nuako *et al.*, 2007).

Preparation of wet mounts

One drop of sediment was placed on slide and mixed with drop of saline and iodine solution and covered with cover slip. Smear was examined under 100X objective lens microscope (WHO, 2017).

Trichrome stain technique

Trichrome staining was done by fixing the smear in schaudins's fixative for 30 minutes followed by immersion in iodine-alcohol solution for one minute followed by washing with 70% ethanol solution. After that the slides were transferred into trichrome stain for 8 minutes followed by acetic acid-alcohol solution for 5 minutes. After de-staining with the help of acetic acid-alcohol solution, slides were further washed with 95% ethanol for 2 second twice and at last the slides were transferred to the absolute xylene for 2 minutes followed by microscopic examination (Kaplan, 1992).

Stool sample collection and preservation

Gastroenteritis positive patients were visited and about 200 fecal samples were collected from different age groups from both genders. Samples were collected in sterile disposable plastic containers and further processing was done at Bolan Medical Hospital microbiology laboratory. Data regarding age, sex, name and stool parameters (consistency, blood, mucus) were also recorded.

Stool samples were stored in 20% glycerin, 10% formalin, and 70% distilled water containing solution till further processing of samples. 10.20 ml preservative was added in each sample and stored at cool temperature (Feenstra *et al.*, 2000).

Temporary mounting of fecal samples

Preserved samples were mixed with one drop of Lugol's iodine and normal saline mixture on a glass slide and observed under microscope at 40X (Khalil *et al.*, 2012).

Procedure

Thin smear was made by taking a drop of stool sample and fixed with methanol for 30 seconds followed by staining with carbol fuchsin for one minute. Washing was done

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with distilled water and further de-stained with acid alcohol for 2 minutes followed by washing with distilled water. After that the slides were further processed for counter staining with the help of Malachite green for five minutes and followed by examination at 200-300 field using higher objectives (Morgan *et al.*, 1998).

***Entamoeba histolytica* culture preparation**

Entamoeba histolytica culturing was done by using L.E.M: lock egg media. For this purpose, 2ml egg solution was mixed with lock solution and 8ml of that mixture was poured into sterilized culture tubes and further examined for pH value. Sterilization was done in oven at 80°C for 30 minutes and further processed for cooling at room temperature and slant preparation. Positive samples along with starch serum were added into culture tubes in 1:8 ratio (Al-Idrrise *et al.*, 2008).

Statistical analyses

Descriptive stat was used for frequency distribution of protozoal parasites in drinking water and fecal samples. Chi- square test was applied using SPSS software for determination of prevalence difference of water-borne protozoal diseases between water and fecal samples at significance level of 0.05.

Results and Discussion

The overall prevalence of protozoan parasites in stool samples (22.5%) was significantly higher than in drinking water (15%). The only protozoa found in the drinking water was *E. histolytica*. The prevalence of protozoan parasites was significantly higher in the drinking water of the Sohbat Pur district (20%) than in Quetta (10%). A non-significant association of protozoan parasites of drinking water with different months was observed. However, different kinds of water bodies showed a significant association with protozoal parasites. The frequency distribution of protozoa in drinking water is given in Table 1.

Two species of protozoa i.e., *E. histolytica* (17%) and *G. lamblia* (5.5%), were found in stool samples. The prevalence of *E. histolytica* was significantly higher than *G. lamblia*. The stool samples of study districts showed a non-significant association with protozoal infection. The district Sohbat Pur (27%) showed a little higher prevalence of protozoa than district Quetta (18%). Similar to drinking water, the prevalence of protozoa in stool samples showed a non-significant association with months. The prevalence of protozoa in the different age groups was also found non-significant. The frequency distribution of protozoa in stool samples is given in Table 2. The prevalence of protozoa in drinking water and stool samples is given in Figure 1.

Current study showed higher prevalence of *E. histolytica* followed by *G. lamblia* in two districts and similar findings (2.3% *E. histolytica* from water wells*)* were reported by Yousefi and Mohammadpour (2007) in the Mazandaran. Overall, 92% infection rate (71.8% *E. histolytica and* 17.5% *G. lamblia*) was observed in United Arab Emirates (Dash *et al.*, 2010). This difference may be due to change in climatic conditions, sample size and season. This study showed the potential of transmission of protozoal parasites through different sources of drinking water.

Similar findings were reported by Ghasemi (2012) in the city of Shush, Khuzestan Province with some higher prevalence (3.6%) of *Entamoeba histolytica* in drinking water. Costa *et al.* (2018) reported 6% prevalence in Brazil.

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Table 1. The frequency distribution of protozoa in drinking water of selected districts of Baluchistan, Pakistan.

	Levels	Examined	Infected	Prevalence	Chi-square	P-Value
District	Quetta	100	10	10.00	3.922	0.048
	Sohbat Pur	100	20	20.00		
Month	April	40	5	12.50	0.784	0.941
	May	40	7	17.50		
	June	40	5	12.50		
	July	40	6	15.00		
	August	40	7	17.50		
Water bodies	Metropolitan water supply	94	5	5.32	25.94	0.000
	Ponds	32	7	21.88		
	Canal system	35	14	40.00		
	Tube wells	39	4	10.26		
Total Sample	Drinking Water	200	30	15.00		

Table 2. The frequency distribution of protozoa in stool samples of selected districts of Balochistan, Pakistan.

	Levels	Examined	Infected	Prevalence	Chi-square	P-Value
District	Quetta	100	18	18.00	3.321	0.128
	Sohbat Pur	100	27	27.00		
Month	April	40	8	20.00	1.434	0.838
	May	40	10	25.00		
	June	40	9	22.50		
	July	40	7	17.50		
	August	40	11	27.50		
Age	1-10	32	9	28.13	4.052	0.542
	11-20	45	14	31.11		
	21-30	35	6	17.14		
	31-40	28	5	17.86		
	41-50	31	6	19.35		
	51-60	29	5	17.24		
Protozoan parasite	*Entamoeba histolytica*	200	34	17.00	13.246	0.000
	Giardia lamblia	200	11	5.50		
Total Sample	Stool	200	45	22.50		

In Pakistan, prevalence of *E. histolytica* is checked in various areas and data showed that *E. histolytica* frequently present. Hussain *et al.* (1997) reported 8% prevalence of *E. histolytica* in Northern areas of Pakistan. In Karachi prevalence of intestinal parasites was recorded in humans and 48.86% *E. histolytica* was reported (Siddiqui *et al.*, 2012). In Muzaffarabad 5.95% prevalence was recorded in children under 15 years of age (Chaudhry *et al.*, 2004). Ayaz *et al.* (2011) reported even higher prevalence of *E. histolytica* (14.4%) in Pakistan. Similar study was conducted in Sukkur, Sindh and they recorded higher prevalence of two protozoa *G. lamblia* in 380 patients (36.19%), followed by *E.*

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histolytica in 195 (18.57%) (Shaikh *et al.*, 2009). This similarity is attributed due to same socio-economic conditions, warm weather zone, absence of health education and poor sanitation.

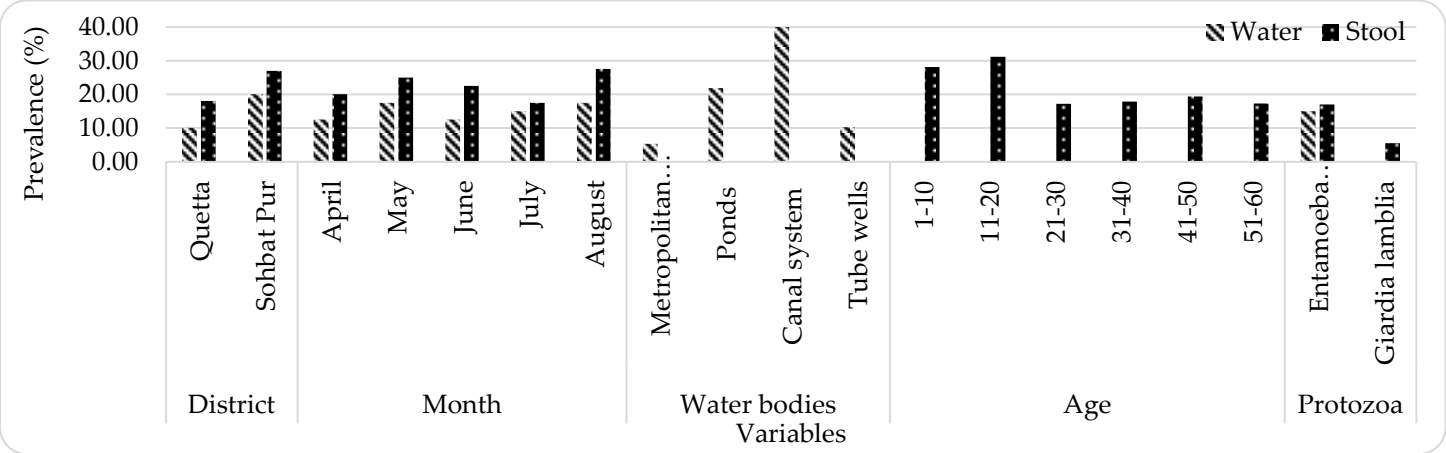

Figure 1. The prevalence of protozoa in drinking water and stool samples of selected districts of Balochistan, Pakistan.

Based on month wise prevalence July and August was found to have high infection rate and similar findings were reported by Zeb *et al.* (2018) in Khyber Pakhtunkhwa showing higher prevalence in July (27.6%) and least in February (16.9%). In a previous study conducted in Columbia higher prevalence was recorded during September and October (42% and 43%) and lowest in February (22%) (Amin, 2002). Similarly, Amin (2006) conducted a study and reported higher prevalence in September (23%) and lowest in February (13%). Intestinal parasites infective stages are linked with temperature, rain fall and humidity (Altizer *et al.*, 2006).

Current study shows higher prevalence rate in children and young people followed by adults and these findings are in line with the finding of Bernawi *et al.* (2013) and they reported higher prevalence in 3.64% higher in young age group. Tasawar *et al.* (2010) reported 27% prevalence in children under 15 years of age in Northern areas of Pakistan which are in line with the findings of the current study. In Malaysia a survey was conducted on prevalence of intestinal parasitic diseases and higher prevalence (52%) was recorded in children from age group of 0-19 years (Yusuf *et al.*, 2007). These findings may be due to the fact that children are having low resistance as compared to adults and parasitic infection mostly occur in youngers as they are more exposed to the overcrowded places e.g., schools, collages, parks, play grounds (Oguntibeju, 2006). Besides that, children are more susceptible due to immature immune system, lack in proper adaptation of preventive measures and personal hygiene (Nematian *et al.*, 2004).

Conclusion

Intestinal parasitic protozoa are re-emerging infections that spread when find suitable environment and host. Proper water treatment can reduce the chances of their prevalence. Proper identification and confirmation of responsible factors, health education, and treatment of carrier individuals is necessity of time to reduce the spread

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of these intestinal parasitic diseases in community.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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