Prevalence and Molecular Detection of Giardia and Cryptosporidium spp in Communities Consuming Different Drinking Water Sources in Kohat, Khyber Pakhtunkhwa Pakistan

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Abstract: A study was conducted to determine the presence of Giardia and cryptosporidium parasites in drinking water sources of three different communities in kohat, Khyber Pakhtunkhwa. A total of 150 water samples from three different sources such as tap water (effluent), open well water, stream water (influent) were examined by polymerase chain reaction (PCR). The average concentration of the parasites in drinking water sources were ranged from 0.0 - 12% Giardia cyst and cryptosporidium oocyst per litter in the concentrated pellet of the volume of one litter. The DNA of the parasites was extracted through vivantis Tissue DNA extraction kit, USA. A 163 bp *Giardia spp* and 256bp cryptosporidium spp gene were amplified. overall prevalence was 12% (18/150), amongst these *Giardia* Spp. and *Cryptosporidium* Spp. were 5.33% (8/150) and 6.66% (10/150) in tap, well and spring water in Kohat, Jauzara and Sheikhan Sherkot respectively. The presence of Giardia and cryptosporidium spp in the examined water sources through PCR may needs the purification and filtrations of the drinking water before its consumption in kohat, Khyber Pakhtunkhwa.

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1. Introduction

Giardia lamblia and cryptosporidium parvaum are the most common waterborne protozoa that cause infection in human and animals (Fayer, 2004). *Giardia* spp is one of the parasites among those which caused the diarrhoeal disease worldwide in humans and animals. A zoonotic and an anthroponotic type of transmission are reported worldwide. (Slifko *et al*, 2000, Thompson, 2000). Drinking water has since long been documented as a possible vector for the transmission of infectious diseases (Andersson and Stenstrom, 1986; Craun, 1991). A great portion of the inhabitants in emergent countries suffers from health problems linked with either deficiency of drinking water or due to the occurrence of microbiological pollution in water (Van Leeuwen, 2000). Poor water quality is liable for the death of an anticipated 5 million children in the emergent countries (Holgate, 2000). Diseases caused due to contaminated water remain one of the major health problems in the world (WHO, 2004). Estimates show that more than 3 million Pakistanis go through waterborne diseases each year of which 1.2 million die (WSP, 2005).

In Pakistan, 30 percents of all diseases and 40 percents of all mortalities are because of poor water quality (Global Water Partnership, Draft South Asia - Water Vision 2025, Country Report – Pakistan, (2000). Diarrhoea is reported as the main cause of mortalities in children in the country while every 5th civilian suffers from diseases caused by the infected water (Kahlown *et al*., 2006). A study carried out by UNICEF found that 20-40% of the hospital beds in Pakistan are in use by patients suffering from water-born diseases (Pak-SCEA 2006).

At least 325 water linked outbreaks of parasitic protozoan diseases have been reported globally (Kramer *et al*., 2001) and contamination of community water systems has the potential to cause disease in large number of residents (Barwick *et al*., 2000).

In developed countries, the commonly occurred human parasitic protozoa transmitted by water belong to the genera *Cryptosporidium* and *Giardia* (Xiao and fayer, 2008). Cysts are commonly found in sewage effluent and surface waters and in superficial springs (Thompson, 2000).

Drinking water resources become polluted when faeces containing the cysts are deposited into water (Smith *et al*., 2007; Reynolds *et al*., 2008). cysts can penetrate surface waters from waste of wastewater treatment plants, biosolids, metropolitan overflow, or agricultural surplus thereby causing infections (Craun *et al*., 2005; Montemayor *et al*., 2005). *G. duodenalis*

cysts can survived in water for up to 2 months at temperatures as low as 8 °C (Meyer and Jarroll, 1980).

Giardia intestinalis is the most usually reported intestinal parasite globally. It can cause acute or persistent diarrhea (Ali and hill, 2003; Carvalho-costa, *et al.,* 2007*)*. In different populations, giardiasis is one of the most common non viral cause of diarrhea among children, which, in turn, gives rise to such problems as malabsorption and weight loss, leading to delayed growth and development (Savioli *et al*., 2006). Keeping the importance of the Giardia and cryptosporidium parasites in the water sources and its ill effect, the present research study was designed to determine the *Giardia and cryptosporidium spp* presence in drinking water sources of different community in kohat, Khyber Pakhtunkhwa.

2. Materials and Methods

2.1. Study population

Kohat is a district of the Khyber-Pakhtunkhwa province of Pakistan, covering total area of 2545 km^2 (982.6 sq mi). It is located at 33°35'13N 71°26'29E with an altitude of 489 meters (1607 feet). It consists chiefly of a bare and intricate mountain region east of the Indus, deeply touch with the war tribal zone of Orakzai agency and Darra Adam Khel. The predominant language is Pashto, which is spoken by 77.54 percent of the total population, while Hindku is mostly spoken and understood in Kohat city and adjacent areas (SMEDA, 2009).

2.2. Water collection and processing.

A total of 150 water samples were randomly collected in a sterilized and clean bottle having capacity of 1 liter directly from the water sources in KDA (effluent tap water), Jauzara (stream influent water) and Sheikhan Sherkot (well influent water). The sampling was continued in summer, winter, autumn and spring March,2011 to March ,2012 .Samples were labeled with date of collection, site and nature and transported to laboratory of Zoology for further process. Samples were filtered through Filta-Max filters with a pump on the inlet side of the filter according to the recommendation of the manufacturer. The filtrates were centrifuge at 800 x g for 5 minutes. The debris was collected and mixed with buffer solution and stored at -20^{0}C refrigerator for further uses.

2.3. DNA Extraction:

The pallet sample was subjected for DNA extraction through DNA Extraction Kit (Vivantis) as per the prescribed protocol of the manufacturer.

According to prescribed protocol 50 ul Proteinase K was added into 200 ul of sample and mixed thoroughly. Then 215 ul of buffer VL was added and mixed by pulsed vortexing. Incubated at 65°C for 10 min. Next 280 ul absolute ethanol was added and mixed immediately. The mixture was transferred into given column. Then centrifuged at 5000 rpm for 1 min and flow-through was discarded. 500 ul wash buffer 1 was added and centrifuged at 5000 rpm for 1 min. flow-through was again discarded. Then 500 ul wash buffer 2 was added to column and centrifuged at 5000 rpm for 1 min and again the flow-through was discarded. Again 500 ul wash buffer 2 was added and centrifuged at 14000 rpm for 3 min. Flow through was discarded again. Then column was transferred to new micro centrifuge tube and 30 -50 ul Elution buffer or water was added. Tube was left for 2 min to stand and then centrifuged 5000 rpm for 1 min.

2.4. DNA Amplification:

The target DNA was amplified in 20 µl reaction mixture containing 2.2 µl 10 X PCR Buffer, 0.2 mM (1 µl) deoxynucleoside Triphosphate, 25 mM (2.4 µl) $MgCl_2$, 1 µM (1 µl) primers, target DNA 5 µl and 0.5 unit of Taq DNA polymerase. DNA amplification at initial denaturation at 96 $^{\circ}$C for 5 min, 35 cycles of 96 $^{\circ}$C for 30 sec annealing at 65 $^{\circ}$C for *Cryptosporidium* and 57 $^{\circ}$C for *Giardia* and extension at 72 $^{\circ}$C for 60 sec. Primers used for *Cryptosporidium were AWA72F (AGTGCTTAAAGCAGGCAACTG), AWA1235R (CGTTAACGGAATTAACCAGAC)* targeting the 18S rRNA and those used for *Giardia* were *ABB97F (AGGGCTCCGGCATAACTTTCC)* and *ABB220R (GTATCTGTGACCCGTCCGAG)* targeting HSP (Heat Shock Protein) gene. The PCR products were electrophoresed on 2.0% agarose gels containing 0.5mg/ml ethidium bromide in 0.5 x Tris-acetate-EDTA buffer at 120 volts for 15-20 minutes at room temperature. Each well contained 12 µl of PCR product and 2 µl loading dye. Required bands were measured with 12 µl ml of 0.1 µg/µl Fermentas GeneRuler 50 base pairs DNA ladder (#SM0373). Amplicons were visualized under UV light.

2.5. Prevalence Rate:

The prevalence rate of parasites in water samples was determined with the following formula as described by (Ayaz *et al*., 2011).

Prevalence Rate = (No. of parasite detected in water Sample/Total no. of water samples Examined) ×100,

3. Result.

A total of 150 drinking water samples were examined from tap, well and spring sources located at Kohat, Jauzara and Sheikhan Sherkot during the period of May 2011 to October 2011. The prevalence (%) of *Giardia* and *Cryptosporidium,* in each category of water samples was determined using PCR based detection techniques. Bands of 163 bp and 256 bp were identified of *Giardia* and *Cryptosporidium* respectively (Fig 1 & 2).

In the present study, overall prevalence was 12% (18/150), amongst these *Giardia* Spp. and *Cryptosporidium* Spp. were 5.33% (8/150) and 6.66%

(10/150) in tap, well and spring water in Kohat, Jauzara and Sheikhan Sherkot respectively (Table 1).

3.1. By source wise prevalence:

It was observed during the study that *Giardia* was found in all water sources whereas *Cryptosporidium* was not detected in the well water. The presence of *Giardia* in tap water was 6% **(**3/50**),** in spring water 8% (4/50) and in well water 2% (1/50) while that of *Cryptosporidium* in tap water was 6% (3/50), spring water 14% (7/50) (Table 2).

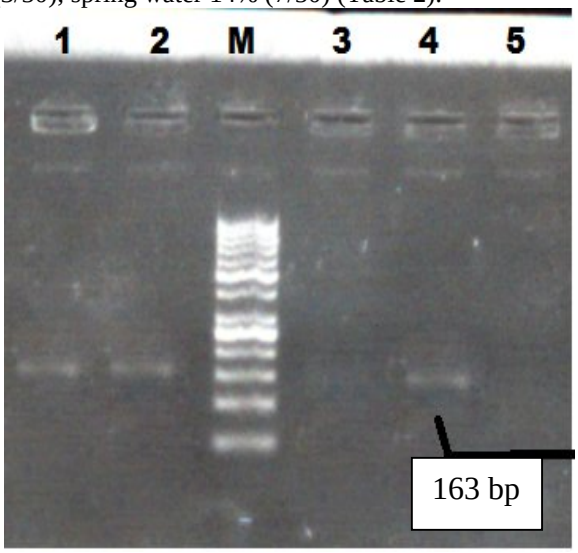

Fig 5: PCR detection of *Giardia* in Environmental water samples. M: Gene marker 50bp. Lane 1 and 2: Positive samples Showing 163 bp band. Lane 3: Negative sample. Lane 4: Positive control. Lane 5: Negative control.

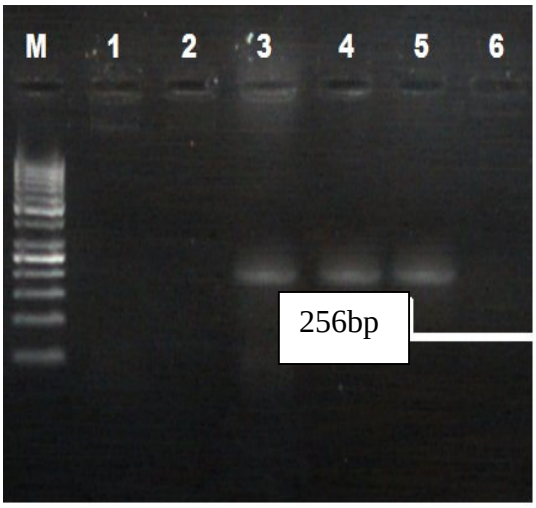

Fig 1. PCR detection of *Cryptosporidium* in environmental water samples.
M: Gene marker 50 bp, Lane 1 and 2: Negative samples. Lane 3 and 4: Positive samples showing 256 bp bandsLane 5: Positive control. Lane 6: Negative control.

3.2. By month wise prevalence:

Water samples were collected during the period of May 2011 to October 2011. It was revealed that in case of *Giardia* highest prevalence was recorded in the month of July i.e. 11.11 % (3/27), followed by June i.e.7.40% (2/27) while lowest prevalence was recorded in the month of October i.e. 0%(0/24). In case of *cryptosporidium* highest prevalence was recorded in the months of June and July i.e. 11.11% (3/27), followed by month of September i.e. 8.33% (2/24) and lowest prevalence was recorded in the month of May i.e. 0% (0/24) (Table 3).

4. Discussion.

In present study a total of 150 drinking water samples were examined from tap, well and springs sources in Kohat during the period of May 2011 to October 2011. In the present study, overall prevalence was 12% (18/150), among these *Giardia* Spp. and *Cryptosporidium* Spp. were 5.33% (8/150) and 6.66% (10/150) respectively. Similar study was conducted microscopically by Ayaz *et al*., 2011, in which they also detect the *Giardia* and *Cryptosporidium* in the drinking water but that study was a preliminary study however it confirmed the finding of our studies.

It was reported from Sri Lanka that the intensity of contamination of water sources was much higher than finding of the present study (WHO, 2004) The differences may be due to the change of environment and geography of the both the country.

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Table # 1 Overall Prevalence of *Giardia* and *Cryptosporidium in Kohat*

Locality	No. of samples tested	*Giardia* +ive samples (%age) a	*Crypto* +ive samples (%age) b	Total (%age) a+b
KDA	50	4(8%)	2(4%)	6(12%)
Jauzara	50	2(4%)	4(8%)	6(12%)
Sheikhan	50	2(4%)	4(8%)	6(12%)
Total	**150**	**8(5.33%)**	**10(6.66%)**	**18(12%)**

Table # 2 Source Wise Prevalence

Parasites.	Tap water	Spring water	Well water	Total
Giardia	3/50 (6%)	4/50 (8%)	1/50 (2%)	**8/150 (5.33%)**
Cryptosporidium	3/50 (6%)	7/50 (14%)	0/50 (0%)	**10/150 (6.66%)**

TABLE # 3 *Giardia* and *Cryptosporidium* Month Wise Prevalence

MONTHS		*Giardia*				*Cryptosporidium*			
		KDA	Jauzra	Sherkot	total	KDA	Jauzra	Sherkot	Total
May	Total	8	8	8	**24**	8	8	8	**24**
	+ive	1	0	0	**1**	0	0	0	**0**
	%age	12.5%	0%	0%	**4.16%**	0%	0%	0%	**0%**
June	Total	9	9	9	**27**	9	9	9	**27**
	+ive	1	0	1	**2**	1	1	1	**3**
	%age	11.1%	0%	11.11%	**7.40%**	11.11%	11.11%	11.11%	**11.11%**
July	Total	9	9	9	**27**	9	9	9	**27**
	+ive	0	2	1	**3**	0	2	1	**3**
	%age	0	22.2%	11.11%	**11.1%**	0%	22.22%	11.11%	**11.11%**
Aug	Total	8	8	8	**24**	8	8	8	**24**
	+ive	1	0	0	**1**	0	0	1	**1**
	%age	12.5%	0%	0%	**4.16%**	0%	0%	12.5%	**4.16%**
Sept	Total	8	8	8	**24**	8	8	8	**24**
	+ive	1	0	0	**1**	1	1	0	**2**
	%age	12.5%	0%	0%	**4.16%**	12.5%	12.5%	0%	**8.33%**
Oct	Total	8	8	8	**24**	8	8	8	**24**
	+ive	0	0	0	**0**	0	0	1	**1**
	%age	0%	0%	0%	0%	0%	0%	12.5%	**4.16%**
GRAND TOTAL		**8/150(5.33%)**				**10/150(6.66%)**			

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