World Applied Sciences Journal 33 (10): 1644-1654, 2015
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2015.33.10.10119

Analysis of Drinking water in District Hangu for the Prevalence of Entamoeba Histolytica, KPK, Pakistan

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Abstract: *Entamoeba histolytica* is an intestinal parasite that causes amoebiasis. About 500 million people are infected by *E. histolytica* and the deaths occurring annually 40,000-110,000 throughout the world. *E.histolytica* is placed in 3rd position as main parasitic cause of death worldwide. The study was conducted in District Hangu, Khyber Pakhtunkhwa for the detection of *E.histolytica* parasite in different locations of drinking water sources. To investigate the presence of *E. histolytica* in different water of District Hangu, a total of 300 water samples were collected from three different water sources including well water, tap water and pond water of District Hangu from 1st September to 30th November 2013. The water samples were processed for the prevalence of *E. histolytica* through microscope and PCR. The water samples were filtered through Whattman filter paper 42 in water filtration assembly and the filtered residues were collected in eppendorf tubes by scratching the filter paper. The filtered residues were centrifuged and stained with Gram iodine for microscopic examination. After that DNA was extracted by NucleoSpin Tissue DNA Extraction Kits. The target DNA was amplified through PCR. In microscopic examination the highest percentage of *E. histolytica* was recorded in pond water 21.82%, followed by 6.43% in tap water while the lowest prevalence rate was recorded in well water 2.86%. The data regarding PCR based detection techniques, the highest prevalence rate was recorded in pond water 32.73% due to the open source to environment, followed by tap water (9.29%) and the lowest prevalence rate was recorded in well water 4.76% because the wells are not covered properly. The high prevalence rate was recorded by PCR based technique because PCR is more specific and sensitive than microscopic technique. From the present study, it was concluded that prevalence of *E. histolytica* was highest in Pond water, while the least prevalence was observed in well water samples and PCR technique for the detection of *E. histolytica* was more accurate and sensitive than microscopy.

Key words: Amoebiasis · Detection Techniques · Water Sources · District Hangu

INTRODUCTION

Water is a main component of all cells and has greatest significant essentials for life. The main sources of drinking water are ground water, spring water, lake water, canal water. Water can be contaminated by various microorganisms like bacteria, fungi and protozoa [1]. Contaminated water causes a large number of water-borne diseases in human and animals [2, 3]. Drinking water quality has always been an important issue in most of the developing countries including Pakistan. In Pakistan due to impurity and microbial contamination and other pollutants, more than half of the population uses impure water for drinking [2, 4].

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Among the water-borne diseases, diarrhoea is the most important, which causes morbidity and mortality in human beings. Poor sanitary and hygienic condition is the main source of diarrheal disease. About 3.5 million people die all over the world of which 3 million children die due to water-borne diseases. In the developing countries, about 98% deaths occur where water-borne outbreaks are prevailing. More than 1.5 million deaths per year are caused only due to diarrheal diseases [5]. Besides other causes of diarrhoea, *Entamoeba histolytica* is considered to be the most common intestinal parasite that causes acute diarrhoea [6]. If attention is not given to the pure drinking water, it is estimated that about 135 million people may die throughout the world from water-borne illnesses in 2020 [7].

Entamoeba histolytica belongs to domain *Eukaryota*, Phylum *Amoebozoa*, Class *Archamoebae*, Order *Amoebida*, Genus *Entamoeba* and Species *E. histolytica*. The genus *Entamoeba* includes six different species, which live in the intestinal lumen, namely *histolytica, dispar*, *moshkovskii, coli, polecki* and *hartmanni* [8]. The first three species are morphologically indistinguishable but under experimental work these show different properties biochemically and genetically [9]. The cysts size of *E. histolytica* is 8-20µm and trophozoites are 20-40µm [10]. *E. histolytica* has a simple life cycle, in which the transmission is via the faecal-oral route. Infection occurs through ingestion of infective cysts or invasion of motile trophozoites leading to dysentery [10].

Symptoms of Amoebiasis: The development of intestinal amoebiasis period takes time from a few days to months or years [11]. Raza *et al.* [12] demonstrated that amoebiasis has two forms, intestinal and extra-intestinal form and the intestinal amoebiasis is sub-divided, into dysenteric and non-dysenteric amoebic colitis. The extra-intestinal amoebiasis is the type that includes the liver, brain, spleen, as well as other organs of the human body. Amoebic liver abscess (ALA) disorders are most commonly observed. Medical appearance of the intestinal infection may include abdominal discomfort, weakness, malaise, constipation that may alternate with diarrhoea, dysentery with the passage of exudates, blood and mucus as well as colicky abdominal pain. General signs of infection include fever, rigors and polymorph nuclear leukocytes.

Chees brough [13] demonstrated that the identification of erythrophagocytic trophozoites in the dysenteric specimens is still considered the basic tools for the diagnosis of amoebic dysentery and the microscopic documentation of *E. histolytica* in stool, mostly iodine stain is used. Koontz and Weinstock, [14] also demonstrated that, for the microscopic documentation of *E. histolytica* different stains could be used to outline the intestinal amoebas by stopping the motility of the trophozoites. The routine diagnosis of amoebic dysentery is still based on the identification of erythrophagocytic trophozoites in dysenteric specimens [15]. After filtration and centrifugation, cyst of *E. histolytica* stained histochemically with eosin exclusion, are detected subsequently by microscopy [16]. *Entamoeba histolytica* can be cultured by taking 200-300µL of pus wound followed by inoculation into the fresh National Institute of Health (NIH) medium and the cultured medium is incubated and checked for the presence of *E. histolytica* motile trophozoites, after 24, 48 and 72 hours [17].

In the serum of patient, parasite can be detected by using anti-amoebic commercial ELISA kit [20]. Other techniques are also used, like microscopy and molecular techniques for the recognition and documentation of the *E. histolytica* [18].

However, PCR based techniques are more suitable for precise detection and differentiation. PCR-based molecular techniques have been established and broadly used to distinguish *Entamoeba* species because of their high sensitivity [19]. PCR is 100 times more sensitive than ELISA.

Currently, so many anti-amoebic medicines are presented in marketplace, in which the 5-nitromidazoles containing metronidazole and tinidazole are the most common products. Some nonimidazoles medicines such as paramomycin, niridazole and nitazoxanide have also be seen that show good action against *E. histolytica*. These medicines show less activity against the cyst while good action against the trophozoites of *E. histolytica*. Diarrhoea, nausea, vomiting and hypersensitivity are the various side effects of these drugs [20].Gill and Beeching [21] stated that luminal amebicides including paramomycin and diloxanide should be used to treat the asymptomatic intestinal infection caused by *E. histolytica*. They also stated that these medications will eliminate the luminal amebae and would avoid further tissue invasion and would stop infection by inhibiting the cysts.

The risk factors of amoebiasis include unawareness, overpopulation, poor and unclean water supplies and unhygienic conditions, toilet habit [22], low socio-economic position [23] and insufficient sanitation practices [24]. The prevalence is also associated with climate, environmental conditions, status and grade of

knowledge [25]. *E. histolytica* is highly endemic throughout poor and socio-economically deprived communities in the tropics and subtropics. Environmental, socio-economic, demographic and hygiene-related behaviour is known to influence the transmission and distribution of intestinal parasitic infections [26].

The prevalence of *E. histolytica* has been recorded 500 millions of asymptomatic diseases and 40,000-110,000 death occurs throughout the world. *E. histolytica* is on 3rd number in the parasitic death throughout the world [27, 31]. *E. histolytica* is a serious health problem for the most part of rising countries [32]. Among children, the intestinal parasitic infection is still a common problem and a major public health problem in Saudi Arabia. El-Sheikh [33] instigated the prevalence of *E. histolytica* in Jeddah (Saudi Arabia), from December 1995–October 1996; he found that 2.2% of 576 children (0–5 years old) are suffering from acute diarrhoea [33]. Harthi [34] reported the prevalence of *E. histolytica*, *E. dispar* and *Giardia lamblia* in Makkah. He noticed that a high prevalence of intestinal parasitic infections was 70.5% among the studied patients. Braiken [35] also demonstrated the prevalence of *E. histolytica* and *E. dispar* in Jeddah at two major public hospitals, at a prevalence of 8.3% in hospitalized patients and 5.9% in non-hospitalized patients. Matthysohion [36] also demonstrated the prevalence of *E. histolytica* and *E. dispar* (25.9%).

In Pakistan, Murtaza [37] reported that the prevalence of *E. histolytica* at a private fertilizer company hospital was 50.9 %. According to that study the prevalence of *E. histolytica* was 28.9 % in female and 22.0 % in male hosts. Jamil[38] also demonstrated that the highest prevalence rate was 26.2 % in age group of 1 day-5 years and 2nd leading prevalence rate was14.3 % in age group of 6-15 years and the 3rd leading prevalence rate was 10.3 % in age group of 16-59 years. The present research was initiated with following objectives; to investigate the prevalence of *E. histolytica* in drinking water sources of District Hangu and compare the microscopy and PCR based techniques for the precise detection of *E. histolytica*.

MATERIALS AND METHODS

The study was carried out in District Hangu Khyber Pakhtunkhwa to detect *Entamoeba histolytica* parasites in drinking water in different localities. The sites from where the water samples were collected were Durri Banda, Kahi, Khazina Banda, Khisari Banda and Zargiri, Naryab and Hangu city area from 1st September to 30th November 2013.

Table 1: Water samples collected from different areas of District Hangu

Location	Well Water	Tap Water	Ponds Water	Total samples
Durri Banda	15	20	15	50
Kahi	15	20	15	50
Khazina Banda	15	20	7	42
Khisari Banda	15	20	6	41
Zargiri	15	20	0	35
Naryab	15	20	6	41
Hangu city area	15	20	6	41
Total	105	140	55	300

Sample Collection: A total of 300 water samples were collected from well water, tap water and pond water. One litre of each water sample was collected in sterilized bottle, labelled (date of collection, name of area and type of water) and transported to the Laboratory of Microbiology Department, Kohat University of Science and Technology Kohat.

Processing of Water Samples: The water samples were filtered through Whattman filter paper in water filtration assembly and the filtered residues were collected in eppendorf tubes by scratching the filter paper.

Microscopy: The water samples which were collected in eppendorf tubes were centrifuged at 12000 rpm for 2minutes. The residues (10 µl) were collected from samples and placed on the slides and thin and thick smears were made and placed in air to get dry. The slides were kept in 95% ethanol for the fixation followed by staining with Gram Iodine. Ten slides were prepared from each sample and stained with Gram iodine for *E. histolytica* detection. A small drop of oil emergent was put on slides and these slides were observed under microscope at 10X, 40X and 100X magnifications.

PCR

DNA Extraction: DNA was extracted by NucleoSpin Tissue DNA Extraction Kits Pakistan with the standard procedure of the manufacturer.

DNA Was Collected and Kept at -20°C

DNA Amplification (PCR): After DNA extraction, the target DNA was amplified in 25µl reaction tubes in a thermal cycler and the PCR reaction was made along with Taq DNA polymerase. The PCR product was amplified by mixing of 2 µl of extracted DNA with 0.5 µl of forward and 0.5 µl reverse primers. The sequence of forward primer of *E. histolytica* EH-1 was GTACAAAATGGCCAATTCATTCAATG while that of

reverse primer EH-2 was TACAAAGTGGCCAA TTTATGTAAGTA. The predictable amplicon size was 439bp and the target gene was small unit of RNA [8].

PCR Cycles Conditions for Entamoeba Histolytica: For an initial denaturation, the sample was heated at 96°C for 2 minutes, followed by 30 cycles of 92°C for 1 minute (denaturation), 56°C for 1 minute (annealing), for extension, the sample was heated at 72°C for 1 minute and 30 seconds and for final extension the sample was heated for 7 minutes at 72°C [8].

Gel Electrophoresis: The agarose gel was prepared by dissolving 0.75 gram of agarose in 50 ml of 1X TBE buffer in flask and boiled for 2 to 3 minutes. Then the flask was kept at room temperature till the temperature of flask come down to 40 45 degree and after that 25 µl Ethidium bromide (1 µg/L) was added and mixed it well. After that fixed the combs in gel tray and poured the gel in to gel tray. Once the gel solidified the combs were removed. The gel tray was placed in gel tank containing 1X TBE buffer. After that 10 µl of PCR product mixture was mixed with 2 µl of loading dye. Then the 12 µl of each sample was loaded in each well and 12 µl of DNA Ladder (500 bp). The gel was run at voltage of 120 volts and 400 Ma current for 25 minutes. Then the gel was examined by UV transilluminator and Gel documentation for picture. The specific DNA amplified product for *E. histolytica* was determined by identifying the 439-bp bands respectively comparing it with 450-bp DNA Ladder which is used as a marker.

Statistical Analysis: The data was analysed statistically by using SPSS.v16.0 and Student T Test value for the determination of p value.

RESULTS

A total of (n=300) water samples were collected from seven different areas of District Hangu for the microscopic and molecular detection of *Entamoeba histolytica.* The water samples were collected from three different sources including well water (105), tap water (140) and pond water (55) as shown in

Microscopic Based Prevalence of *Entamoeba Histolytica* in Different Water Sources of District Hangu

Prevalence of Entamoeba Histolytica in Pond Water: Table 2 shows the microscopic results for the prevalence of *E. histolytica* pond water, with the highest prevalence of 21.82% found in pond water. Regarding the area wise prevalence of *E. histolytica*, the highest i.e., 33.33% prevalence was found in Hangu city area, followed by Durri Banda with 26.67%. The lowest prevalence was observed in pond water samples of Khazina Banda, where it was recorded as 14.29%.

Prevalence of Entamoeba Histolytica in Tap Water: Table 3 shows the microscopic results for the prevalence of *E. histolytica*. in tap water, the highest prevalence was recorded in the samples collected from Durri Banda which was 15.00%, followed by the both Kahi and Khazina Banda with 10.00%. The lowest prevalence was in the samples collected from Zargiri and Hangu city area, which was 5.00%. The overall prevalence of *E. histolytica* was recorded in the tap water samples was 6.43%.

Prevalence of *Entamoeba histolytica* in Well Water: Table 4 shows the microscopic results for the prevalence of *E. histolytica*. The highest prevalence was observed in well water samples from Durri Banda, Kahi and Zargiri at the same prevalence which was 6.67%. While from the rest of the areas, no *E. histolytica* was detected in well water samples. The overall prevalence of *E. histolytica* was recorded in the well water samples was 2.86%.

Overall Prevalence of Entamoeba Histolytica in Water Sources: Table 5 shows the microscopic results for the overall prevalence of *E. histolytica*. Regarding the areas wise prevalence of *E. histolytica* the highest prevalence was found in Durri Banda which was 16.00%, followed by Kahi with 12.00%. The Hangu city area shows prevalence of *E. histolytica* which was 7.31%. The lowest prevalence was in the samples collected from Khazina Banda and Naryab which was recorded as 2.43%. The overall prevalence of *E. histolytica* was recorded in the well water samples with 8.00%.

PCR Based Prevalence of *Entamoeba histolytica* in Different Water Sources of District Hangu

Prevalence of Entamoeba histolytica in Pond Water: Table 6 shows the PCR results for the prevalence of *E. histolytica* of area where the highest prevalence in pond water was 32.73%. Regarding the area wise prevalence of *E. histolytica*, the highest i.e., 50.00% prevalence was found in Khisari Banda, followed by Khazina Banda with 42.86%. The lowest prevalence was observed in pond water samples of Naryab, where it was recorded as 16.67%. The overall prevalence of *E. histolytica* recorded in the pond water samples was 32.73%.

Table 2: Prevalence of *Entamoeba histolytica* in Pond Water of Different Areas of District Hangu by Microscopy

Location	Pond Water	Positive	Percent (%)
Durri Banda	15	4	26.67
Kahi	15	3	20.00
Khazina Banda	7	1	14.29
Khisari Banda	6	1	16.67
Zargiri	0	0	0.00
Naryab	6	1	16.67
Hangu city area	6	2	33.33
Total	55	12	21.82

Table 3: Prevalence of *Entamoeba histolytica* in Tap Water of Different Areas of District Hangu by Microscopy

Location	Tap Water	Positive	Percent (%)
Durri Banda	20	3	15.00
Kahi	20	2	10.00
Khazina Banda	20	2	10.00
Khisari Banda	20	0	0.00
Zargiri	20	1	5.00
Naryab	20	0	0.00
Hangu city area	20	1	5.00
Total	140	9	6.43

Table 4: Prevalence of *Entamoeba histolytica* in Well Water of Different Areas of District Hangu by Microscopy

Location	Well water	Positive	Percent (%)
Durri Banda	15	1	6.67
Kahi	15	1	6.67
Khazina Banda	15	0	0.00
Khisari Banda	15	0	0.00
Zargiri	15	1	6.67
Naryab	15	0	0.00
Hangu city area	15	0	0.00
Total	105	3	2.86

Table 5: Overall Prevalence of *Entamoeba histolytica* in Water Sources of Different Areas of District Hangu by Microscopy

Location	Total samples	Positive	Percent (%)
Durri Banda	50	8	16.00
Kahi	50	6	12.00
Khazina Banda	42	3	7.14
Khisari Banda	41	1	2.43
Zargiri	35	2	5.71
Naryab	41	1	2.43
Hangu city area	41	3	7.31
Total	300	24	8.00

Table 6: Prevalence of *Entamoeba histolytica* in Pond Water of Different Areas of District Hangu by PCR

Location	Pond Water	Positive	Percent (%)
Durri Banda	15	5	33.33
Kahi	15	4	26.67
Khazina Banda	7	3	42.86
Khisari Banda	6	3	50.00
Zargiri	0	0	0.00
Naryab	6	1	16.67
Hangu city area	6	2	33.33
Total	55	18	32.73

Table 7: Prevalence of *Entamoeba histolytica* in Tap Water of Different Areas of District Hangu by PCR

Location	Tap Water	Positive	Percent (%)
Durri Banda	20	4	20.00
Kahi	20	3	15.00
Khazina Banda	20	2	10.00
Khisari Banda	20	0	0.00
Zargiri	20	2	10.00
Naryab	20	0	0.00
Hangu city area	20	2	10.00
Total	140	13	9.29

Table 8: Prevalence of *Entamoeba histolytica* in Well Water of Different Areas of District Hangu by PCR

Location	Well water	Positive	Percent (%)
Durri Banda	15	2	13.33
Kahi	15	2	13.33
Khazina Banda	15	0	0.00
Khisari Banda	15	0	0.00
Zargiri	15	1	6.67
Naryab	15	0	0.00
Hangu city area	15	0	0.00
Total	105	5	4.76

Table 9: Overall Prevalence of *Entamoeba histolytica* in Water sources of Different Areas of District Hangu by PCR

Location	Total samples	Positive	Percent (%)
Durri Banda	50	11	22.00
Kahi	50	9	18.00
Khazina Banda	42	5	11.90
Khisari Banda	41	3	7.31
Zargiri	35	3	8.57
Naryab	41	1	2.43
Hangu city area	41	4	9.75
Total	300	36	12.00

Prevalence of Entamoeba Histolytica in Tap Water: Table 7 shows the PCR results for the prevalence of *E. histolytica*. In tap water, the highest prevalence was recorded in the samples collected from Durri Banda which was 20.00%, followed by the Kahi which showed 15.00%. The lowest prevalence was shown by the water samples of Khazina Banda, Zargiri and Hangu city area, which was 10.00%. The overall prevalence of *E. histolytica* recorded in the tap water samples was 9.29%.

Prevalence of Entamoeba Histolytica in Well Water: Table 8 shows the PCR results for the prevalence of *E. histolytica*. The highest prevalence was observed in both the Durri Banda and Kahi, water samples which was 13.33%, followed by the Zargiri which showed 6.67%. While from the rest of the areas, no *E. histolytica* was detected in well water samples through PCR based detection technique. The overall prevalence of *E. histolytica* recorded in the well water samples was 4.76%.

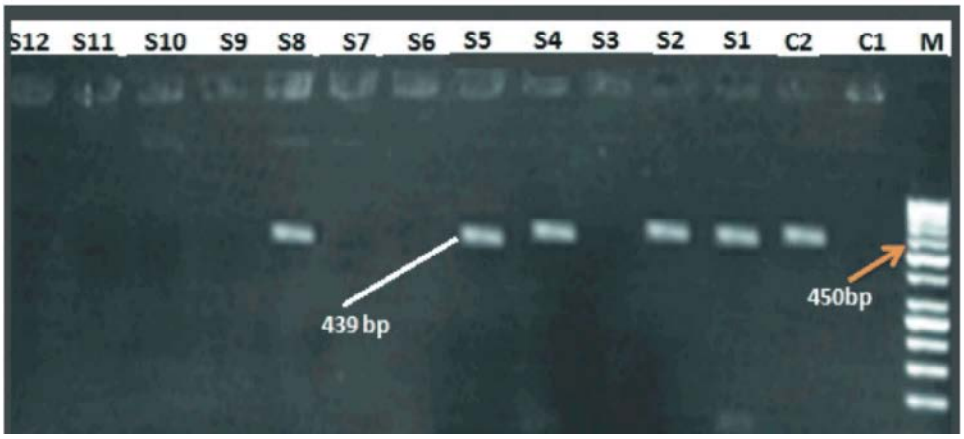

Fig. 1: PCR showing amplified439bp fragment of DNA of *Entamoeba histolytica*

Overall Prevalence of Entamoeba Histolytica in Water Sources: Table 9 shows the PCR results for the prevalence of *E. histolytica*. Regarding the areas wise prevalence of *E. histolytica* the highest prevalence was found in Durri Banda which was 22.00%, followed by Kahi which was 18.00%. The Khazina Banda Hangu city area showed prevalence of 11.90% and the Hangu city area 9.75%. The lowest prevalence was observed in water samples of Naryab which was recorded as 2.43%. The overall prevalence of *E. histolytica* was recorded in the well water samples which was 12.00%.

Comparison of Microscopy and PCR Techniques for the Detection of *Entamoeba histolytica*

Comparison of Microscopy and PCR Results of Pond Water Samples: Table 10 shows the comparison of microscopy and PCR methods for the detection of *E. histolytica* in pond water. The mean prevalence of *E. histolytica* was found in pond water with 21.82%. The highest prevalence of *E. histolytica* was found in Hangu city area 33.33%, followed by Durri Banda with 26.67%. The lowest prevalence was observed in pond water sample of Khazina Banda, where it was recorded as 14.29%. While the PCR results showed that the mean prevalence of *E. histolytica* in pond water was 32.73%. The highest prevalence of *E. histolytica* found in Khisari Banda 50.00%, followed by Khazina Banda with 42.86%. The lowest prevalence was observed in pond water samples of Naryab, where it was recorded as 16.67%.

Comparison of Microscopy and PCR Results of Tap Water Samples: Table 11 shows the comparison of microscopy and PCR methods for the detection of *E. histolytica* in tap water. In tap water, the highest prevalence was recorded in the samples of Durri Banda which was 15.00%, followed by the both Kahi and Khazina Banda with 10.00%. The lowest prevalence was in the samples collected from Zargiri and Hangu city area, which was 5.00%. The mean prevalence of *E. histolytica* was recorded by microscopic based detection technique in the tap water samples was 6.43%. While by the PCR based detection technique, the highest prevalence was recorded in the samples collected from Durri Banda which was 20.00%, followed by the Kahi which showed 15.00%. The lowest prevalence was shown by the water sample of Khazina Banda, Zargiri and Hangu city area, which was 10.00%. The mean prevalence of *E. histolytica* was recorded by PCR method in the tap water samples was 9.29%.

Comparison of Microscopy and PCR Results of Well Water Samples: Table 12 shows the comparison of microscopy and PCR methods for the detection of *E. histolytica* in well water. The highest prevalence was observed in well water samples from Durri Banda, Kahi and Zargiri at the same percentage which was 6.67%. While from the rest of the areas, no *E. histolytica* was detected in well water samples. The mean prevalence of *E. histolytica* recorded by microscopy method in the well water samples was 2.86%. While with PCR based detection technique, the highest prevalence was observed in both the Durri Banda and Kahi, water samples which was 13.33%, followed by the Zargiri which showed 6.67%. While from the rest of the areas, no *E. histolytica* was detected in well water samples through PCR based detection technique. The mean prevalence of *E. histolytica* recorded in the well water samples was 4.76%.

Overall Comparison of Microscopic and PCR Based Techniques: Table 13 shows the overall comparison of microscopy and PCR based detection techniques for the

Table 10: Comparison of Microscopy and PCR Methods for the Detection of *Entamoeba histolytica* in Pond Water

	Comparison (%)	
Location	Microscopy	PCR
Durri Banda	26.67	33.33
Kahi	20.00	26.67
Khazina Banda	14.29	42.86
Khisari Banda	16.67	50.00
Zargiri	0.00	0.00
Naryab	16.67	16.67
Hangu city	33.33	33.33
Mean prevalence (%)	21.82	32.73

Table 11: Comparison of Microscopy and PCR Methods for the Detection of *Entamoeba histolytica* in Tap Water

	Comparison (%)	
Location	Microscopy	PCR
Durri Banda	15.00	20.00
Kahi	10.00	15.00
Khazina Banda	10.00	10.00
Khisari Banda	0.00	0.00
Zargiri	5.00	10.00
Naryab	0.00	0.00
Hangu city	5.00	10.00
Mean prevalence (%)	6.43	9.29

Table 12: Comparison of Microscopy and PCR Methods for the Detection of *Entamoeba histolytica* in Well Water

	Comparison (%)	
Location	Microscopy	PCR
Durri Banda	6.67	13.33
Kahi	6.67	13.33
Khazina Banda	0.00	0.00
Khisari Banda	0.00	0.00
Zargiri	6.67	6.67
Naryab	0.00	0.00
Hangu city	0.00	0.00
Mean prevalence (%)	2.86	4.76

Table 13: Overall Comparison of Microscopic and PCR based techniques for the detection of *Entamoeba histolytica* in Different Areas of District Hangu

	Comparison (%)	
Location (n)	Microscopy	PCR
Durri Banda (50)	16.00	22.00
Kahi (50)	12.00	18.00
Khazina Banda (42)	7.14	11.90
Khisari Banda (41)	2.44	7.31
Zargiri (35)	5.71	8.57
Naryab (41)	2.43	2.43
Hangu city (41)	7.32	9.75
Mean prevalence (300)	8.00	12.00

detection of *E. histolytica*. Analysis of the data recorded that the mean prevalence 12.00% in PCR based detection was greater than microscopic method of detection, which was observed as 8.00%. For the individual location, PCR showed greater prevalence as compared to microscopic for the sampled areas except Naryab, where both the techniques showed same prevalence 2.43%.

Overall Comparison of Microscopy and PCR in Different Water Sources: In the current study the microscopic and PCR techniques were compared with each other which showed that the PCR was more sensitive as compared to microscopy as shown in Table 14. In the pond water highest prevalence of *E. histolytica* 50% was detected by PCR method while through microscopy the prevalence of *E. histolytica* was 16.67% in Khisari Banda. In tap water the prevalence of *E. histolytica* was 20% followed by 15% in Durri Banda and Kahi respectively by means of PCR and 15 and 10% by means of microscopic examination. The lowest prevalence of *E. histolytica* was 6.67% observed in the Well water of Zargiri by both techniques i.e., PCR and Microscopy. Similarly the prevalence of *E. histolytica* was observed 13.33% in Well water of Durri Banda and Kahi while by microscopic examination the result showed 6.67% in the both area.

Table 14: Overall Comparison of Microscopy and PCR in Different Water Sources of Different Areas in District Hangu

	Pond water		Tap water		Well water	
Location	Microscopy (%)	PCR (%)	Microscopy (%)	PCR (%)	Microscopy (%)	PCR(%)
Durri Banda (50)	26.67	33.33	15.00	20.00	6.67	13.33
Kahi (50)	20.00	26.67	10.00	15.00	6.67	13.33
Khazina Banda (42)	14.29	42.86	10.00	10.00	0.00	0.00
Khisari Banda (41)	16.67	50.00	0.00	0.00	0.00	0.00
Zargiri (35)	0.00	0.00	5.00	10.00	6.67	6.67
Naryab (41)	16.67	16.67	0.00	0.00	0.00	0.00
Hangu city area (41)	33.33	33.33	5.00	10.00	0.00	0.00
Total prevalence (Source wise)	21.82	32.73	6.43	9.29	2.86	4.76

DISCUSSION

Prevalence of intestinal parasites in a population is generally related to the level of poverty, type of living conditions, personal and environmental hygiene, inadequacy of health services, poor sanitation and unavailability of clean water supply [39].

It is possible that negative results may be due to the small amounts of *Entamoeba histolytica* DNA that are lower than the detectable level of the assay, due to problem in handling or it is also possible that these samples may not contain *E. histolytica* they belonged to other *Entamoeba* species. Microscopic examination was not enough to detect low level of infection but amplification of DNA can reveal it. So this study was entirely based on the detection of *E. histolytica* through the PCR diagnosis.

In the present study regarding the microscopic observation, the highest prevalence rate was found in the pond water which was 21.82%, followed by the tap water which was 6.43% and well water showed 2.86%. The overall prevalence rate through microscopic techniques was found as 8%.

By using PCR as detective tool, the highest prevalence observed in the pond water i.e. 32.73%, while the lowest prevalence was recorded in well water (4.76%). The overall prevalence rate found through PCR techniques was 12%.This study revealed that PCR based detection technique was found more precise and accurate for the diagnosis of *E. histolytic* as compared to the microscopy.

[40-41] conducted study on the prevalence on *E. histolytica* through microscopy. It was found that 9.80% samples were positive, which is closely related to the current study. In our study, 8% samples were found positive in microscopic detection. Similarly, Ayaz [42] recorded 14.4% prevalence rate through microscopy in their study conducted in Kohat division, Pakistan. In our study, 8% samples were found positive in microscopic detection. Nyenke [43], recorded 11% prevalence rate of *E. histolytica* through microscopic observation, which is slightly higher.

Ejazet [44] recorded 52% prevalence rate of *E. histolytica* in fecal samples, through microscopic technique. This value was too high as compared to our current study because they were directly related to fecal samples. Another study was conducted in urban and rural areas of Iran, out of 16,592 stool samples 226 were positive bearing 1.36% of the total samples for *E. histolytica* through microscopy [21].

Ejazet [44] conducted a study for the prevalence of *Entamoeba histolytica*, a total of 100 stool specimens from the patients were diagnosed through PCR based detection techniques, where (26 %) were positive for *E. histolytica*. This value was too high as compared to our current study which was (12%) because it was directly related to fecal samples.

Gonin and Trudel [45] also conducted a similar study. There were 95 samples tested for the detection of *E. histolytica* in which 68 (71.57%) were positive through PCR from patients. In contrast to our study this value was too high as compared to the current study which was (12%) because it was directly related to fecal samples.

Another similar study was conducted in Iran, in which 35 out of 116 (30.2%) water samples were contaminated in the rural areas through microscopy [46]. This value was too high as compared to the current study which was 8% by microscopic detection.

Another study was conducted in urban and rural areas of Iran, out of 16,592 stool samples 226 were positive bearing 1.36% of the total samples for *E. histolytica* through microscopy [47], which was too much low than our results 8%.

Noor [48] also carried out work on the prevalence of *E. histolytica* in different areas of District Peshawar. In their study, overall prevalence of *E. histolytica* was 11.33%. The tube well water showed 3.57% followed by bore well showed 2.73% and tap water showed 14.41% which were very close to our findings. In the current study, overall prevalence was 12%. The ponds water showed 32.73% followed by 9.29% and4.76% in tap water and well water respectively.

Outcomes in this study, point out that water is a probable cause for transmission of *Entamoeba* to human host. Cysts can survive for long time in the external environment and are responsible for transmission because of the protection by their cell wall. It is possible that negative results may be due to the small amount of *Entamoeba* DNA that is lower than the detectable level of the assay or they belonged to other *Entamoeba* species. The present results can be helpful in the prevention and supervision of amoebiasis. Further, PCR based detection method is crested among the local people to avoid the use of pond water, also suggest the authority to provide pure or treated water to the residents of district Hangu.

CONCLUSIONS

From our findings, it was concluded that PCR techniques for the detection of *Entamoeba histolytica* was more accurate and sensitive than microscopy.

Regarding sources, prevalent of *E. histolytica* was highest in Pond water, while the least prevalence was observed in well water samples. Durri Banda showed the highest prevalence of *E. histolytica* among all study areas.

Following are important suggestions in order to keep *E. histolytica* prevalence rate at low level.

- PCR based technique is recommended for the precise detection of *E. histolytica*.
- The local people may be educated to boil the pond water.
- It is also suggested that water filtration plants may be installed at different locations.

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