Original Article

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Prevalence of *Entamoeba Histolytica* in Stool Samples of Diarrheal Patients

Muhammad Barkhurdar, Sadullah Jan, Zunera Tanveer, Nargis Haider Kakar, Bushra Shaheen, Muhammad Shahzad Farooq, Muhammad Yousaf, Abdul Samad, Asad Ullah

ABSTRACT

Objective: The aim of this study was to determine the prevalence of *Entamoeba histolytica* in patients suffering from diarrhoea in Quetta. **Study Design:** Microscopic examination of stool samples and analysis through the formalin ethyl-acetate sedimentation technique. **Duration:** Six months (from 13-2-2017 to 21-7-2017). **Methodology:** A total of 235 stool samples randomly collected from females, males and children having abdominal discomfort who visited outpatient departments (OPDs) in different hospitals of Quetta. **Results:** Out of a total of 235 patients, *E. histolytica* was found in 45 (19.1%) patients (males = 55.6% and females = 44.4%). **Conclusion:** Infections of *E. histolytica* can be controlled by protecting food from flies, use of boiled drinking water, proper washing of fresh vegetables, hygienic conditions and a well-established sanitation system. It has been concluded that the use of animal compost for fertilizer, cleansing of vegetables with water contaminated by animal faeces, and a poor sewage system can enhance the risks of infections caused by this parasite.

Keywords: *Entamoeba histolytica*, Faecal samples, Microscopy, Prevalence in Quetta.

Corresponding Author

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DR. MUHAMMAD SHAHZAD FAROOQ, Professor of Biochemistry, Faisalabad Medical University, Faisalabad-Pakistan.
Contact / Email: +92 321-9147488, shehzadfarooq38@yahoo.com

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INTRODUCTION

Gastrointestinal infections caused by parasites prevail all over the world. There is high incidence of these infections in overcrowded regions with little income resources, a contaminated environment, improper waste disposal, a lack of clean water and an unhygienic lifestyle resulting in illness and mortality.[1,2]

Infectious diseases are caused by bacteria, viruses and parasites. *Entamoeba histolytica* (*E. histolytica*) is one of the most important protozoan parasites that causes intestinal amoebiasis. *Histolytica* is a Latin word, "Histo" means tissue and "Lytica" means destruction.[3] Infection is asymptomatic, and clinical indications include amoebic dysentery and extra intestinal diseases.[4] Morphologically *E. histolytica* has two forms, i.e. cyst and trophozoite. Infectious cysts are ingested by eating contaminated food or contaminated drinking water and settle in the colon, every cyst produces eight trophozoites. Sometimes infection is not self-limiting, leading to amoebic dysentery and liver abscesses. Ninety percent of infections with *E. histolytica* are asymptomatic and self-limiting.[5] According to World Health Organization (WHO) reports, every year approximately 50 million cases of invasive infection are caused by *E. histolytica*. *Entamoeba* is 3rd among the parasites which are responsible for 100,000 deaths annually.[6] Infections of the intestine caused by protozoan parasites are closely related to contaminated water or contaminated food or unhygienic conditions. The prevalence rate is therefore higher in developing countries.[7] The WHO has estimated that currently around 3.5 billion people are infected with intestinal parasites and 450 million people suffer from the results of those infections, including abdominal discomfort and diarrhea.[8] *E. histolytica* is a protozoan parasite, distributed worldwide and the second leading agent causing death. Nearly 500 million people are infected with *E. histolytica* per year which causes liver inflammation and inflammation of the inner lining of the colon in 0.05 billion and results in the loss of an expected $40x10^3$ and $100x10^3$ people every year. [9,10]

E. histolytica can also cause liver abscesses, but their occurrence is less than 5%. Mostly extra intestinal infections found in Mexico, Indonesia, Subcontinent, sub-Saharan and hot countries of Africa and some regions of South and Central America are caused by *E. histolytica*.[11] More common infections are found in subtropical, hot zones such as Mexico, South America, South Asia and Western Africa.[12]

METHODOLOGY

Study Design: Cross sectional study.
Settings: Outpatient departments (OPDs) of different hospitals within Quetta city Pakistan.
Duration: February to July, 2017
Sample Technique: Non-probability consecutive sampling.
Sample Size: 235 fresh stool specimens.
Specimen Collection: Samples were collected from patients evident of abdominal pain and diarrhoea. A total of 235 fresh stool specimens were collected from the patients of different age groups and gender into sterile disposable plastic containers. Samples were immediately transported to the parasitology lab of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan (UoB), Quetta. Samples were processed using the formalin-ethyl

acetate sedimentation method to separate the parasites from debris. This technique is reported to be sensitive for the detection of parasites when these are in small numbers.[13]

Microscopic Identification of Parasites: Samples were processed immediately for microscopy without preservation. Direct wet films were prepared for each sample using normal saline (0.85%) for the detection of active motile trophozoites while Lugol's iodine (5%) was used for cysts in order to clarify their organelles.

Microscopy was done at 10× and 40× magnification to identify trophozoites and cysts of *E. histolytica*. Each sample was checked microscopically in triplicate. The following criteria were used to declare a sample positive for *E. histolytica*. Cysts were showed the appearance of four or a lesser number of nuclei, while trophozoites were identified as having ingested RBCs.

RESULTS

A total of 235 patients from OPDs of different hospitals in Quetta presenting abdominal discomfort and diarrhoea were examined for *E. histolytica* infection. Out of these 235 samples, 45 (19.1%) were positive, of which 25 (55.6%) were male patients while 20 (44.4%) were female patients (Table 1 and Figure 1).

Table 1: Prevalence of E. Histolytica among genders

Total samples	Total infected %	Male infected %	Female infected %
235	45 (19.1%)	25(55.6%)	20 (44.4%)

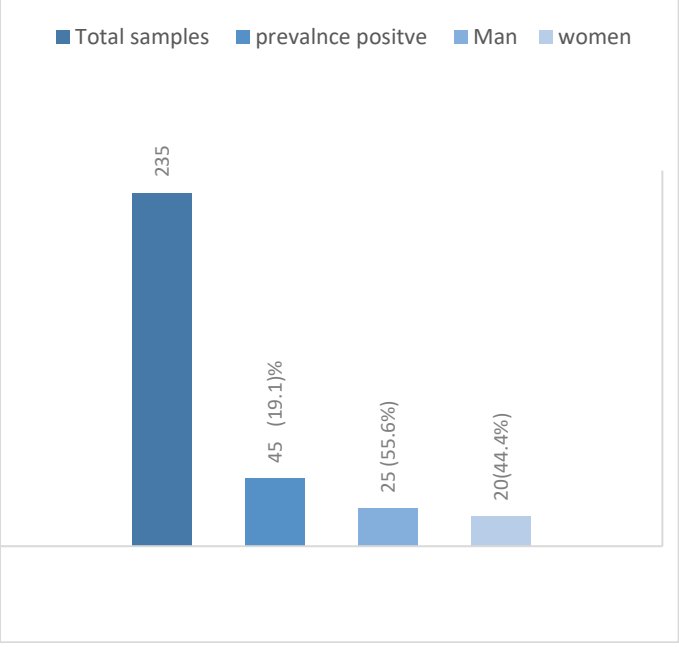

Figure 1: Gender wise prevalence of E. Histolytica

E. histolytica prevalence was also checked on a month wise basis which was observed to be highest in the month of July (30%) and lowest in February (12.5%). During the months of March, April, May and June, the prevalence rate was 14.3%, 16.7%, 17.8% and 21.9%, respectively (Table 2).

Table 2: Monthly prevalence of Entamoeba Histolytica

Month	Samples collected	Infected
February	32	4 (12.5%)
March	35	5 (14.3%)
April	42	7 (16.7.5%)
May	45	8 (17.8%)
June	41	9 (21.9 %)
July	40	12 (30%)
Total	235	45 (19.1 %)

The reason for high prevalence in July could be the warm weather and lower prevalence in February could be due to the lower temperature.

Table 3: Prevalence of E. Histolytica among various age groups

Age (years)	Total Samples	Infected	Percentage
0-5	38	08	21.05 %
6-10	50	13	26 %
11-20	54	10	18.51 %
21-40	50	06	12%
>40	43	08	18.6%

The age wise prevalence of *E. histolytica* was higher in the 6-10 years age group (26%) and lower in the 21-40 years age group (12%). In the 0-5 years age group the prevalence of *E. histolytica* was 21.05%, in the 11-20 years age group it was 18.51% and in the >40 years age group a 18.6% prevalence was found (Table 3 and Figure 2).

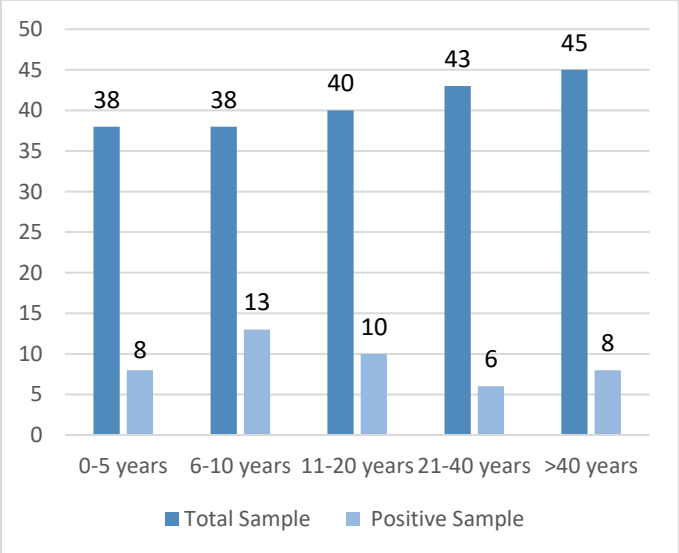

Figure 2: Age wise prevalence of E. Histolytica

DISCUSSION

The prevalence of intestinal parasites in a population is generally related to the level of poverty, type of living conditions, personal and environmental hygiene, adequacy of health services, sanitation and availability of clean water supply. Gastrointestinal infections caused by parasites are found all over the world, mostly high prevalent rates are found in those humans that are living in congested regions with little income resources, contaminated environment, improper waste disposal, unsecure water supply and a filthy personal lifestyle.[14] In our study, a total of 235 stool sample were collected from gastrointestinal patients having abdominal discomfort, dysentery and fever. Out of the 235 samples the prevalence rate was 45 (19.1%) identified microscopically.

In a study conducted by Kaur *et al*. (2002) the prevalence of *E. histolytica* was 11% in school going children. The same result was reported by Blessmann *et al*. (2003) where 11.2% out of 383 randomly collected samples from endemic zones of amoebiasis in Vietnam were positive for *E. histolytica*.[15, 16] A study published in the Biomedical Journal by Hussain *et al*. (1997) reported that in the Northern areas of Pakistan the prevalence rate was 8%.[17] A study by Chaudhry *et al*. (2004) in Pakistan reported a 5.95% occurrence of *E. histolytica*.[18] The prevalence rate of *E. histolytica* is higher in present study as compared to the other studies mentioned above. This higher prevalence rate could be due to a number of risk factors such as overcrowded population areas, poverty, untreated water, unhygienic food, raw vegetables and a poor sanitary system.

A study by Tasawar *et al*. (2010) in Multan showed a 21.7% prevalence rate of *E. histolytica* while another study by Mehmood *et al*. (2009) conducted in Vehari revealed a 20.2% occurrence of this parasite.[19, 20]

A study by Okafor & Azubike (1991) from rural areas of Nigeria reported 22.7% and 19.7% prevalence's of *E. histolytica* in males and females respectively,[21] which is to some extent comparable to the findings of our study.

In contrast to the findings of our present study, other reasons are males have more outdoor works in villages and are exposed to the more unhygienic environment. A study from Pakistan by Ejaz *et al*. (2010) reported prevalence's of 31.5% and 19.6% for *E. histolytica* in females and males, respectively,[22]

According to the outcomes of the current study, the prevalence rate of *E. histolytica* in the age group of 6-10 years was 26% and in the 0-5 years age group it was 21.05%. Lower prevalence's were found in age groups of 11-20 years and 40 years of 18.51% and 18.6%, respectively. In contrast to findings of this study a higher incidence rate of up to 41% among the age group of 6-14 years was reported by Subbannayya *et al*. (1989).[23] Noor Azian *et al*. (2007) reported a 52% occurrence of *E. histolytica* in the age group of 19 years.[2] Children are at higher risk of infection as compared to adults due to a weaker immune system and lack of personal hygiene.

In the present study the highest prevalence of *E. histolytica* was observed during the month of July (27.6%), whilst it was lowest (16.9%) in the month of February. These observations are comparable to the findings of study by Natividad *et al*. (2008), who reported higher prevalence in the months of May and July.[24] A higher occurrence of this parasite during warmer months of the year makes sense because there are optimum conditions of temperature, salinity and humidity for the growth of microbes.

CONCLUSION

These results demonstrate that *E. histolytica* can cause enteric infections when it finds favourable environmental (particularly poor sanitation) and host conditions. It is concluded that *E. histolytica* infection is the most dominant intestinal parasitic problem and serve as the main source of infections among humans. It is now time to develop an effective vaccine and management system against *E. histolytica* for controlling these infections.

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CONFLICT OF INTEREST

There is no conflict of interest involved.

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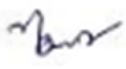
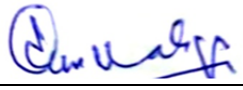
AUTHORSHIP AND CONTRIBUTION DECLARATION

AUTHORS	Contribution to The Paper	Signatures
Dr. Muhammad Barkhurdar M. Phil Scholar, Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta Pakistan	Perform Experimental Work, Data Collection, Paper Write up	
Dr. Sadullah Jan Associate Professor, Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta Pakistan	Conceptualized and Supervised the research	
Dr. Zunera Tanveer Demonstrator, Department of Physiology, Bolan University of Medical and Health Sciences, Quetta Pakistan	Data Analysis and Compiled the paper	
Dr. Nargis Haider Kakar Assistant Professor, Physiology Bolan University of Medical & Health Sciences, Quetta Pakistan	Assist in Research work and Data analysis	
Dr. Bushra Shaheen Associate Professor, Biochemistry Independent Medical College, Faisalabad Pakistan	Experimentation, Review the discussion	
Prof. Dr. Muhammad Shahzad Farooq Professor of Biochemistry, Faisalabad Medical University, Faisalabad Pakistan	Review the paper	
Dr. Muhammad Yousaf M. Phil Scholar, at Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta Pakistan	Result analysis Reference writing	
Dr. Abdul Samad Assistant Professor, at Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta Pakistan	Reference write up	
Dr. Asad Ullah Assistant Professor, at Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta Pakistan	Review of literature