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Full Length Research Paper

Determination of the prevalence of *Entamoeba histolytica* in human at a private fertilizer company hospital in Pakistan using microscopic technique

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Amoebiasis is still a big problem of human civilization at the beginning of 21st century, so every study from this field is valuable. The present study elaborated the prevalence of *Entamoeba histolytica* in Pakistani human. During the study, 4712 fecal samples of human were studied by using microscope from October 2008 to January 2010 at a private fertilizer company hospital, Engro Fertilizers, Pakistan. Out of 4712 hosts, 52.1% were infected with *E. histolytica*. The relationship between sex and *E. histolytica* in human was calculated. According to these results, the infection of *E. histolytica* was more prevalent (31.5%) in female hosts as compared (19.6%) in male hosts. The relationship between age and *E. histolytica* in human was also determined. According to these results, *E. histolytica* had highest prevalence (28.7%) in age group of 1 day - 5 years, 13.9 % in age group of 6 - 15 years and the infection was 12.1% in age group of 16 - 59 years. High prevalence of *E. histolytica* in females, young ones and older people is due to low immunity, low level of hygiene, poor sanitary conditions and other socio-economical factors.

Key words: *Entamoeba histolytica*, prevalence, sex, age, microscopic analysis.

INTRODUCTION

Amoebiasis is still a big problem of human civilization at the beginning of 21st century, so every study from this field is valuable. Amoebiasis is caused by *Entamoeba histolytica*, a parasitic protozoan, which infects predominately human and other primates (Lebbad and Svard, 2005) . Approximately 40 million people, world wide suffered from this disease per year and 40,000 died due to dysentery, intestinal diseases and liver abcess. About 5% of the US population is affected with this problem (Al-Harthi, 2004; Haq et al., 2006). Human are the definitive hosts. There is indistinguishable specie, *Entamoeba*. *dispar*, which is not usually, if ever, distinguished clinically. Therefore, the majority of the quoted prevalence, especially in the US, may actually be attributable to this benign species rather than *E. histolytica*.

Other mammals such as dogs and cats can become infected but usually do not shed cysts with their feces and thus do not contribute significantly to transmission. The transmission of amoebiasis occurs by fecal contamination of various edibles and by direct contact with dirty hands or objects as well as by fecal exposure during sexual contacts. Theoretically, the ingestion of one viable cyst

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is an infectious dose. Housefly and the use of the human feces as fertilizer is an important mode of spread. The infection is not uncommon in the tropic and Polar Regions, but also occurs in other crowded situations of poor hygiene in temperate urban environments. The most dramatic incident in the USA was the Chicago world's fair outbreak in 1933 caused by contaminated drinking water (Tanyuksel and Petri, 2003) . Defective plumbing permitted sewage to contaminate the drinking water.

Recently, food handlers are suspected of causing many scattered infections, but there has been no single large outbreak (Chintu et al., 1995). All people are believed to be susceptible to infection, but individuals with a damaged or undeveloped immunity may suffer more severe forms of the disease. The homosexuals and the citizens of industrialized countries are mainly at risk of this disease (Hart et al., 1990). Although some work has been done in various parts of the world, no published report is found in Pakistan. So keeping in view the importance of this parasite, the project was designed with following aims: (1) To study the overall prevalence of *E. histolytica* in human.

(2) To study the relationship between sex and *E. histolytica* in human. (3) To study the relationship between age and *E. histolytica* in human.

The tested individuals belong to various levels of life and commune conditions of a well known textile city of Pakistan with two major pollutions: air and under-ground water pollutions (Haq et al., 2006). Actually this city is a mixture of many rural territories with a main crowded city with bad sanitary and water supply conditions except some modern housing areas. The cattle are also kept in residential premises that are big source of infections. Here, textile industry job oriented persons are usually male however almost 15 - 20% female community is also concerned with this profession. About 10% tested individuals belong to a well educated community who generally have clean life-style, approximately 30% are casually educated with moderately clean environment and remaining 60% are illiterate people provided with least sanitary conditions.

MATERIALS AND METHODS

The present study was conducted from October 2008 to 2009 in order to collect information on the overall and monthly prevalence of *E. histolytica* in humans and relationship between age, sex and *E. histolytica* in human. A total of 4712 fecal samples, were collected from humans. The collected samples were transferred to bottles containing 5% formalin. The specimen bottles were labeled with host name, age and sex. Temporary mounts were made in order to diagnose the parasite (Cable et al., 1985).

Statistical analysis

The results were tested statistically by one way ANOVA at 0.01 level of significance using SPSS version 13.0.

Preparation of temporary mounts of fecal samples

A small drop of preserved fecal material was mixed with a small drop of staining agent that is, iodine solution and studied using microscope. The fecal samples were preserved in normal saline solution till identification.

Composition of normal saline and iodine solution

Normal solution

Sodium chloride = 8.5 g; Distilled water = quantity sufficient to make volume up to 1000 ml.

Iodine solution

Iodine crystals = 5 g; Potassium iodide = 10 g; Distilled water = quantity sufficient to make volume up to 1000 ml. Potassium iodide and iodine were dissolved in water and then filtered into a brown bottle and stored in the dark.

RESULTS

The results of microscopy for the fecal parasite are summarized in Tables 1 - 3. During this study, 4712 fecal samples of human were examined for the parasite *E. histolytica* and different parameters studied. The overall prevalence of *E. histolytica* was calculated (Table 1). The relationship between sex and *E. histolytica* in human was calculated (Table 2). According to these results, the infection of *E. histolytica* was significantly ($p < 0.01$) more prevalent (31.5%) in female hosts as compared (19.6%) in male hosts. The relationship between age and *E. histolytica* in human was also determined (Table 3). According to these results, *E. histolytica* had significantly ($p < 0.01$) highest prevalence (28.7%) in age group of 1 day - 5 years, 13.9 % in age group of 6 - 15 years and the infection was 12.1% in age group of 16 - 59 years.

DISCUSSION

In Pakistan, the morbidity and mortality of amebiasis is still a health problem in recent years. Nevertheless, it is elaborated that prevalence in rural people is higher than in urban areas; amebiasis often influences people of low socioeconomic status that favors fecal- oral transmission. The classical method for diagnosis has been microscopy, although that test does not discriminate between *E. histolytica* and *E. dispar* (Lebbad and Svard, 2005).

In the present study, 3317 fecal samples of human were examined for the parasite *Entamoeba histolytica* and the parameters studied are given below.

The overall prevalence of *Entamoeba histolytica* in human at a private fertilizer company hospital in Pakistan

The overall prevalence of *E. histolytica* was calculated

Table 1. The overall prevalence of *Entamoeba histolytica* in human at a private fertilizer company hospital, Engro Fertilizers, Pakistan.

Name of parasite	No. of hosts examined	No. of hosts infected	Prevalence (%)
Entamoeba histolytica	4712	2455	52.1

Table 2. Relationship between sex and *Entamoeba histolytica* in human at a private fertilizer company hospital, Engro Fertilizers, Pakistan.

Name of parasite	Male			Female		
	Examined	Infected	Prevalence (%)	Examined	Infected	Prevalence (%)
Entamoeba histolytica	2176	427	19.6	2536	799	31.5

Table 3. Relationship between age and *Entamoeba histolytica* in human at a private fertilizer company hospital, Engro Fertilizers, Pakistan.

Name of parasite	No. of hosts examined	Age of the host observed		
		1 day to 5 years	6 years to 15 years	16 years to 59 years
Entamoeba histolytica	4712	n=1353 (28.7 %)	n=655 (13.9 %)	n=570 (12.1 %)

(Table 1). According to these results, the overall prevalence of *E. histolytica* was 52.1%. Literature shows various studies on prevalence of *E. histolytica* in different parts of the world. Gonzalez et al. (1995) studied prevalence of *E. histolytica* in human of Mexico by using ELISA's technique. He examined 60,538 patients. The overall prevalence of *E. histolytica* was 4.49%. Oyerinde et al. (1998) studied the metropolitian logos. Total 2,825 people were studied and the overall prevalence of *E. histolytica* was 11.2%. River et al. (2000) studied prevalence of *E. histolytica* in the people of Phillipine. Total 1,872 human cases were examined. Out of which, 18 stools were infected and overall prevalence of *E. histolytica* was 0.961%. Nimri and Meqdam (2003) obtained fecal samples from Taiwan. Total 1,569 samples were examined and overall prevalence of *E. histolytica* was 8.2%.

In the present and other studies, the high prevalence of *E. histolytica* in human suggest the wide spread contamination of environment with cysts of the parasite. Amoebiasis is transmitted by fecal contamination of drinking water and foods and by direct contact with dirty hands or objects as well as by fecal exposure during sexual contact in which case not only cysts, but also trophozoites are infectives. Theoretically, the ingestion of one viable cyst is an infectious dose. Houseflies spread the cysts and the use of human feces as fertilizer on fruit and vegetable crops is an important mechanism of spread. The infection occurs in crowded situations of poor hygiene in temperate urban environments. Food handlers are suspected of causing many scattered infections (Chintu et al., 1995).

Relationship between sex and *Entamoeba histolytica* in human at a private fertilizer company hospital in Pakistan

The relationship between sex and *E. histolytica* in human was calculated (Table 2). According to these results, the infection of *E. histolytica* was significantly ($p < 0.01$) more prevalent (31.5%) in female hosts as compared (19.6%) in male hosts. Kim et al. (1997) reported *E. histolytica* in human from Korea. Total 2,247 people were studied. Out of which, 1,101 males and 1,146 were females. Cross et al. (1975) studied *E. histolytica* in human from Java and Indonesia. Total 695 people were studied. Out of which, 439 were males and 256 were females. The prevalence of *E. histolytica* was higher in females (2.7%) as compared to males (1.6%). Ohnishi and Murata (1997) studied prevalence of *E. histolytica* in the area of Tokyo. Total 28 study cases were proposed. Out of which, 26 were males and zero percent females were infected. Nimri and Meqdam (2003) studied 1,569 human fecal samples in Taiwan. In this study, the prevalence of *E. histolytica* was higher in females (11.75%) as compared to males (6.9%).

The results of the present study resemble to that of Kim et al. (1997) and Nimri and Meqdam (2003). While two other studies conducted by Cross et al. (1975) and Ohnishi and Murata (1997) do not support present results. The low immunity of females may account for higher prevalence of *E. histolytica* in this sex group. As already mentioned, the tested individuals belong to rural polluted areas where major job of females is to carry out following jobs: house work, cattle cultivation and dusting

of their full homes including wash rooms, sleeping rooms, lawns and occasionally the relevant streets. These females rarely feel any need after exposure to such activity. All these job responsibilities expose these females to garbage and contamination due to which they retain various pathogens with their body and become their victim. These persons are very courageous and do not take serious action to treat such problem. When the disease affects seriously their health, then they go to physician for medication. Additionally, they do not take proper medication for proper period. They quit the use of medicines as soon as they feel the end of disease symptoms. Whereas many medicines demand their use for longer period even after the vanishing of disease symptoms e.g. the use antibiotic is instructed for 7 - 14 days which causes a reduction in immunity in such people.

Relationship between age and *Entamoeba histolytica* in human at a private fertilizer company hospital in Pakistan

The relationship between age and *E. histolytica* in human was also determined (Table 3). According to these results, *E. histolytica* had significantly ($p < 0.01$) highest prevalence (28.7%) in age group of 1 day-5 years, 13.9% in age group of 6 - 15 years and the infection was 12.1% in age group of 16 - 59 years. Haque and Petri (2000) studied the prevalence of *E. histolytica* in Dhaka, Bangladesh. The high prevalence was 5% in age group of 2 - 5 years children age group. Lucia et al. (2001) detected *E. histolytica* using ELISA based antigens in the people of Brazil. Total 735 peoples were studied. The high prevalence of *E. histolytica* was 14.9% in age group of 1 - 5 years group. Astal (2004) proposed prevalence of *E. histolytica* in Khan Younis, Government Hospital. Total 1,370 peoples were studied. The high prevalence of *E. histolytica* was 34.2% in age group of 6 - 11 years children.

The reason of this high prevalence of *E. histolytica* in age group of 1 day - 5 years and older people may be due to the low immunity against various pathogens as these age groups are comparatively less resistant to diseases as described in a previous study (Haq et al., 2006). All young children are mostly susceptible to infections due to immaturities in their immune mechanisms and a lack of prior antigen exposure which results in a high frequency of infections and more severe infections than youth ages. Inherited primary deficiencies of the immune system may result not only in infections with common pathogens, but also atypical infections with opportunistic organisms (Davies, 2006). Additionally, the children feel free to play any where irrespective of the cleanliness or dustiness due to the absence of separate play grounds. The playing areas are main sources of diseases because waste materials of homes and industries are thrown there. Moreover, the sale of adulterated and impure edibles also affects the health adversely of young ones as there is least observance of healthy policies and laws in the country due to irrational sale of impure products/low quality products.

Conclusion

The results indicate that the parasite is more prevalent in female, younger and eldest hosts as compared to middle-age hosts. The high prevalence of this parasite could be due to low resistance of hosts in the present study, moreover due to low level of hygiene and poor sanitary conditions.

REFERENCES

Al-Harthi SA (2004). Prevalence of intestinal parasites in school children in Makkah, Saudi Arabia. The New Egypt. J. Med., 31: 37-43.

Astal Z (2004). Epidemiological survey of prevalence of parasites among children in Khan Younis Government Hospital, Palestine. Parasitol. Res., 10: 449-451.

Cable RM (1985). An illustrated laboratory manual of parasitology. J. Parasitol., 5: 269-275.

Chintu C, Luo C, Khumalo-Ngwenga B (1995). Intestinal parasites in HIV seropositive Zambian children with diarrhea. J. Trop. Ped., 41: 149-152.

Cross JH, Irving GS, Cunawan S (1975). The prevalence of *Entamoeba histolytica* and Toxoplasma gondii antibodies in Central Java, Indonesia. J. southeast Asian Trop. Med. Pub. Health, 6: 467-471.

Davies EG (2006). Impaired immunity in children. Curr. Pediatr., 16:16-28.

Gonzalez CR, Isibasi A, Ortiz-Navarrete V, Paniagua J, Garcia JA, Ramirez A, Salvatierra B, Tapia R, Sepulveda J, Gutierrez G (1995). Prevalence of antibodies against *Entamoeba histolytica* in Mexico measured by ELISA. Epidemiol. Infect. J. Parasitol., 115: 535-543.

Haque R, Ali IM, Petri WAJ (2000). Prevalence and immune response to *Entamoeba histolytica* infection in preschool children in Bangladesh. Am. J. Trop. Med. Hyg., 60: 1031-1034.

Haque R, Mondal D, Duggal P, Kabir M, Roy S, Farr BM, Sack RB, Petri WA (2006). *Entamoeba histolytica* Infection in Children and Protection from Subsequent Amebiasis. Infect Immunity, 74: 904–909.

Hart AS (1990). Novel organism associated with chronic diarrhea in AIDS. J. Parasitol., 335: 169-170.

Kim CH, Park CH, Kim HJ, Chun HB, Min KK, Koh TY, Soh CT (1997). Prevalence of intestinal parasites in Korea. Korean J. Parasitol., 9: 25-38.

Lebbad M, Svard SG (2005). PCR differentiation of *Entamoeba histolytica* and *Entamoeba dispar* from patients with amoeba infection initially diagnosed by microscopy. Scand J. Infect Dis., 37: 680-685.

Lucia-Libanez BCB, Manuela LG, Melissa WS, Clece P, Andrea S, Barbara JM (2001). *Entamoeba histolytica* and *Entamoeba dsipar* infections as detected by monoclonal antibody in an urban slum in Fortaleza, Northeastern Brazil J. Euk. Microbiol., 40: 340-344.

Nimri LF, Meqdam M (2004). Enteropathogens associated with cases of gastroenteritis in rural population in Jordan. J. Clin. Microbiol. Inf., 10: 634-639.

Ohnishi K, Murata M (1997). Present characteristics of symptomatic amoebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. J. Epidemiol. Inf., 119: 363-367.

Oyerinde JP, Alonge AA, Adegbite-Hollist AF, Ogunbi O (1979). The epidemiology of *Entamoeba histolytica* in Nigerian urban population. Int. J. Epidemiol., 8: 55-59.

Rivera WL, Tachibana H, Kanbara H (1998). Field study on the distribution of Entamoeba histolytica and Entamoeba dispar in the northern Philippines as detected by the polymerase chain reaction. Am. J. Trop. Med. Hyg., 59: 916-921.

Tanyuksel M, Petri WA Jr (2003). Laboratory Diagnosis of Amebiasis. Clin. Microbiol. Rev., 16: 713-729.