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Entamoeba Moshkoviskii as a causative agent of diarrhea in children

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ABSTRACT

The present investigational study was intentionally aimed to detect of *E.moshkoviskii* in stool as the first study and new record in Iraq by using polymerase chain reaction technique. Additionally understand the prevalence of these three parasites (*E.moshkoviskii*, *E.dispar,* and *E.histolytica*) in the human population. Stool sampling of 190 specimens was performed from clinically recognized patients suffering diarrhea with an inflammatory feature from Al-Diwaniya Teaching Hospital, Maternity and Children Hospital, Afak general Hospital and some Hospitals in the province of Iraq. All samples undergo full history was distributed according to name, age, gender, address, bloody diarrhea and clinical symptoms. General stool examination was done to samples within 30 minutes. The samples were divided into two sterile containers the first one grown in media and the second two were kept frozen at -20 Ċ (deep freezing) for DNA extraction used for PCR. From extracted DNA was added 5µl in PCR tube (master mix) with 1.5 µl forward primer and 1.5µl reverse primer, then 12µl from nuclease-free water, all volume was completed to 20 µl. One hundred ninety stool samples were collected and examined by general stool examination positive for *Entamoeba* spp The present study showed the high prevalence of amoebiasis in rural area more than urban area, the result revealed that the infected patients in rural area 119 (62.6%) while in urban area 71 (37.3%) The present study revealed the high percentage (25.2%) of infection with gastrointestinal symptoms under the age of 14 years old and above the age of 45 years. In the present study was showed the PCR product was detected in 182 (96 %) samples, and 8 (4 %) were detected as negative utilizing PCR test. The present study concluded that presence of three-identical species of *Entamoeba* utilizing PCR to target stool-based DNA of patients expressing gastrointestinal symptoms (*E.moshkoviskii, E. dispar,* and *E.histolytica*) could be grown in modified Lock's – egg slant medium . The single round PCR described in this study is a specific and sensitive method for distinguishing between those species.

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INTRODUCTION

The intestines of humans are considered as a normal habitat for many microorganisms such as Entamoeba that has six species, Entamoeba histolytica, *E. dispar, E. moshkovskii, E. poleki, E. coli,* and *E. hartmanni*. Some of these species are morphologically similar to each other such as *E. histolytica, E. dispar,* and *E. moshkovskii*; however, they react differently to biochemical tests. Known-pathogenic species such as *E. histolytica* is well-pathogenically understood, but others such as *E.moshkoviskii* and E.dispar are not well-identified for such pathogenicity (Hamzah et al., 2006). In such cases caused

by the pathogenic E. histolytica, Entamoeba moshkovskii is not diagnosed in the cystic and trophozoitic stage. E. moshkovskii has been isolated and identified from environmental samples (Clark and Diamond, 1997).

E. moshkovskii could be cultivated at ambient temperature and is well-known for its resistance to emetine that differs from E. histolytica and E. dispar (Dreyer et al., 1961). E. moshkovskii, unrecognized isolates for pathogenicity with few exceptions, has been retrieved from North America, Italy, South Africa, India, Iran, and Bangladesh (Haque et al., 1998; Robinson and Sargeanat, 1969). For clinical and treatment purposes, differentiation between E. moshkovskii and E. histolytica becomes an important issue. Limited information is present about E. moshkovskii due to the absence of successful identification tools which mostly rely on non-useful methodology such as cultivation. For the first time, the report recognizes direct tools to identify E. moshkovskii in children from Bangladesh-endemic regions with E. histolytica and E. dispar (Haque et. al, 1998).

In 1941 and for the first time, the species of E. moshkovskii was identified in humans from countries of different economic levels. Despite limited information regarding the pathogenicity of E. moshkovskii, some recent literature referred that this species could have an impact on intestine of people (Clark and Diamond, 1991). Entamoeba moshkovskii, Entamoeba histolytica, and Entamoeba dispar from a human being sampled were used as targets for a modified-single-round PCR. A middle-region of the small-subunit rRNA gene was followed to design the forward primer, but a signature-based specific-sequence was targeted to design the reverse primer for each species. PCR-resulted products were at 166bp, 580bp, and 752bp respectively. The degrees of recognition sensitivity was at 10pg for the first two species and at 20pg for the last one. Using multiple targets of bacteria and protozoa, the method was highly identified for its accuracy (Gonzalez et al.,1994). According to the findings of E. moshkovskii–based illnesses in different countries (Clark et al., 1991), it was highly needed to identify the responsibility of this species of such infections in the affected humans (Hossain et al.,2003). The first identification of environmental samples containing E. moshkovskii was in 1941 (Tshalaia, 1941; Clark and Diamond, 1991). Then, a patient with diarrhea was detected for the presence of a species similar to E. histolytica, Laredo strain (Dreyer, 1961). Same-temperature growth and treatment criteria are necessary when cultivating and treating Laredo strain and E. moshkovskii (Clark and Diamond, 1997). So the present investigational study was intentionally aimed to detect of E.moshkoviskii in stool as the first study and new record in Iraq by using polymerase chain reaction technique. Additionally, understand the prevalence of these three parasites (E.moshkoviskii, E.dispar, and E.histolytica) in the human population.

PATIENTS AND METHODS

Stool sampling of 190 specimens was performed from clinically recognized patients suffering diarrhea with an inflammatory feature from Al-Diwaniya Teaching Hospital, Maternity and Children Hospital, Afak general Hospital and some Hospitals in the province of Iraq.

All samples undergo full history was distributed according to name, age, gender, address, bloody diarrhea and clinical symptoms, general stool examination was done to samples within 30 minutes. The samples were divided into two sterile containers the first one grown in media and the second two were kept frozen at -20 Ċ (deep freezing) for DNA extraction used for PCR. From extracted DNA was added 5µl in PCR tube (master mix) with 1.5 µl forward primer and 1.5µl reverse primer, then 12µl from nuclease-free water, all volume was completed to 20 µl. The worker must avoid any type of contamination, this by taking the solution with separated tips under septic conditions control for DNA must be made by taking 3µl from primer with 17µl from PCR water. Control for primer must be made by taking 5µl from extracted DNA with 15µl from PCR water.

Data analyses were performed utilizing SPSS v20. Sensitivity= true positive/ true positive + false negative samples. Specificity= true negative / true negative + false positive.

RESULTS AND DISCUSSION

Microscopic Examination

One hundred ninety stool samples were collected and examined by general stool examination positive for Entamoeba spp. The patients classified according to the gender, age and residence Table (1).

Residence characteristics of patients according to age by using the microscopic examination

The present study showed the high prevalence of amoebiasis in rural area more than urban area, the result revealed that the infected patients in rural area 119 (62.6%) while in urban area 71 (37.3%) this may be due to the infection which depends greatly on lifestyle, age, hygienic awareness, socioeconomic status and personal hygiene figure (1).

Table 1: Demographic characteristic of the patients with gastrointestinal symptoms

Residence	Rural	119	62.6%
	Urban	71	37.3%
Gender	Male	122	64.2%
	Female	68	35.7%
Age/Years	≤ 5	36	18.9%
	(5 -14)	42	22.1%
	(15-24)	11	5.7%
	(25-34)	7	3.6%
	(35-44)	25	13.1%
	(45-54)	21	11 %
	≤65	48	25.2 %

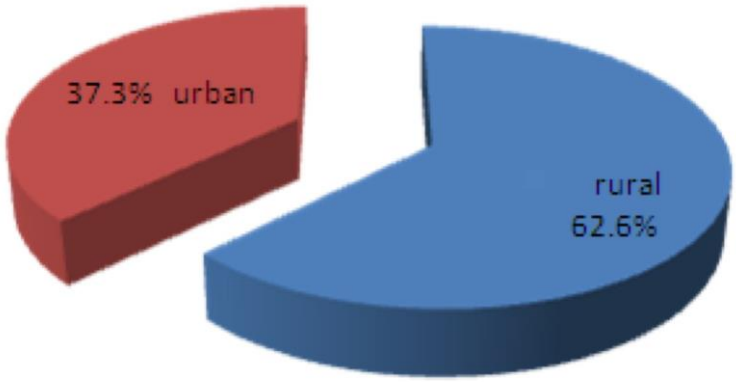

Figure 1: The ratio of rural area to urban area in patients with gastrointestinal symptoms according to the microscopic examination

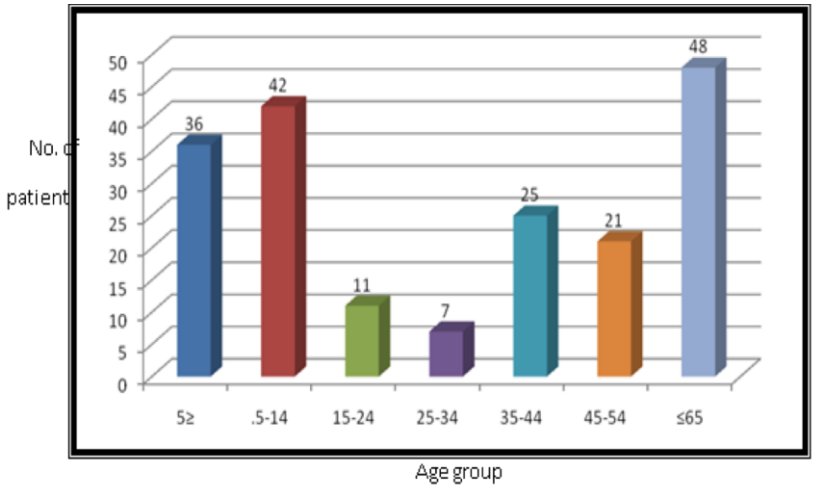

Figure 2: The number and age of patients with gastrointestinal symptoms according to the microscopic examination

This agrees with the study done by Al-Ebrahimi, (2013); Ngui et al., (1996); Lebbad and Svard, (2005) who showed the same result of people in rural area are more susceptible to infection than an urban area.

The present study revealed the high percentage (25.2%) of infection with gastrointestinal symptoms under the age of 14 years old and above the age of 45 years (Figure 2) this agrees with (Fotedar, et. al., 2007); agrees with (Gathiram and Jackson, 1985).

Characteristic of patients with amoebiasis according to the type of diarrhea by macroscopic and microscopic examination

Stool sampling of 190 specimens was macroscopically tested. The result showed Entamoeba species 99 (52.1%) bloody diarrhea, while from non - bloody 91(47.8%) samples Table (2).

The total number of microscopic-positive fecal samples containing the *Entamoeba* species were 190 were exposed to PCR-affirmation purposes of

Table 2: Entamoeba species according the type of diarrhea under Macro and Microscopic examination

Macroscopic Examination	Positive *Entamoeba* species samples in microscopic examination	Percentage %
Bloody diarrhea	99	52.1 %
Non – bloody diarrhea	91	47.8
Total	190	

Table 3: PCR result of stool DNA for three identical Entamoeba species.

PCR result to positive Microscopic examination *Entamoeba* species	Positive	Negative	Total
	182 (96 %)	8 (4 %)	190
Statistical analysis	x^2 =60.4 d.f = 1 P value =0.02		

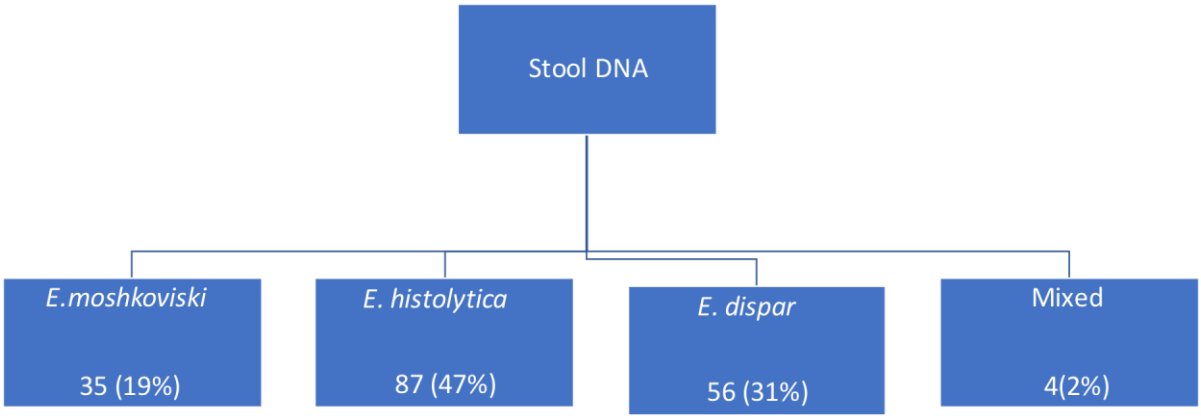

Figure 3: The number and percentage of Entamoeba species result according to the PCR technique to direct stool DNA extraction

Table 4: The number of E.moshkoviskii based on the age according to the PCR result

Age /year	*E.moshkoviskii* No.	Male %	Female %	Total %
≤5	3	2 (5.7%)	1(2.8%)	8.5 %
5-14	7	3 (8.5%)	4(11.4%)	19.9%
15-24	2	1(2.8%)	1(2.8%)	5.6 %
25-34	6	5(14.2%)	1(2.8%)	17 %
35-44	10	7(20%)	3(8.5%)	28.5 %
45-54	4	1(2.8%)	3(8.5%)	11.3 %
≤65	3	1(2.8%)	2(5.7%)	8.5 %
Total	35	20(57.1%)	15(42.8%)	100 %

the diagnosis of Entamoeba *moshkoviskii*. In the present study was showed the PCR product was detected in 182 (96 %) samples, and 8 (4 %) were detected as negative utilizing PCR test. Table (3).

Figure (3) showed that 35 /182 (19.2%) were contain only E.moshkoviskii, 56/182 (31% %) contain E. dispar, 87/182 (48 %) contain E. histolytica and, only 4/182 (2 %) was mixed divided into 3 (1.5 %) E .dispa r and E. histolytic and 1 (0.5 %) E. dispar and E. moshkoviskii (Table 4). This result agrees with Hossain et al, (2003) previously described in Bangladesh who reported the prevalence of E. moshkoviskii in preschool children, and agrees with Fotedar et al., (2007) who detect three Entamoeba species in stool samples in Australia.

According to the researcher's opinion, there were high widespread of infectious illnesses generated via those species that were previously noticed in India, Australia, Tanzania, Iran, Turkey, and Malaysia, may be due to high-risk groups include travelers, immigrants from the area of the endemic city. This agrees with Beck et al., (2008) and Fotedar et al., (2007). Here, the current exploration study revealed that E. moshkoviskii could be a true human parasite as was recognized by many studies from various countries such as South Africa 13% Beck et al., (2008), Australia 24.7% Fotedar et al. (2007), India 1.9% (Khainar et al., 2007), Iran (Kheirandish, 2011) and Tanyuksel et al (2007) in Turkey.

CONCLUSION

The herein identification study was followed to detect the presence of three-identical species of *Entamoeba* utilizing PCR to target stool-based DNA of patients expressing gastrointestinal symptoms. *E.moshkoviskii, E. dispar* and *E.histolytica*) could be grown in modified Lock's – egg slant medium. The

single round PCR described in this study is a specific and sensitive method for distinguishing between those species.

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