

Diagnostic and Epidemiological study of *Blastocystis hominis* in Samarra city, Iraq

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Abstract

The present study included detection of infection with *Blastocystis hominis* in Samarra city / Salah Al-Deen province, from April 2014 until April 2015. 264 stool samples were collected from children less than 15 years old which attending the General hospital and some medical laboratories in the city. Microscopic examination by using trichrome stain was used to detect this parasite. By using trichrome stain, cyst and vacuolar forms the only stages had been distinguished. The results of study revealed the rate of infection was (15.15%) by using microscopic examination. Epidemiological survey demonstrated there is no significant differences between male and female and more frequently infected at the (6-10) years age group (21.9%) and (13.33%). Also the higher rate of infection was during Spring, and the lower rate was in Autumn. Single infection with *B.hominis* was (9.09%), but the mixed infection with the other parasite was (3.78%) with *E. histolytica* and (2.27%) with *G. lamblia*. Diarrhea was the distinguish sign with infection children.

Introduction

Blastocystis sp. is an enteric protozoan parasite of humans and many animals. It was discovered in 1870 by the Russian physician and since then its pathogenic significance has always been uncertain [1]. *Blastocystis* has been ignored as a pathogen due to its association with mild nature of gastrointestinal symptoms and also with many asymptomatic cases [2].

Blastocystis can infect both children and adults and its geographical distribution appears to be global and it is often the most frequently isolated protozoan in parasitological surveys [3]. In developing countries, *Blastocystis* is commonly identified in stool specimens and it is one of the most common parasites that reside in the human intestinal tract. Clinical symptoms attributed to *Blastocystis* infections include recurrent watery diarrhea, mucous diarrhea, vomiting, abdominal cramps and flatulence [4].

Morphologically the parasite has four phases: vacuolar, granular, amoebic and cystic phase. The later phase has been considered a dominant phase found in environment (soil and water) so, it acts as vehicle for transmitting the parasite into the host [5]. Diagnosis of *B. hominis* overlap with other causatives of diarrhea specially *Cyclospora* sp., *Entamoeba histolytica* and other protozoan parasites (Tan, 2004). Routinely direct microscopy by preparing of wet preparation of Logo's iodine, fecal smear staining with trichrome stain can demonstrate *B. hominis* [6,7]. This study aimed to identify *B.hominis* forms in fecal samples of parasite suffering from intestinal ailments including diarrhea by using Trichrome stain to apply in epidemiological study.

Materials and methods

The current study included the examination of 264 faeces sample of patients who suffer from diarrhea or abdominal pain which were attended to the laboratory of parasites in Samarra General Hospital and some private laboratories in the city, Age range 3-15 years old between April 2014 to the end of April 2015. Stool samples collected in plastic jerry size of

approximately 20 ml of a broad nozzle, and sterilized with a tight lid to keep the sample moisture and prevent drying in one of its aspects poster paper to jot down sample number and the name of the patient, has also been taking some information from patients in terms of sex, age, and recorded information on the strength. The sample, according to Form questionnaire. Each sample has been examined by using trichrome stained method for *B.hominis* and other enteric parasite [8].

Statistical analysis

Data were analyzed by using one way ANOVA with the help of SPSS computer software. P value was considered significant when it was $p \leq 0.05$ according to SPSS [9].

Results

This study investigated the occurrence of *B. hominis* as unknown protozoa in Samarra city province of Salah Al-Din for the first time. The total rate of infection with *B.hominis* stained by trichrome stain was 40 (15.15%). The cyst and vacuolar forms was only stages recorded from all examined stool samples (Fig 1, 2).

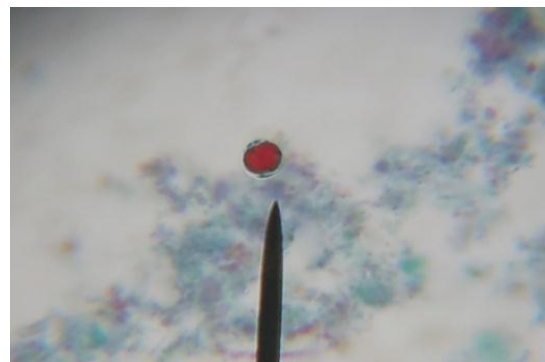


Fig 1: *B. hominis* cyst form stained with trichrome stain

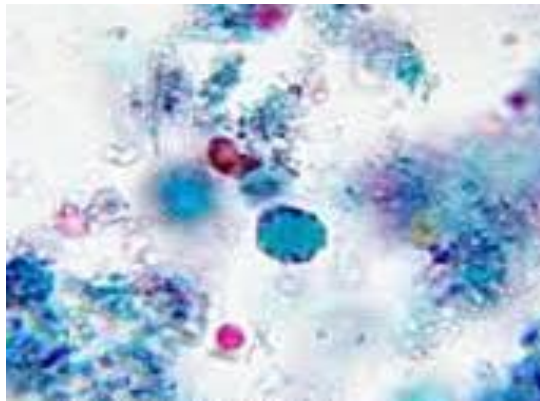


Fig 2: *B. hominis* vacuolar form stained with trichrome stain

This study showed no significant differences between all results. The highest infection rate was in the age group (6-10) (21.9%), by using trichrome stain table (1). *B. hominis* cases occurred more in female (17.75%) than in male (13.37%) (table 2).

Table (3) demonstrated that the incidence of this parasite is wide spread through the year but spring was the more season that the infection occurred in (21.42%).

B. hominis was found in 24 specimens (9.09%) in the absence of other pathogens. The others 10 (3.78%) had co infected with *E. histolytica* and 6 (2.27%) with *G. lamblia*.

Table (1): Prevalence of infection with *B. hominis* according to the age

| Age (year) | Total number of examined case | Trichrome stain | |
|------------|-------------------------------|-----------------|--------|
| | | Number of +ve | % *ns |
| 1-5 | 61 | 6 | 9.83% |
| 6-10 | 105 | 23 | 21.9% |
| 11-15 | 98 | 11 | 11.22% |
| Total | 264 | 40 | 15.15 |

*ns refers no significant differences between the rates of infection

Table (2): Prevalence of infection with *B. hominis* according to the sex

| Sex | Total number of examined case | Trichrome stain | |
|--------|-------------------------------|-----------------|--------|
| | | Number of +ve | % *ns |
| Male | 157 | 21 | 13.37% |
| Female | 107 | 19 | 17.75% |
| Total | 264 | 40 | 15.14% |

*ns refers no significant differences between the rates of infection

Table (3): Prevalence of *B. hominis* according to seasonal variation

| Season | Total number of examined case | Trichrome stain | |
|--------|-------------------------------|-----------------|-------|
| | | Number of +ve | % *ns |
| Summer | 98 | 12 | 12.24 |
| Autumn | 23 | 2 | 8.69 |
| Winter | 101 | 17 | 16.83 |
| Spring | 42 | 9 | 21.42 |
| Total | 264 | 40 | 15.14 |

*ns refers no significant differences between the rates of infection

Discussion

Permanent smears appear to be the procedure of choice for light microscope diagnosis of *B. hominis* [10]. Trichrome staining of permanent smears has been recommended for routine use in the diagnosis of *B. hominis* [11]. Schaudinn's fixative, which is used in this technique, preserves the morphology of parasites forms, and Chromotropic 2R which is an ingredient of trichrome stain has a strong affinity with the protozoa [12]. The parasite also may be difficult to distinguish from leucocytes or from trophozoites or cysts of protozoa [13], therefore we recommended to use molecular diagnosis by using PCR to provides a clear visual indicator of positive or negative results [14] and provides not only high sensitivity when compared with microscopic examinations and the culture method, but also high specificity for the detection of the organism's DNA [15].

The rates of infection almost agreed with many studies in detection this parasite, Sinniah and Rajeswari [16] in Malaysia (18.1%), Culha and Ozar [17] in Turkey (19.8%) and El-Shazly *et al* [18] in Egypt (14%). High rate of infection in the age group (6- 10 years) in this study may return to the fact that children at this age are more vulnerable to different foods, and exercise usually put fingers in the mouth, especially in the children's bags holders, and they are at this age eager for a taste of anything this may increase the rate of infection in this age group and his result agreed Sinniah and Rajeswari [16] in Malaysia (18.8%) in age group (6-10) and with Adel *et al* [19] in Iraq record infection rate in the age group (1-10) 5.02%. Many results showed that the age is a risk factor for the development of long term illness with *B. hominis* infection where the population of many individuals [20]. The present study revealed that there was no significant difference in the prevalence rate of infection with *B. hominis* between male and female groups, this may returned to practicing the same activities and habits. This finding was similar with the results recorded by Yaicharoen, *et al.*, [21] and Leelayoova, *et al.*, [22]. Why females showed a slightly higher infection rate than males is un clear, but could be due to a variety of environmental and behavioral factors, genetic or immunological characteristics [23]. Significant differences in the prevalence of *B. hominis* infection were found between the rainy and cool season [24]. In Egypt El-Masry *et al* [25] reported 445 (31%) proved positive, of these positive cases, 131 (29%) and 130 (29%) were identified during the spring and summer months respectively. Duda, *et al* [26] found that the parasite infections in soldiers were diagnosed in the autumn and the spring. Although, the rates reflect increased transmission during warm and wet summer. These dates do not essentially negate possible spring peaks [27]. Exposure, experiencing symptoms, seeking medical help and testing would probably explain this time lag [27]. Many records disagreed with our result and revealed the incidence of *B. hominis* wide spread

throughout the year. *B.hominis* has been classified as pathogenic by the Centers Diseases Control (CDC) [28]. It is often associated with other protozoa like *E. histolytica* and *G. lamblia*, but some times it may be the only organism present [28], and that agreed with

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دراسة تشخيصية ووبائية لطيفلي *Blastocystis hominis* في مدينة سامراء ، العراق

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الملخص

تضمنت الدراسة الحالية الكشف عن الخمج بطيفلي *Blastocystis hominis* في مدينة سامراء / محافظة صلاح الدين للفترة من شهر نيسان 2014 ولغاية شهر نيسان 2015. جمعت 264 عينة من غائط أطفال دون سن 15 سنة عمراً والذين وفدوا الى مستشفى سامراء العام وبعض المختبرات الأهلية في المدينة. استخدمت طريقة الفحص ألمجهري باستخدام صبغة الترياكروم للكشف عن وجود الطيفلي. باستخدام طريقة المسحات المصبوغة بصبغة الترياكروم تم تمييز الطورين الكيسي والفجوي في عينات الغائط المخمجة بالطيفلي. أظهرت نتائج الدراسة ان نسبة الخمج كانت (15.15%). أظهر المسح الوبائي بالاعتماد على نتائج الفحص ألمجهري عدم وجود فرق معنوي في نسبة الخمج بين الذكور والاناث، وسجلت أعلى نسبة خمج 21.9% و 13.33% في الفئة العمرية (6-10)، وكانت أعلى نسبة خمج في فصل الربيع وأدناها في فصل الخريف. شكل الخمج المفرد بطيفلي *B.hominis* نسبة (9.09%) أما نسبة الخمج المختلط مع مسببات مرضية أخرى فكان (3.78%) مع *E. histolytica* و(2.27%) مع *G. lamblia*. وكان الإسهال العلامة المميزة للأطفال المخمجين بالطيفلي.