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Comparison between different methods of Blastocystis hominis detection in stool samples.

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ABSTRACT:

Blastocystis hominis is common protozoan in human intestinal tract and can cause so-called blastocystosis characterized by diarrhea. Its routine identification in clinical laboratories is made by detection of vacuolar form in stool samples using wet mount smears. The present study was carried out with the aim of evaluating the effectiveness of different techniques for diagnosis of *B. hominis* in the stool samples from the patients attended Brack Hospital and Medical Technology Department, Faculty of Engineering and Technology, Brack**.**

A total of 360 stool samples were collected form randomized patients, presenting different genders and ages (121 males and 239 females ,and aged from less than one year to 90 years), residing in different localities of Wadi Al-shati province. All specimens were examined by direct smear microscopy (normal saline, iodine, and eosin stains), concentration (formalin–ether sedimentation) and two xenic culture systems (Monophasic Jone's medium and Diphasic Boeck and Drbohlav's) for the detection of *B. hominis*. The results highlights the low sensitivity of direct smear microscopy (11.29%) compared to concentration method (15.5%) and *in-vitro* culture methods (22.60%). There was no significant difference ($p>0.05$) between direct smear preparations, and concentration method, meanwhile there was significant difference ($p<0.05$) between direct smear preparations and the two xenic culture systems for the detection of *B. hominis*. There was an almost equal numbers of positive samples in both culture techniques (85 samples in diphasic medium and 78 in monophasic medium), and no significant difference ($P>0.05$) was found between the two culture methods. Diphasic Boeck and Drbohlav's medium produced highest numbers (7.600±6.379) of *B. hominis* cells compared to Monophasic Jone's medium (5.051±4.938) after every passage cultures and only the vacuolar morphologic type of this organism was found in both culture systems. Moreover, a larger size of vacuolar stage of *B. hominis* detected in Diphasic Boeck and Drbohlav's medium, than in Monophasic Jone's medium.

In vitro cultivation does seem worthwhile in the detection of *B. hominis* in diagnostic laboratories. Of all the diagnostic techniques used, diphasic Boeck and Drbohlav's medium was the most sensitive method for detecting *B. hominis* in stool specimens.

The short-term *in vitro* culture methods achieved the best performance with regard to sensitivity with other studied methods. With the advantages in terms of sensitivity, the *in vitro* culture methods could be applied to identify *B. hominis* for both clinical diagnosis and field study purposes, thus indicating the need to include laboratory techniques that enable *B. hominis* detection on a routine basis.

Key Words: Prevalence, intestinal parasites *Blastocystis hominis* , culture methods.

INTRODUCTION

Blastocystis hominis is a type of unicellular protozoan commonly found in the intestinal tract of humans. This parasite can cause blastocystosis with the characteristic diarrhea accompanied by abdominal pain, dizziness, anorexia, nausea, vomiting, intestinal timpanists, and weight loss [1, 2].

B. hominis is a polymorphic parasite, which may present in vacuolar, multivacuolar, avacuolar, granular, amoeboid and cystic forms [2, 3]. As other intestinal parasites, transmission occurs by fecal-oral route, although this has not been confirmed experimentally [3]. It is probable that the cystic rather than the vacuolar form, is mainly responsible for infection by *B. hominis*[2, 3].

The literature has reported that *B. hominis* has a worldwide distribution, mainly in developing countries where the prevalence rate is higher (approximately 30 to 50%) than those observed in developed countries [2, 3]. Groups with lower social-economic level and standards of hygiene tend to present a higher prevalence of infection than other groups in the community. The infection does not appear to have a gender bias, but it may be influenced by the host's age and immunologic condition [2]. The wide range in prevalence of *B. hominis* seen between countries can be attributed to several factors such as socioeconomic conditions, but also to the different diagnostic methods used for the detection of this organism[4]. The most common diagnostic technique used worldwide for identification of *Blastocystis* is the permanent stain. The

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use of xenic cultures, in which *Blastocystis* is grown *in-vitro* with non-specific microorganisms, has been shown to be more sensitive in detecting this organism, but it is not commonly used in the diagnostic laboratories [3, 5, 6].

Direct microscopic examination of fecal material, with or without addition of Lugol's solution, has been suggested for diagnostic purposes [2, 5]. Permanent smears stained with trichrome, iron hematoxylin, Giemsa, Gram and Wright's stains have also been recommended for the diagnosis of *B. hominis* infection [2].

Concentration methods such as zinc sulphate flotation or gravity sedimentation technique are unsuitable for concentration of *B. hominis* because water, as well as several other solutions, can lysate the vacuolar, multivacuolar and granular forms of the organism [2, 7]. Techniques for concentration using formalin-ether may however be suitable because preservative liquids are used for storage and dilution of the faeces.

Infection by *B. hominis* is frequently diagnosed by the finding of typical vacuolar forms, which are recognized by their characteristic appearance and large size in a light microscopy of faecal samples, either directly, as a simple wet mount smears or after some form of concentration [2, 7].

Since, this organism is the most frequent isolate in human stools in Libya [8-10], and so far, only two studies have been carried out to compare sensitivity of direct smear, concentration and xenic culture for the detection of *B. hominis* in stool specimens [10, 11]. Therefore this study was aimed to compare four diagnostic techniques direct smears microscopy, sedimentation in formalin ether and two xenic culture systems (diphasic Boeck and Drbohlav's medium, and monophasic Jone's medium) for the detection of *B. hominis* in stool samples. The two xenic media (diphasic and monophasic) were used to investigate, which culture medium could be more efficient and suitable for diagnosis of *B. hominis* in clinical laboratories.

MATERIALS AND METHODS

Area and population: During the period of October 2010 to end of June 2011, a total of 360 stool specimens, presenting different genders and ages (121 males and 239 females, and aged from less than one year to 90 years) were freshly collected from patients, who routinely submitted their stool samples for routine parasitological analysis to Brack Hospital and Medical Technology Department, Faculty of Engineering and Technology, Brack.

Detection of *Blastocystis hoimins*: Each faecal sample was divided into three parts: one part was submitted to direct saline ,direct iodine and eosin wet mounts [12]. Soon after direct smear microscopy, the second part of samples was concentrated by formalin-ether sedimentation technique as described [12, 13], and the third one *in vitro* culture for the detection of *B. hominis.*

Culture Techniques: The third one part of stool samples were cultured in two different short term *in vitro* culture medium , i.e. in Monophasic Jone's Medium and Diphasic Xenic System Boeck and Drbohlav's inspissated egg medium (B-D) Locke- Egg – Serum (LES) Medium as described by Jones [14] and Zierdt and Swan [15] respectively.

Statistical analysis: Statistical analysis was performed using SPSS, version 11.5(SPSS Inc., Chicago, I L, USA). The results for positive samples of *B. hominis* of the detection techniques were expressed as percentages, and statistical analysis was carried out by using chi square test. A probability (*P*) value of less than 0.05 was considered as significant whenever appropriate.

RESULTS

The results of comparison of diagnostic techniques showed 11.29, 15.5, 21.6 and 23.61% positive rates for *B. hominis* in stools by directed smear microscopy, concentration, Jone's medium and Boeck and Drbohlav's medium respectively. All the stool specimens found positive in direct smears, were also found positive in concentration, and both culture methods (40 of 360). Eighty-five (23.61%) samples were positive in one or more of the diagnostic techniques. Direct smear microscopy, and concentration showed significantly lower sensitivity ($p<0.05$) compared to both culture techniques for identifying *B. hominis*, meanwhile there was significant difference ($p<0.05$) between direct smear preparations and the two xenic culture systems for the detection of *B. hominis*. No significant difference ($p>0.05$) was found between monophasic Jone's medium and diphasic Boeck and Drbohlav's medium (Table 1).

Table 1: Comparison of methods for the detection of *B. hominis* in stools.

Samples examined	Number and percentage of positive samples by different methods					
	Direct smear microscopy			Concentration	Culture	
360	Normal saline	Iodine	Eosin	Formalin-ether sedimentation	Monophasic Jone's Medium	Diphasic Boeck and Drbohlav's Medium
	34 (9.44)	45 (12.5)	43 (11.94)	56 (15.5)	78 (21.6)	85 (23.61)

Figures in parentheses indicate percentages.

Of all the diagnostic techniques used, cultivation of stool samples was the most sensitive method for the detection of *B. hominis*. There were 275 stool samples found to be negative (represented 76.66% of the total samples) and 40 of 360 (11.11%) were found to be positive by all used methods (direct smear microscopy, concentration and culture techniques. 14 (3.8%) samples gave negative results in direct smear microscopy only but were found positive in both formalin-ether concentration, and culture methods. 22(6.1%) stool samples found positive in culture techniques only, but were negative in both direct smear microscopy, and concentration method. Nine stools samples (2.5%) were negative in concentration only, but

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were found positive in both direct smear microscopy, and culture techniques. There was no stool sample, which showed negative results in culture methods, but was found positive in both direct smear microscopy and concentration (Table 2).

Table 2: Comparison of the Detection efficiency (%) of direct smear microscopy, formalin–ether Concentration and culture methods for the detection of *B. hominis*.

Status	Methods					
	Direct smear (-Neg) Concentration (-Neg) Culture (-Neg)	Direct smear (+Pos) Concentration (+Pos) Culture (+Pos)	Direct smear (-Neg) Concentration (+Pos) Culture (+Pos)	Direct smear (-Neg) Concentration (-Neg) Culture (+Pos)	Direct smear (+Pos) Concentration (-Neg) Culture (+Pos)	Direct smear (+Pos) Concentration (+Pos) Culture (-Neg)
Number of samples	275	40	14	22	9	0
Detection efficiency (%)		11.11	3.80	6.10	2.50	0.00

(+Pos) = Positive, and (- Neg) = Negative

Growth profiles of *B. hominis* in monophasic and diphasic medium are shown in Table 3. Diphasic Boeck and Drbohlav's medium produced higher numbers (7.60 ± 6.38) of cells compared to monophasic Jone's medium (5.05 ± 4.94) but this difference was not statistically significant (p>0.05).

Table 3: Growth profile of *B. hominis* in Monophasic Jone,s Medium and Diphasic Boeek Drbohlav,s Medium .

Culture Methods	Number and Percentage of *B. hominis* cases	Number of *B. hominis* cells		P. value
		Range	Mean ± SD	
Monophasic Jone's Medium	78 (21.6)	2-20	5.05±4.94	>0.05
Diphasic Boeck and Drbohlav's Medium	85 (23.61)	3-25	7.60±6.38	

Figures in parentheses indicate percentages.

Table 4 shows the different methods used for the identification of *B. hominis* in stool samples in Libya.

DISCUSSION

Blastocystis hominis is a common human intestinal protozoan, reported in children and adults in developing countries [16]. Diagnosis of *B. hominis* public health centers, and clinical laboratories is mostly made by the demonstration of typical vacuolar form (approximately 10 to 15µm in diameter), with a large central vacuole and 1 to 4 nuclei in the peripheral cytoplasm. The small forms of *B. hominis* like multivacuolar, avacuolar, and cysts are not used for detection in the routine microscopy, which are also present in the stool samples and usually are missed during laboratory examinations in direct smear microscopy. Moreover, asymptomatic infections are also common in the communities and no doubt these cases are frequently undetected or underestimated [6]. Missed diagnosed patients or shedding of *B. hominis* from asymptomatic cases may be a vast potential source of infection humans in the region.

So far, only two studies have been carried out in Libya to compare sensitivity of direct smear, concentration and xenic culture for the detection of *B. hominis* in stool specimens. A prevalence of 26.21%, 34.10% and 42.31% by using direct smear, concentration and Boeck and Drbohlav's Medium respectively were reported from patients attending Central Laboratory in Sebha [11]. However, prevalence of 14.0%, 21.0 % and 35.5% by direct smear, concentration and Boeck and Drbohlav's Medium respectively were reported from food handlers in Sirte [10]. A prevalence of 18.30% and 21.20% in community population in Wadi Al-Shati were reported by using direct smear and concentration respectively [9]. Al-Fellani *et al* [8], reported prevalence of 18.55% in patients attending Central Laboratory in Sebha; Salem *et al* [17], reported prevalence 29.6% in Libyan patients in Sirte; Sadaga and Kassem [18], reported prevalence 6.7% School children in Derna ; Saleh [19], reported prevalence 22.69% in patients attending Central Laboratory in Sebha; Kassem *et al* [20], reported prevalence 12.57% in Children and neonates admitted to Ibne-Sina Hospital in Sirte and Gelani *et al* [21] , reported prevalence 20.21% in patients attending Brack Hospital and Brack Medical Laboratory by only using direct smear method .

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Table. 4: Comparison of the prevalence and diagnostic methods used for the detection of *Blastocystis hominis* in Libya.

Reference	Locality/Category	Detection Methods (Prevalence %)			
		Direct smear	Concentration	Culture Techniques	
				Jone's Medium	Boeck and Drbohlav's Medium
Al-Fellani *et al* [8]	Patients attending Central Laboratory in Sebha.	18.55	ND*	ND	ND
Ruqaia [11]	Patients attending Central Laboratory in Sebha.	26.21	34.1	ND	42.31
Salem *et al* [17]	Libyan patients in Sirte	29.6	ND	ND	ND
Sadaga and Kassem [18]	School children in Derna	6.7	ND	ND	ND
Saleh [19]	Patients attending Central Laboratory in Sebha.	22.69	ND	ND	ND
Kassem *et al* [20]	Children and neonates admitted to Ibne-Sina Hospital in Sirte.	12.57	ND	ND	ND
Awatif *et al* [9]	Community population in Wadi Al-Shati.	18.3	21.2	ND	ND
Gelani *et al* [21]	Raudam Poplation of Rural ares of Wadi Al-Shati.	20.21	ND	ND	ND
Fathy [10]	Food handlers in Sirte	14	21	ND	35.5
Present study	Patients attending Brack Hospital and Medical Laboratory Department, Brack, Wadi Al-Shati.	11.26	15.55	21.66	23.61

*ND = Not Determine

In the present study, the culture methods (diphasic Boeck and Drbohlav's medium and monophasic Jone's medium) detected significantly (23%) infection of *B. hominis* than direct smear microscopy (11.27%). This finding is similar to results of Zierdt [22], Zhang *et al* [23], Mohammed *et al* [24]and Fathy [10], who reported that culture method (Boeck and Drbohlav's medium) was more sensitive than microscopy direct smear, and /or permanent stained smears of stool specimens. This observation is also similar to Zaman and Khan [5], Vennila *et al* [25], Suresh *et al* [26] and Yakoob *et al* [27], who found monophasic Jone's medium most effective than direct smear microscopy, and / or concentration technique. Similarly, Dogruman *et al* [28] compared direct smear microscopy in iodine stain, permanent stained smears in trichrome, immunofluoresence assay and monophasic Ringer's culture medium for the detection of *B. hominis* in stool samples. Moreover, Roberts *et al* [4] compared the sensitivity of diphasic Boeck and Drbohlav's and monophaisc tryptone, yeast extract, glucose methionine–9 medium with permanently stained smears using a modified iron–hematotoxylin stain. Results showed low sensitivity of permanent stained smears compared to two culture systems.

The results of present study, demonstrate that both culture methods (diphasic Boeck and Drbohlav's and monophasic Jone's medium) detected almost equal number of positive samples for *B. hominis* . The results are in accord with Roberts *et al* [4], who reported that both diphasic Boeck and Drbohlav's medium and monophasic tryptone, yeast extract, glucose, methionine-9 medium) showed equal effectiveness for the detection of *B. hominis* in clinical stool samples. The results from this study also showed higher growth profile of *B. hominis* in diphasic medium compared to monophasic medium .Similar observation has been reported by Roberts *et al* [4] , who observed high numbers of *B. hominis* cells growth in diphasic medium than monophasic medium. The increased in the numbers of positive samples of *B. hominis* using stool culture methods may be attributed to the organism needing more time to grow and replicate and appeared to be due to change of cyst and multivacuolar forms into vacuolar forms during cultivation of stools (as mostly vacuolar stages were seen in both culture systems). Moe *et al* [29], also observed that cyst forms of *B. hominis* isolated from human faeces, developed into a large number of vacuolar forms in short term *in-vitro* culture.

In the present study, increased in numbers and size of this organism was also observed during the culture of stool samples .These results were the same as those of Boreham and Stenzel [1], Stenzel [30], Moe *et al* [29] , Leelayoova *et al* [6] and Zhang *et al* [23]. Although the examination of direct wet smears is convenient and inexpensive, but it frequently leads to a false-negative results. The main problem with simple direct smear is that small numbers of *B. hominis* are present in the stool samples may go undetected or at least unrecognized.

The better sensitivity, and higher yield growth profile of *B. hominis* in diphasic Boeck and Drbohlav's medium may be due to that of egg slant of this medium,

providing more surface area for the growth of this organism. The results showed that concentration method was found relatively more sensitive (15.5%) than the average of direct smear microscopy (11.27%). This increased in the detection efficiency of *B. hominis* in concentration method, compared with direct smear microscopy is probably due to presence of small numbers of *B. hominis* cells in the faecal specimens, which are missed during routine direct smear microscopy. The results are in accordance with Qadri *et al* [31], Adeen and Hale [32] , Guimaraes and Sogayar [33], Logar *et al* [34]and Taamasri *et al* [35], who reported that concentration methods are beneficial, compared to direct smear microscopy. However, others have reported that concentration methods have no advantages over direct smear microscopy for the detection of *B. hominis* in the stool specimens [3, 6, 36-38]. They assumed that low detection efficiency appears due to necessary steps of shaking and centrifugation in formalin–ether technique that lead to rupture of the vacuolar, multivacuolar and granular forms of *B. hominis* during the procedure.

In the present study, there is also increased in the positive samples of *B. hominis* in concentration negative stool samples in both xenic culture methods. This may be due to presence of smaller forms (other than vacuolar) of *B. hominis* in the faecal materials, which successfully grow, and multiply in diphasic and monophasic medium. Similarly, Leelayoova *et al* [6], Suresh and Smith [3] and Tungtrongchitr *et al* [37]also reported that stool specimens found negative in formalin–ether concentration technique were found positive for *B. hominis* in stool culture.

This study demonstrated that *B. hominis* detection efficiency was found to be more in culture methods, followed by faecal concentration in formalin–ether sedimentation and direct smear microscopy. Similar observations have been reported by others (Leelayoova *et al* [6]; Tungtrongchitr *et al* [37]; Yakoob *et al* [27] and Rugaia[11]). These workers have reported that *in-vitro* cultivation of faecal material is effective and had advantages over direct smear microscopy, and concentration method.

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