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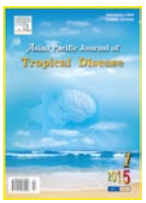

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Prevalence and diagnostic approach for a neglected protozoon *Blastocystis hominis*

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PEER REVIEW

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Comments

This is a good study in which the authors confirmed the pathogenic potential of *B. hominis* in children, and also The MZN staining technique is also higher sensitivity than iodine and SMB stains, and was more feasible to be used in laboratories with limited funds although IFA is the most sensitive stains.

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ABSTRACT

Objective: To determine the prevalence of *Blastocystis hominis* (*B. hominis*) among pre-school children and evaluate the different staining techniques for its detection in stool.

Methods: A total of 300 children of both sexes aged 2-5 years were included in this study. Each child was subjected to examination of stool sample using iodine, safranine methylene blue (SMB) and modified Ziehl-Neelsen (MZN) stains. Immunofluorescence antibody (IFA) stain was applied on stool specimens of 30 cases only.

Results: Results showed a total prevalence rate of 53%. A higher prevalence was detected among children aged 4-5 years and most of them were males. The majority of cases (82.8%) infected with *Blastocystis* alone were symptomatic. Fecal parasite burden was significantly higher in symptomatic than asymptomatic cases, so *B. hominis* should be considered as a pathogenic parasite. IFA stain was proved to be the most sensitive (73.3%) followed by MZN (68.2%), iodine (59.1%) and SMB (50%) stains.

Conclusions: This study confirmed the pathogenic potential of *B. hominis* and shed light on the great need to promote the hygienic standards among the risky group of children. IFA staining technique though having the highest sensitivity, it is suitable for use in large hospitals or public health laboratories in developed countries owing to its high cost. The MZN staining technique had a significantly higher sensitivity than iodine and SMB stains, and was more feasible to be used in laboratories with limited funds as in Egypt, because it is cheap, rapid, easy to perform and clarify the definitive morphological details of the parasite.

KEYWORDS

Prevalence, Diagnosis, Stain, Immunofluorescence, *Blastocystis hominis*

1. Introduction

Blastocystis is a prevalent enteric protozoon that infects a variety of vertebrates. It is probably the most common protozoan found in the human gut worldwide[1]. The parasite has been described since the early 1900s, but only in the last decade there have been significant advances in the understanding of *Blastocystis* biology. However, the pleomorphic nature of the parasite has hindered laboratory diagnosis and efforts to understand its mode of reproduction, life cycle, prevalence, and pathogenesis[2].

The possibility of *Blastocystis* being a pathogen has long been a matter of debate. Although recent accumulation of clinical evidence suggests the pathogenic potential

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of *Blastocystis*, it should be put in mind that it is still not proven conclusively till date[3-5]. Certain populations may be susceptible to *Blastocystis* infection including immunocompromised persons and young children[6,7].

Blastocystis hominis (*B. hominis*) is an extremely ubiquitous parasite with a worldwide distribution[8]. It is not uncommon for this parasite species to be among the most frequently isolated parasites in epidemiological surveys and clinical parasitology[9]. Prevalence varies widely from country to country and within various communities of the same country. In general, developing countries have higher prevalence than the developed ones, and this has been linked to poor hygiene, contact with animals, and consumption of contaminated food or water. In some countries, the prevalence can be rather variable, depending on the subpopulation studied. Such variations within the same country could reflect true differences between communities, especially if the same techniques were employed to identify the parasite[2].

Few authors studied the prevalence of *B. hominis* in Egypt including Qualyobia Governorate, Ismailia City, Menoufiya Governorate and Cairo Governorate[10-13]. However, until now no study was done on the prevalence rate of this parasite in any area of Gharbeya Governorate.

Diagnosis of *Blastocystis* is considered a challenge to diagnostic laboratories leading to underestimation, difficulty of identifying *Blastocystis* in stool that was attributed to its irregular shedding from day to day, the variety of its morphological forms (vacuolar, granular, amoebic or cystic), and the large size variation exhibited by this organism or its similarity in appearance to fat cells, white blood cells or yeast. So, several studies have strongly recommended the need for reliable tests for diagnosis of *Blastocystis* infection[14-16].

Therefore, the aim of this study was to estimate the prevalence of *B. hominis* among pre-school children in Gharbeya Governorate, Egypt and also to compare the sensitivity and specificity of different direct diagnostic techniques for detecting this protozoon in stool samples of positive cases.

2. Materials and methods

This study was carried out in the period from July 2012 to June 2013 in the Medical Parasitology Department, Faculty of Medicine, Tanta University. A total of 300 children of both sexes aged 2-5 years in three nurseries in Shobra Elnamla Village, Gharbeya Governorate, Egypt were included in this study. The nature of the study was explained to one of the parents of the child or the legal guardian. Informed consents were obtained and ethics committee approved the protocol in compliance with the current standard laws. A clinical questionnaire focusing on age, sex, drinking water supply, personal hygiene and complaints, was fulfilled for each child. Children under anti-parasitic treatment were excluded from this study.

2.1. Stool collection

Three fresh stool samples were collected from each child on three alternative days within a week in clean containers and labeled with the child name and number.

2.2. Stool examination

All stool samples were concentrated using Sheather's sugar floatation concentration technique[17]. Using Sheather's sugar technique resulted in destruction of *B. hominis* cells. So the different stains were applied on unconcentrated stool samples (only simple sedimentation in normal saline was used for all samples). After sieving, the stool samples were preserved in 10% formalin until used for staining by the following techniques:

-Iodine stain: Lugol's iodine was applied to non-fixed smears. The stained slides were examined microscopically using 10×, 40× and 100 objectives[18].

-Safranine methylene blue (SMB): 3% HCl in 100% methanol for 3-5 min was applied to the air fixed stool smears; then 1% aqueous safranine was used as a stain for 60 seconds followed by 1% methylene blue as a counterstain for 30 seconds. The stained slides were examined microscopically using 40× and 100 oil immersion objectives to confirm internal morphology[19].

-Modified Ziehl-Neelsen (MZN) stain: fixation of the smears with absolute methanol for 30 seconds was done, then samples were stained with Kinyoun's carbolfuchsin for 1 min, decolorized using acid alcohol for 2 min then counterstained using malachite green for 2 min[20]. The stained slides were examined microscopically using 40× and 100 oil immersion objectives to confirm internal morphology.

-Immunoflurescent antibody (IFA) stain: IFA stain was applied to 30 cases using ParaFlor B reagent (Boulder Diagnostics, Boulder, CO) that was obtained by personal communication from USA. This product line identifies parasites directly from stool samples in one step, using fluorescently labeled monoclonal antibodies that will react with the targeted parasite antigen. Inclusion criteria for the examined cases: cases of mixed parasitic infection were excluded, so cases infected by *B. hominis* alone were chosen for this stain. Also, cases diagnosed as positive or negative by all the three other stains (iodine, MZN and SMB) were excluded, so cases which were a matter of controversy when using the above mentioned 3 stains were subjected to the IFA stain to confirm their

diagnosis. IFA stain was undertaken as the gold standard to which other stains (iodine, MZN and SMB) were compared to get true positive/false positive and true negative/false negative cases diagnosed by each stain. This step was undertaken as a preliminary one to calculate sensitivity and specificity of each stain. Formalin preserved *B. hominis* cell suspension was applied as a positive control. IFA stain was run as follow: 100 μL of a well-mixed stool sample was combined with one drop of ParaFlor B reagent and was mixed in a microfuge tube. If the stool sample was firm, 100 μL of phosphate buffer solution was added to 100 μL of stool sample and mixed, and then one drop of the ParaFlor B reagent was added. About 10 μL aliquot of positive control was diluted in 100 μL of phosphate buffer solution and then one drop of the ParaFlor B reagent was added to confirm the positivity of the samples. The samples were incubated at room temperature. Once during incubation, the samples were remixed by flicking the tube with a finger. A volume of 10 μL of the tested samples and 10 μL of the positive control samples were placed separated on a slide and then each was covered with a cover slip and the slide was examined using a fluorescein isothiocyanate compatible fluorescent filter (Excitation/Emission=493 nm/518 nm)[21].

-Intensity of infection: parasite load was assessed by calculating the mean number of parasites in 15 examined high power fields (×100)[22].

-Criteria for assessment of different stains include: *B. hominis* recovery and staining quality of the recovered parasite *i.e.* clarification of morphological details, color, differentiation of the recovered *B. hominis* from background fecal debris (contrast). The sensitivity and specificity of the different stains for detection of *B. hominis* were calculated and compared statistically[23].

2.3. Statistical analysis

Statistical presentation and analysis of the present study were conducted using the mean standard deviation and *Chi*-square test by SPSS V.16.

3. Results

Out of the examined 300 stool samples of pre-school children, 159 children were positive for *B. hominis* infection with a prevalence rate of 53%. In the majority of the children (145, 91.2%), *B. hominis* was the only detected parasite. Out of the 159 positive specimens, 14 samples (8.8%) showed mixed infections, where *B. hominis* was found in association with *Entamoeba histolytica* (*E. histolytica*) in 11 samples (6.9%) and with *Giardia lamblia* (*G. lamblia*) in 3 samples (1.9%).

This study showed an increasing risk of acquisition of *Blastocystis* infection with age. A higher prevalence of *B. hominis* (80.8%) was recorded in children aged 4-5 years than those aged 2-3 (19.6%) and 3-4 (48.2%) with high statistically significant difference between the different age groups. Moreover, a significantly higher prevalence was reported in males (59.8%) compared to females (44.9%). Additionally, drinking tap water (90% of the positive cases) and bad personal hygiene (70%) were found to be important risk factors. The highest prevalence of *Blastocystis* was detected during summer (85.1%), followed by autumn (53.9%), spring (37.7%) then winter (19.7%). There was a significant difference between different seasons of the year.

Concerning the clinical manifestations in the examined children infected with *B. hominis* alone, 120 cases (82.8%) were symptomatic, while 25 cases (17.2%) were asymptomatic. The most common symptom among these patients was abdominal pain (71.8%) followed by diarrhea (40%), anorexia (32.5%), failure to gain weight (27.5%), constipation (13.3%) and vomiting (4.16%). It was found that 25.3% of the symptomatic children had two or more gastrointestinal symptoms while 74.7% had only one symptom. The mean number of *B. hominis* cells per high power field (HPF) was found to be significantly higher ($P<0.05$) in the symptomatic infections compared to asymptomatic ones.

MZN was the most superior stain where it detected 159 positive cases out of examined 300 samples (53%). Iodine and SMB detected 131 (43.7%) and 110 (36.7%) respectively. There was a significant difference between the three stains regarding the detected number of the positive cases ($X^2=4.638$, $P<0.05$).

In reference to IFA stain, it was proved to be significantly more sensitive in detecting *B. hominis* than the other stains. It could identify 22 positive cases out of 30 (73.3%) as compared to 15 (50%), 13 (43.3%) and 11 (36.7%) positive cases identified by MZN, iodine and SMB stains respectively. Also MZN stain was significantly more sensitive than iodine and SMB stains ($P<0.05$). There was no statistically significant difference between iodine stain and SMB stain ($P>0.05$).

Table 1 shows true and false cases diagnosed by the three stains in comparison to IFA stain, 8 (26.7%) out of the examined 30 cases were diagnosed negative for *B. hominis* infection using IFA stain, where 22 cases (73.3%) were proved to be positive for this infection. Using IFA stain as the gold standard, 15 cases (68.2%) were proved to be true positive using MZN stain, on the other hand 7 cases (31.8%) were proved to be false negative ones. Regarding iodine stain, 13 cases (59.1%) were diagnosed as true positive while 9 cases (40.9%) were proved to be false negative when compared to IFA stain. SMB stain was proved to diagnose 11 (50%) as true positive and 11 cases (50%) as false negative ones. No case was diagnosed as false positive when comparing the three stains to the gold standard IFA stain.

As regards different forms of *B. hominis* demonstrated by the different stains other than IFA stain, the vacuolar form was the most common to be detected by all methods. The amoeboid form was only detected by MZN stain.

Table 1

True and false cases diagnosed by each stain compared to IFA stain among 30 selected cases.

Examined stain	Cases diagnosed by each stain	Cases diagnosed by each stain compared to IFA stain	Cases diagnosed by IFA stain	
			Positive	Negative
MZN	Positive 15 (50.0%)	True positive15 (68.2%)	22 (73.3%)	8 (26.7%)
	Negative 15 (50.0%)	False negative 7 (31.8%)		
Iodine stain	Positive 13 (43.3%)	True positive 13 (59.1%)		
	Negative 17 (56.7%)	False negative 9 (40.9%)		
SMB	Positive 11 (36.7%)	True positive 11 (50.0%)		
	Negative 19 (63.3%)	False negative 11 (50.0%)		

From the comparison between the four used stains regarding the morphological details of the recovered *B. hominis* cells, it was found that IFA stain clearly identified *B. hominis* cells which appeared as brightly fluorescent rounded cells of varying size distinct from the dark background (Figures 1 and 2). This method was a rapid procedure and the smears could be easily detected at low magnification but it was difficult to visualize their definitive morphology.

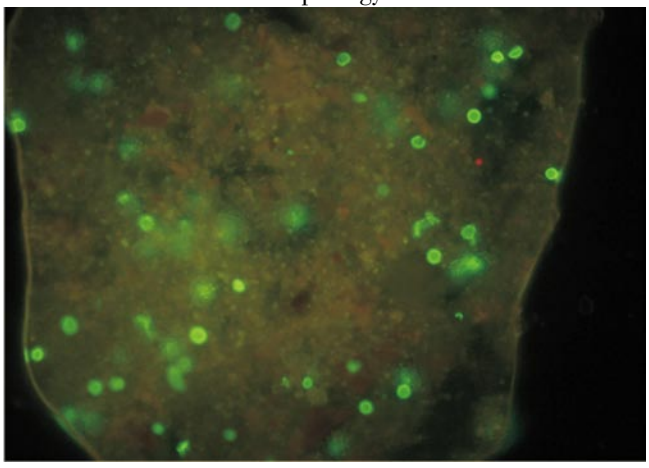

Figure 1. An IFA-stained fecal smear showing multiple brightly fluorescent rounded *B. hominis* cells (×400).

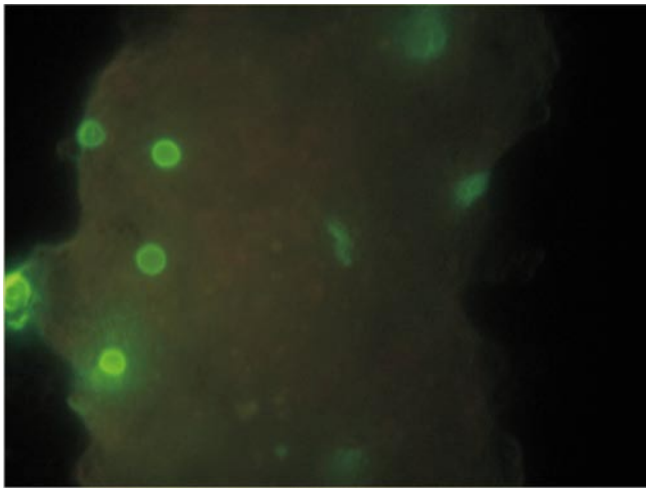

Figure 2. An IFA-stained fecal smear showing multiple brightly fluorescent rounded *B. hominis* cells (×1 000).

Using MZN staining technique, nearly all forms of *B. hominis* were identified as vacuolar, cystic, multi-vacuolar and amoeboid forms. The vacuolar form appeared rounded and moderately greenish blue in color on a pale greenish blue background. The central vacuole stained pale greenish blue surrounded by a dark greenish blue rim of cytoplasm containing deeply stained nuclei. As regards the cystic form, it appeared rounded or oval, small in size with faintly-stained center containing one or two dark-stained nuclei and dark-stained cell wall which was easily distinguished from the pale greenish blue background (Figure 3). A thick surface coat was seen surrounding the cyst wall in some cysts (Figure 4). The multi-vacuolar form contains multiple vacuoles at the center and a peripheral band of cytoplasm with multiple deeply-stained nuclei. A thick surface coat was seen surrounding some multi-vacuolar cells. The amoeboid form was small in size, irregular in outline and possessed a distinct pseudopod-like extension (Figure 5). This stain was easy, rapid and cheap.

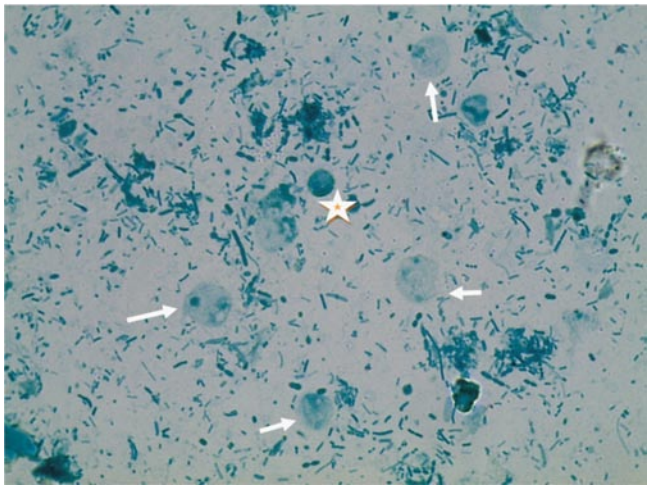

Figure 3. A MZN-stained fecal smear showing multiple vacuolar forms (arrows) and a single cystic form (star).
The cystic form appears rounded, smaller in size with dark-stained cyst wall and faintly-stained center containing two dark stained nuclei (×1 000).

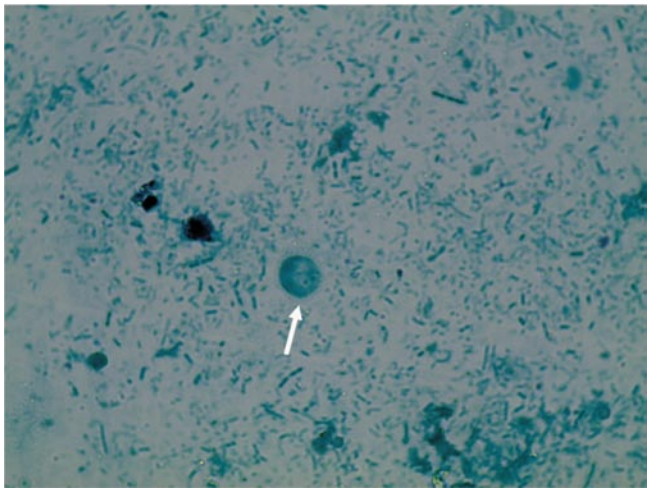

Figure 4. A MZN-stained fecal smear showing cystic form of *B. hominis* with a thick loose surface coat surrounding the cyst wall (×1 000).

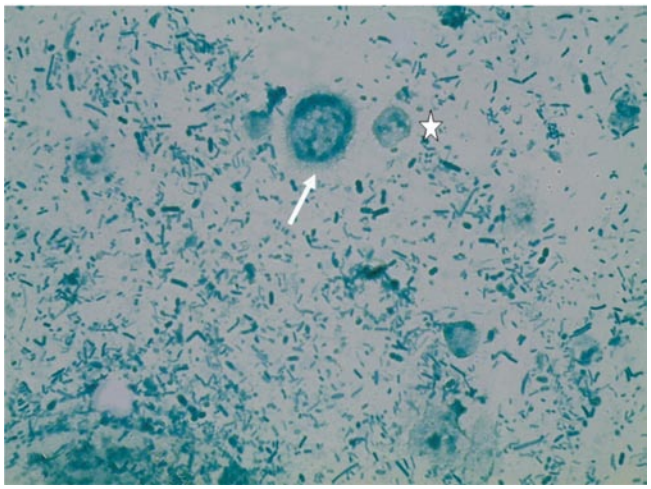

Figure 5. A MZN-stained fecal smear showing a large multi-vacuolar form and an amoeboid form of *B. hominis*.
arrow: A thick loose surface coat; star: One distinct pseudopod-like extension (×1 000).

In iodine staining technique, the vacuolar, cystic and granular forms were identified. Concerning the vacuolar form, it appeared refractive, rounded or ovoid and varied greatly in size. The central vacuole appeared yellowish surrounded by a thin band of cytoplasm containing deeply stained brown nuclei. The parasite was easily distinguished from the grayish yellow background (Figure 6). The granular form appeared similar to the vacuolar form but containing multiple deeply stained yellowish granules (Figures 7 and 8). As regards the cystic form, it appeared smaller than other forms, refractive yellow with a well defined cyst wall and one or two deeply stained nuclei. Some cysts showed multiple vacuoles at their centers (Figure 8). This stain was the easiest and the most rapid. Moreover, the recognition of the definitive morphological details of the different forms was easier than the other stains.

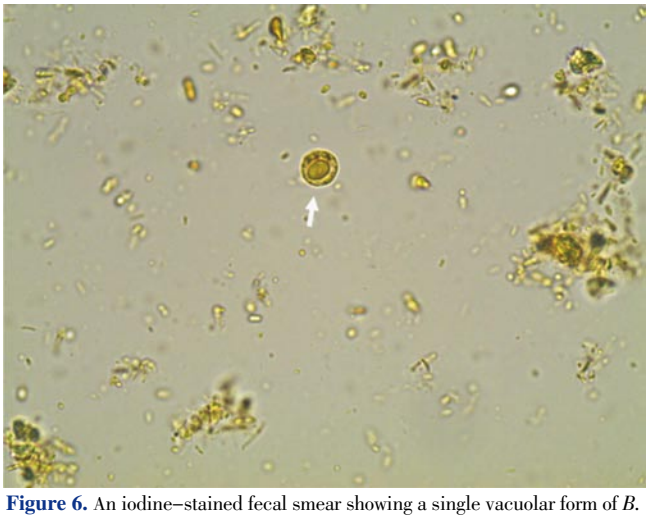

Figure 6. An iodine-stained fecal smear showing a single vacuolar form of *B. hominis* (×1 000).

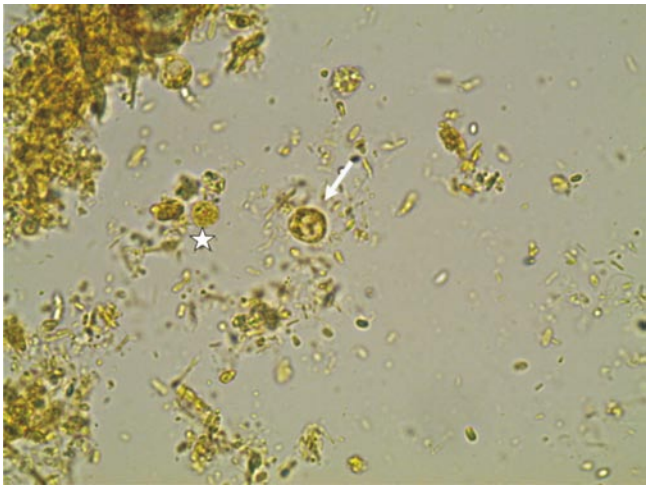

Figure 7. An iodine-stained fecal smear showing vacuolar (arrow) and granular forms (star) of *B. hominis* (×1 000).

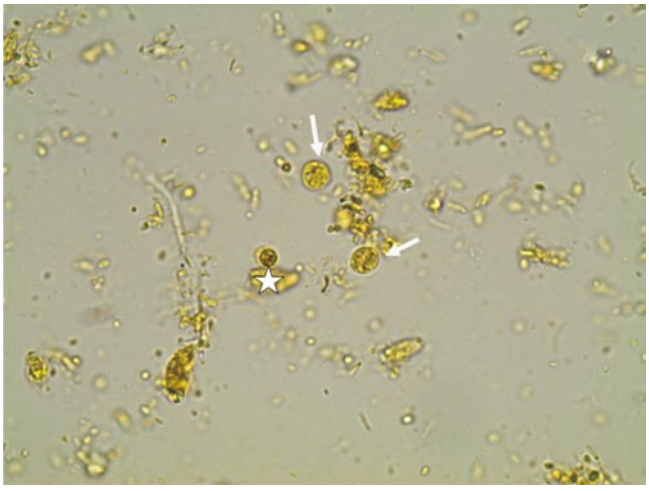

Figure 8. An iodine-stained fecal smear showing granular (arrows) and cystic forms (star) of *B. hominis* (×1 000).

Using SMB staining technique, only the cystic form was identified. It appeared as blue rounded or oval bodies, with faintly-stained center containing one or more deeply stained nuclei. The background appeared pale blue (Figure 9). This stain was cheap, rapid and easy.

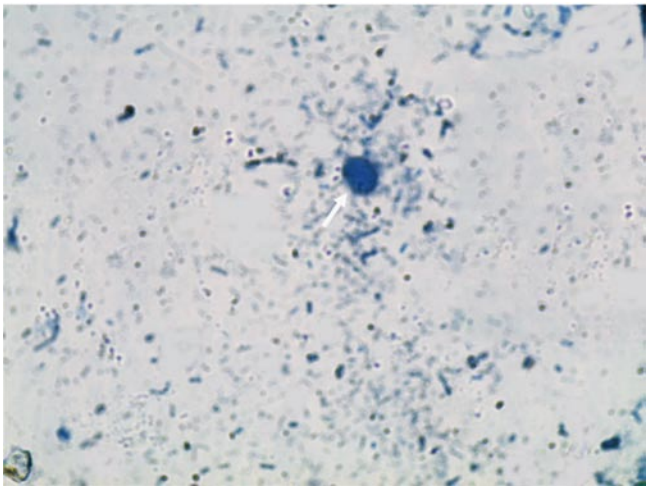

Figure 9. A SMB-stained fecal smear showing a cystic form (arrow) which appears as blue oval body with dark-stained wall and faintly-stained center containing deeply-stained nuclei (×1 000).

Regarding sensitivity and specificity of the studied stains, IFA stain was taken as the gold standard to which all other stains were compared. The sensitivity of MZN stain, iodine stain and SMB stain was 68.2%, 59.1% and 50% respectively. While the specificity was 100% for all stains (Table 2).

Table 2
Sensitivity and specificity of different stains.

	MZN stain	Iodine stain	SMB stain
Sensitivity	68.2%	59.1%	50.0%
Specificity	100%	100%	100%
PPV	100	100	100
NPV	53.3	47.1	42.1
Accuracy	76.7%	70.%	60.%

PPV: positive predictive value (probability that the disease is present when the test is positive); NPV: negative predictive value (probability that the disease is absent when the test is negative).

4. Discussion

The emergence of, the previously considered commensal, *B. hominis* as the most common protozoon reported in human fecal samples with a prevalence that often exceed 5% in the general population of the industrialized countries and can reach to 30%-60% in the developing countries[2,21]. The accumulating *in vivo* and *in vitro* studies that strongly suggested its pathogenicity have given this parasite a greater interest in recent years[4,5,24]. This increased interest in *Blastocystis* infection has brought light into two major problems facing medical practitioners. Firstly, the physicians are lack of experience. This parasite is a causative agent for gastrointestinal troubles and therefore its diagnosis is not routinely requested. Secondly, *Blastocystis* was ignored by most laboratory parasitologists and technicians either due to inexperience or use of insensitive unsuitable methods to detect this parasite[14,25].

In the current study, the prevalence rate of *B. hominis* infection was 53% (159/300). This could be considered the highest one as compared to the previous prevalence reported in Egypt. In Ismailia city, many authors reported prevalence rates of 10.0% in school children, 33.3% and 10.0% in population respectively[11,26]. A prevalence of 46.6% was reported in Shibin El Kom, Menoufiya Governorate among 250 food handlers[12]. Also, another study reported a prevalence rate of 24.4% in Dakahlia Governorate[27]. In Cairo, a prevalence rate of 34.5% was recorded and found that it was significantly higher (54.2%) in the iron deficiency anemia group. This high prevalence reported in the present work may be referred to the young age (2-5 years) of the studied group of the children in a rural area and their presence together in crowded settings in nurseries where bad personal hygiene facilitate feco-oral transmission. Moreover, most of those children use unfiltered water for drinking[13].

Moreover, this high prevalence in Egypt is in accordance with other findings of other studies in developing countries as Senegal, where the prevalence of *B. hominis* was the highest prevalence ever recorded worldwide (100%), Brazil (40.9%), Cuba (38.5%) and Argentina (27.2%)[28-31]. This high prevalence in the developing countries is referred to the low socioeconomic status, the unsafely of drinking water, the consumption of contaminated food and the bad personal hygiene. On the other hand, the prevalence of *B. hominis* is low in developed countries such as United States (23%), Japan (0.5 to 1%) and Singapore (3.3%)[32-34].

In the current study *B. hominis* was detected alone in 145 samples (91.2%). Double infections were found in 14 samples (8.8%), where it was found in association with *E. histolytica* in 11 samples (6.9%) and with *G. lamblia* in 3 samples (1.9%). In Egypt, *G. lamblia*, *E. histolytica* and *Cryptosporidium* were found also mixed with *Blastocystis* in 250 food handlers[12]. The prevalence of *Blastocystis* was studied among 400 Egyptian and Libyan food handlers and reported that 8.5% of total cases were mixed with other parasites mainly *G. lamblia*, *E. histolytica* and *Entamoeba coli*. All these co-infecting parasites are transmitted by feco-oral route which emphasizes the importance of this route for transmission of *B. hominis*[22].

In this study, a higher prevalence was detected in children aged 4-5 years old than younger groups, and in males (59.8%) than females (44.9%). This may reflect a higher risk of acquisition of *Blastocystis* infection in older children than younger ages and in males than females. This risk factor is probably related to the older children's behavior such as playing with their collegues on unclean floor areas or playgrounds, poor toilet training and handling of contaminated food. In contrast, younger children had been taken care of by their childcare workers. Also, female children are less active with less contact with contaminated things than males who are more prone to contaminate their hands during exploration of their environment. These results are similar to those reported in several studies, where *B. hominis* infection was more prevalent in older than younger children and in males than females[32,35,36]. On the contrary, a study reported a higher prevalence in females than males, another one reported significant reduction in *B. hominis* infection prevalence rate in older children when compared with younger children[37,38]. A third study reported high prevalence of this infection among all age groups (6-14 years old)[39].

In respect to the association of the prevalence of *B. hominis* with the different seasons of the year, it was found that the highest prevalence was in the summer (85.1%). In accordance with this finding, is the higher prevalence reported in hot climates[40]. On the other hand, no variation at different periods all over the year was reported in another one[41].

With respect to the pathogenicity of *B. hominis*, two findings in the current study favor the pathogenic nature of *B. hominis*. The first is the high percentage of symptomatic cases (82.8%) in comparison to the asymptomatic cases (17.2%). The second is

the positive correlation between the intensity of infection and the clinical presentation (mean 5.25 cells/HPF) in symptomatic cases compared to (mean 2.12 cells/HPF) in asymptomatic cases. These findings are in accordance with other studies who reported a higher parasite burden in symptomatic cases than asymptomatic cases and all of them accepted intensity of *B. hominis* above 5 organisms/HPF as a pathogenic criterion[42,43]. However, a number of studies reported a lack of such a correlation[3,44]. The reasons for this discrepancy may be due to genotype differences among *Blastocystis* isolates or to host factors such as age and genetic background variations in the population studied.

Concerning the efficacy of different stains for detection of *B. hominis* in stool samples, MZN stain could be considered cheap, rapid and good stain in relation to the number of positive cases (159/300, 53.0%). Also, all forms of *Blastocystis* were identified easily. Moreover, it is the only staining method that detected the amoeboid form and the surface coat (around the cystic and the multi-vacuolar forms). SMB staining techniquewas also rapid and cheap, but it recovered a lower number of positive cases (110/300) and detected the cystic form only. This may be due to the heating step used in this technique which may damage the other forms of the parasite. Two studies compared different methods for identification of *B. hominis* including MZN and SMB stains and concluded that both were rapid, simple, permanent, cheap and easy to perform and had the advantage of staining the cyst and the rare amoeboid form. Using MZN and SMB stains, the color contrast wasn't good (the parasite and the background had the same color but with different degrees)[12]. This could be interpreted by the fact that *B. hominis* isn't an acid fast parasite and therefore stained with the counterstain used in MZN or SMB stain (malachite green or methylene blue respectively)[45].

Regarding iodine stain, it was the most rapid, the simplest, the cheapest and the easiest stain. Moreover, it facilitated the detection and identification of different forms of the parasite. However, *Blastocystis* may be confused with yeast, fat globules or artifacts using this stain. This could be attributed to that it isn't a permanent stain, where using a permanent stain was supposed to increase the chance of recovering protozoa[23].

With regard to the IFA stain, it was applied on 30 cases only because of its high cost. This stain proved to be the most sensitive method detecting 22 positive cases out of 30 (73.3%). All samples found positive by other methods were also positive by IFA stain. Moreover, this method detected a number of positive cases that were false negative by other stains. *Blastocystis* cells appeared as brightly fluorescent rounded cells of varying sizes distinct from the dark background, making the diagnosis very rapid, easy and reliable even at low magnification. This may help to put an end to the inconsistency between different laboratories as great differences in the results regarding the detection of *Blastocystis* have been found among laboratories using a shared set of stool samples[46]. This result coincides with the only study used IFA stain for detection of *Blastocystis* in humans[21]. These authors compared the efficacy of IFA stain, trichrome stain, iodine stain and PCR for detection of *Blastocystis* in 30 stool specimens diagnosed positive for *B. hominis* by *in vitro* culture and stated that the IFA stain had the advantage of providing a positive result in a short time and had a clearer visual indicator of positive and negative status. These authors found no cross reactivity with *G. lamblia*, *Entamoeba coli*, *Candida*, human leucocytes and human erythrocytes.

The present study declared that the vacuolar form was the most common to be detected by all stains other than IFA stain. This coincides with other studies[12,47], who noted that the vacuolar form was the most common form. Moreover, the vacuolar form was considered as the typical form used to diagnose *B. hominis*[42].

Comparing the sensitivity of different stains with the IFA stain (as a gold standard), it was found that MZN stain had the highest sensitivity (68.2%) then iodine staining (59.1%) and lastly SMB stain (50.0%). With respect to the feasibility for use as a routine test for diagnosis of *Blastocystis*, MZN stain is more feasible than other techniques especially in laboratories with limited funds as in most of the developing countries as in Egypt. This is because this stain had a higher sensitivity than iodine and SMB stains. Moreover, it is cheap, easy to perform and the definitive morphological details of the variable forms of *Blastocystis* were easy to be found.

Although having the highest sensitivity and facilitating easy identification of *Blastocystis* cells which appeared brightly fluorescent against a dark back ground at a low magnification within a short time, the IFA stain can be suitable only for use in large hospitals or public health laboratories in developed countries because of its high cost.

This study confirmed the pathogenic potential of *B. hominis* on the basis of the higher percentage of symptomatic cases together with the observed significant increase in the parasite burden in these patients compared to the asymptomatic ones. So, there is a great need to promote the hygienic standards among the risky group of children as well as to create health awareness of their parents and the child care workers about modes of infection and the prophylactic measures to avoid this infection. Moreover, IFA staining technique though having the highest sensitivity, is suitable only for use in large hospitals or public health laboratories in developed countries because of its high cost. The MZN staining technique had a significantly higher sensitivity than iodine and SMB stains and is more feasible to be used in laboratories with limited funds as in Egypt and other developing countries because it is cheap, rapid, easy to perform and clarify the definitive morphological details of the parasite. Further studies are suggested to estimate the prevalence of *Blastocystis* among other vulnerable populations and determine the subtypes

among the different isolates.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Few authors studied the prevalence of *B. hominis* in Egypt including Qualyobia Governorate, Ismailia City, Menoufiya Governorate and Cairo Governorate. However, until now no study was done on the prevalence rate of these parasites in any area of Gharbeya Governorate. And also, diagnosis of *Blastocystis* is considered a challenge to diagnostic laboratories leading to underestimation, difficulty of the identification.

Research frontiers

The authors investigated the prevalence of *B. hominis* among pre-school children and evaluate several staining methods for the detection in stool. The majority of cases infected with *Blastocystis* alone were symptomatic. *B. hominis* shoud be considered as a pathogenic parasites, and IFA stain was probed to be the most sensitive stains.

Related reports

The possibility of *Blastocystis* being a pathogen has long been a matter of debate. Although recent accumulation of clinical evidence suggests the pathogenic potential of *Blastocystis*, it should be put in mind that it is still not proven conclusively till date. Certain populations may be susceptible to the parasite infection including immunocompromised persons and young children.

Innovations & breakthroughs

Results showed a higher prevalence was detected among children aged 4-5 years and most of them were males. The majority of cases infected with *Blastocystis* alone were symptomatic. These results confirm that *B. hominis* shoud be considered as a pathogenic parasites, and also IFA stain was probed to be the most sensitive stains.

Applications

The research is useful for parasitologists, lab personnel, paediatricians, and public health specialists. It gives clear differentiations among various diagnostic techniques.

Peer review

This study confirmed the pathogenic potential of *B. hominis* in children. The hygienic standards among the risky group of children are needed to promote. Although IFA is the highest sensitivity, its cost is high. The MZN staining technique is also higher sensitivity than iodine and SMB stains, and was more feasible to be used in laboratories with limited funds.

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