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Evaluation of Jones' Medium Culture versus Locke Egg Medium in Diagnosis of *Blastocystis Hominis*

Laila A. Hegazy[1], Marwa A. Salama[1], Eman M. Fawzy[1], Amira A. Saleh[1], Amany A. Maghawry[1]
[1]Department of Medical Parasitology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Abstract

Background: *Blastocystis* is a widely distributed protozoa. *Blastocystis hominis* suspected to be possible cause of irritable bowel syndrome, and chronic diarrhea. It is transmitted via fecal-oral route through ingestion of cysts. Laboratory diagnosis of *Blastocystis* infection is usually via microscopic examination of freshly stained or chemically preserved stool samples but it lacking the sensitivity. The current study is concerned with evaluation of sensitivity and specificity of Jones' medium culture in comparison to Locke egg medium for diagnosis of *Blastocystis hominis*.

Subjects and Methods: Stool samples were collected from seventy two people, complaining of different gastrointestinal symptoms and attending to different Outpatient Clinics of Zagazig Hospitals. All samples were examined microscopically by wet mount, saline, iodine then preserved for staining by Modified Ziehl-Neelsen (MZN) and trichrome stain and were cultured on Boeck and Drbohlav Locke egg serum medium (LE) and Jones' medium , then examined by light microscope with objectives x10, x40 and x100.

Results: *Blastocystis hominis* infection was common in younger aged males (14-35years). Usual methods for diagnosis of *Blastocystis* e.g simple saline, iodine stained smears, MZN and trichrome stain are simple, specific, non-invasive methods but with low sensitivity. Stool culture was more accurate and sensitive for detection of *Blastocystis* than direct microscopy. LE medium was more accurate and gave better result than Jones' medium culture.

Conclusions: LE medium culture offers better sensitivity than other ordinary methods and Jones' medium culture for detecting *B. hominis* infection in stool samples. It is appropriate for use in large hospitals or public health laboratories.

Keywords: *Blastocystis hominis,* , Jones' medium culture ,Boeck and Drbohlav Locke egg (LE) serum medium

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List of abbreviations:

MZN: Modified Ziehl-Neelsen.

LE: Boeck and Drbohlav Locke egg serum medium.

IFA: immunofluorescence.

Blastocystis hominis: *B.*hominis.

LJ: Lowenstein-Jensen medium.

1. Introduction:

Blastocystis, anaerobic enteric parasite, is one of the commonly detected parasite in a variety of vertebrates throughout the world **[1].** Its prevalence ranged from 10-70% in different countries with adults being more infected than children **[2, 3].** High infection rates are related with low socio-economic standard, inadequate, increased population density, unhygienic disposal of faeces, clean water sources and immune-compromised patients predominantly those infected with human immunodeficiency virus **[4].**

The pathogenic role of *Blastocystis* remains arguable. Some scholars say about the harmfulness of *Blastocystis* in humans due to its link with asymptomatic carriers **[5]**, though others are with the faith of the possible pathogenic role it may play in symptomatic cases **[6].** *Blastocystis* has been related with irritable bowel syndrome, inflammatory bowel disease, chronic diarrhea and ulcerative colitis **[7, 8].**

Blastocystis hominis has multiple morphological forms: vacuolar, granular, amoeboid, cyst, multivacuolar, and avacuolar forms **[9]** and a proposed precyst form **[10].** So, it provides significant challenges for laboratory diagnosis because of the possibility of confusion with yeast, *Cyclospora* spp. or fat globules **[11].** A better finding method to distinguish therapies which eradicate the parasite from those providing short-term symptomatic improvement is an urgent requirement **[12].**

Diagnosis of Blastocystis infection is usually via microscopic examination of stained, or chemically preserved faecal specimens, however, they are not often detected because of their variable shedding and polymorphic nature that may possibly lower the sensitivity of direct examination of stool samples with ordinary staining techniques **[13].**

Many scholarships recommended that stool culture may be the most sensitive technique for detection of *Blastocystis* **[16, 17],** together with immunofluorescence (IFA) assay and enzyme-linked immunosorbent assay (ELISA) **[18].** Short-term (24 to 72 hrs.) in-vitro cultivation rise *Blastocystis* detection sensitivity in comparison with microscopic methods **[14].**

In vitro techniques would be an brilliant choice being economically better in screening large sample size **[4],** in addition obtaining a higher concentration of the requested genetic material for molecular testing **[19].** Due to the light infection with *Blastocystis* spp. is common and organisms can be simply lost by direct microscopy, combination of simple smear and culture method may give the standard method for detecting *B.hominis* in patient specimens **[20].**

In this work we aimed to evaluate the sensitivity and specificity of culture test (LE medium) in comparison with Jones' medium for diagnosis of *Blastocystis hominis*.

2. Methods:

The study was performed in the Parasitology Department, Faculty of Medicine, Zagazig University, during the period from March to July 2019

2.1 Samples collection:

72 fresh faecal specimens were collected from patients with/without gastrointestinal symptoms attending Zagazig hospitals. From all patients, information were obtained including full history taking (age, sex, residence, sanitary habits, source of food/water, GIT troubles, and history of previous illness or receiving drugs), patients with diarrhea were asked about period of illness, number of motions/day, character of stool and previous similar attacks. Patients receiving antiparasitic or immunosuppressive drugs were omitted. Samples were collected in clean dry labeled cups, with tight fitting cover.

2.2 Processing of samples:

Each collected sample was separated into two parts, one part saved fresh to be examined microscopically by wet mount, and iodine stain using objective lenses X10 and X40 respectively **[21]** and for culture. The second part saved in formalin 10% then prepared for staining by Modified Ziehl-Neelsen stain **[22]** and trichrome stain **[21]** to exclude the possibility of a mixed infection with other parasites.

2.3 Isolation & Cultivation of *Blastocystis:*

Stool samples were cultivated immediately in 5ml screw caped tube containing a previously prepared LE media and Jones' medium supplemented with 10% fetal bovine serum (Biowest), antibiotic mixture ready-made antibiotic mixture of penicillin (10000 U/ml) and streptomycin (10000 µg/ml) (Biowest) and antifungal (diflucan) at 37°C for 2-3 days **[20].** Culture tubes were diagnosed for Blastocystis multiplication rate by light microscopy every 48hr-72hr. The sediment was examined with or without iodine by (×10, ×40 and ×100) objectives, when the characteristic vacuolar/granular forms of Blastocystis were detected, a sub-cultured in a new medium was performed. Sub-cultivation was performed every 3days.

2.4Ethical Considerations: The study was allowed by Institutional Review Board, Faculty of Medicine, Zagazig University. The purpose and procedures of the study were clarified to all participants, and a written well-versed consent was obtained from all cases and from parents on

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behalf of their children.The study was permitted by Institutional Review Board, Faculty of Medicine, Zagazig University.

2.5Statistical analysis:

Data were recorded, calculated, tabulated and statistically analyzed using statistical computer program SPSS version **18.0**. Qualitative data were represented as frequencies and percentages. Chi square test was used to calculate difference between qualitative variables.

3. Results:

Out of 72 studied cases (44 males and 28 females) with an age range of (2 – 60) years, *B. hominis* was detected in 30 cases (41.7%), males were more affected than females with an average age range of 14-35 (table1). Within *Blastocystis* positive cases, only 19 cases (26.38 %) were only infected with *B.hominis*, and 11 cases (15.3%) were mixed infection of *B.hominis* and other protozoa. The most common associated infection was *Cryptosporidia* followed by *Entamoeba hitolytica*, *Entamoeba coli* and *Giardia lambilia* with percentage of infection was (6.94%, 4.2%, 2.8% and 1.4%) respectively (table 2)(fig1,2). When comparing the frequency of detection of *Blastocystis* by microscopic methods and Jones' medium culture in comparison to Locke egg medium, we found that positive cases were (15.3%, 20.8%, 25% and 30.6%, 41.7%) by direct saline, iodine, MZN, trichrome stain and LE medium respectively (table 3)(fig 4). Concerning the sensitivity, specificity and accuracy of two culture tests for detection of *B.*hominis with LE medium as gold slandered, trichrome staining was the most sensitive method followed by MZN, iodine staining and direct saline with a sensitivity of 73.3%, 60%, 50%, 36.7% and specificity 100% for all methods and accuracy of 88.9%, 83.3%, 79.2%, 73.6% respectively (fig 5) with statistical significance difference between them. Comparing between Jones' medium and LE medium, we found that the mean number of live *Blastocystis* on LE medium was more than Jones' medium with statistical highly significance difference. Also there were highly statistical highly significance increase in count in each medium after 24, 48 & 72 hrs. [table (4), fig. (6,7,8)].

4. Discussion:

Blastocystis is the most common protozoa detected in the stools of humans and many animals worldwide **[11].** The pathogenic role of *Blastocystis* in humans not yet proved **[23].** In our study, we found that the prevalence of *B. hominis* was 41.7% (30/72). Other studies in Egypt stated similar finding. **Abd El Wahab and Selim [24],** said a prevalence rate of 46.6% in Shibin El

Kom, Monofiya Governorate, and **Farghaly et al. [25],** who reported that *B. hominis* infection rate was 42.3%. About age groups, we found that the highest prevalence of infection was among the age group 14-35 years old (46.7 %) followed by 36-50 years (26.7%), >50 year's (16.6%) and finally 2-13 years old (10%) with no statistically significant difference between different age groups table (1). our finding was in accordance with **Gabr et al. [26],** who stated that the highest prevalence rate of *B. hominis* infection was observed in two age groups (15-30 years and >30 years) and the age group (< 15 years) showed the lowest positive percent. On the other hand, other studies found that the *Blastocystis spp.* infection was more prominent in the old age group **[27].** Also, **El Safadi et al. [28]** who reported that patients aged 0-14 years were mostly affected (26.3%) followed by patients aged 15-49 years (22.2%) and patients aged over 50 years were the least affected (13.6%). Our finding can be recognized to the more frequent exposure to sources of infections as young adults commonly favor the junk food. Among *B. hominis* infected cases, males (60%) were more exposed to infection than females (40%) with no statistically significant difference between them (table1) .This was in agreement with **Farghaly et al. [25]** who reported that *B. hominis* infection rate among males (69.4%) was higher than females (30.6%) and **Nithyamathi et al. [4]** who reported that *Blastocystis* infection rate among males (12%) was higher than in females (9%). This could be due to the Egyptian customs and traditions outdoor activities that make them more liable to infection than females. On the other hand, higher prevalence rates were recorded in females **[29].**

Diagnosis of *Blastocystis* relies mainly on microscopy using ordinary staining methods (trichrome, MZN, Sufarine methylene blue and iodine), but it is of low sensitivity due to its variable shedding and polymorphic nature **[13].** On microscopic examination of stool samples, we observed that, the vacuolar form of *Blastocystis* spp. was the most common obvious form (fig 3). This was in agreement with **Mehta et al. [30], Darabian et al. [31] and Gabr et al. [26].**The vacuolar form of *Blastocystis* spp. is the diagnostic stage and could be easily illustrious from other protozoa.

In our study, we considered LE culture media as the gold standard for diagnosis of *B. hominis* infection. It was proved to be the most sensitive method detecting 30 positive cases (41.7%) out of 72 examined samples. All positive samples by other methods were also positive by LE media with no false positive detection in all methods. Moreover, this method detected positive cases that were negative by other tests. Trichrome stain was time consuming, this make it unsuitable

for use in the survey study. In the current study, when comparing between Jones' medium and LE medium , we found that the mean number of live *Blastocystis* on LE medium was more than Jones' medium with statistical highly significance difference. Also there were highly statistical highly significance increase in count in each medium after 24, 48 & 72 hrs. [table (4), fig. (6, 7,8)].So, LE medium showed the highest mean number of live *Blastocystis* compared to Jones' medium. This agrees with **Saksirisampant et al. (2010)** who found that the prevalence of *B.hominis* by using simple smear was 0.90% which was not significantly different when compared to the concentration technique (1.05%) while LE medium (43.67%) and Jones' medium (22.15%) and showed statistically significant difference from the Jones' medium. Also with **Padukone et al. (2018)** who found that all positive samples by Jones' medium were positive by LE medium but Lowenstein-Jensen medium (LJ) was able to support the growth of *Blastocystis* in only two samples.

LE medium has the advantage of being less cost effective than that of the Jones' medium, since the hen egg consistent in LE medium is cheaper than imported yeast extract. Moreover, hen egg could eliminate concerns about expiration dates of yeast extracts. This consideration is particularly suitable for laboratories in developing countries.Moreover, the efficient reproduction of *B.hominis* in LE medium may also be suitable for antigen preparation, *in vitro* drug sensitivity studies and organism harvesting prior to PCR **(Saksirisampant et al., 2010).**

The combination of simple smear and cultivation may provide the standard approach for detecting *B.hominis* in patient specimens. Culture technique needs less expertise to perform than those of molecular biological and immunological techniques for laboratory diagnosis **(Termmathurapoj et al., 2004 and Su SL et al., 2007)**.

5. Conclusion:

Locke egg serum medium is simple, rapid, easy to perform with high sensitivity, so it could be an alternative to direct microscopy, for that reason, we recommend its use in large hospitals and public health laboratories in developing countries.

6. Declaration of interest:

The authors affirmed that there is no conflicts of interest.

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7. Funding information:

None declared

Table(1): *B. hominis* positive (+ve) and negative (–ve) cases regarding age and sex

Variable	+ve cases (n=30)		-ve cases (n=42)		χ^2	P
	No	%	No	%		
Age group:						
• 2 – 13 y	3	10	6	14.3	4.11	0.25 NS
• 14 – 35 y	14	46.7	10	23.8		
• 36 – 50 y	8	26.7	16	38.1		
• > 50 y	5	16.6	10	23.8		
Sex:						
• Male	18	60	26	61.9	0.03	0.87 NS
• Female	12	40	16	38.1		

χ2: Chi square test

NS: Non significant (P>0.05)

Table (2): Associated infections among the studied groups:

Variable	(n=72)		χ2	P
	No	%		
No infection (-ve cases)	42	58.33		
(+ve cases)	30	41.7	**18.18**	**<0.001 ****
Blastocystis alone	19	26.38		
Mixed infection	11	15.3		
B. hominis with *Cryptosporidia*	5	6.94		
B. hominis with *Entamoeba histolytica*	3	4.2		
B. hominis with *Entamoeba coli*	2	2.8		
B. hominis with *Giardia Lambilia*	1	1.4		

**: Highly significant (P<0.01)

χ2: Chi square test

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Table (3): Comparison between different diagnostic methods for detection of *Blastocystis* among studied groups.

Variable	(n=72)			
	No +ve	**%**	**No** -ve	**%**
Direct saline wet mount	11	15.3	61	84.7
Direct Iodine stain	15	20.8	57	79.2
MZN	18	25	54	75
Trichrome	22	30.6	50	69.4
Culture on jone's medium	24	33.3	48	66.7
Culture on LE medium	30	41.7	42	58.3

Table (4): Comparison between the mean number of live *Blastocystis hominis* on LE and Jone's medium at different follow up periods:

Live *Blastocystis*	24 Hours	48 Hours	72 Hours	P^
	No. of cell × 10^4 Mean±Sd	No. of cell × 10^4 Mean±Sd	No. of cell × 10^4 Mean±Sd	
LE Medium	105±2	227±4	336±4	<0.001**
Jones Medium	54.33±2.5	86±3	110±3	<0.001**
T	27.3	48.84	78.29	
P	<0.001**	<0.001**	<0.001**	

****: Highly significant (P<0.001)**

All means are count of live *B hominis*.

t: Independent t test P^: Repeated measure ANOVA test

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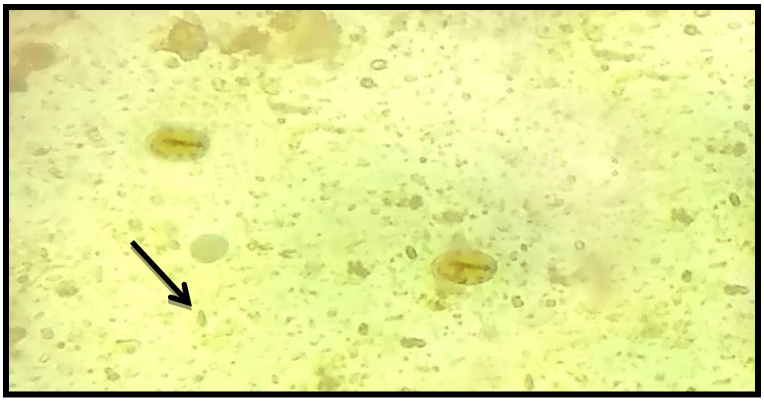

Fig. (1): Stool smear stained with iodine showing cystic form of *Giardia* (X1000).

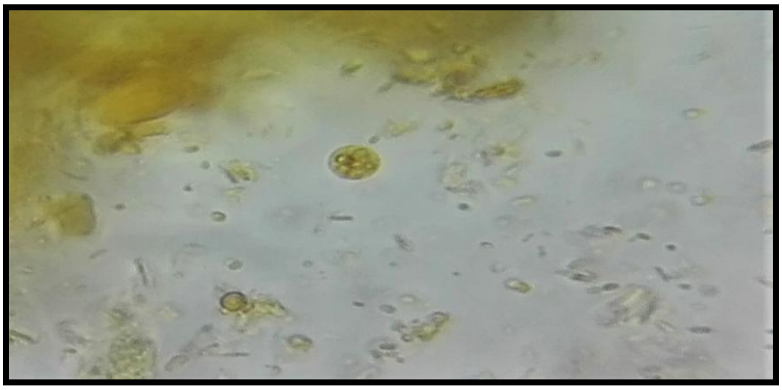

Fig. (2): Stool smear stained with iodine showing *E. coli* cyst (x400)

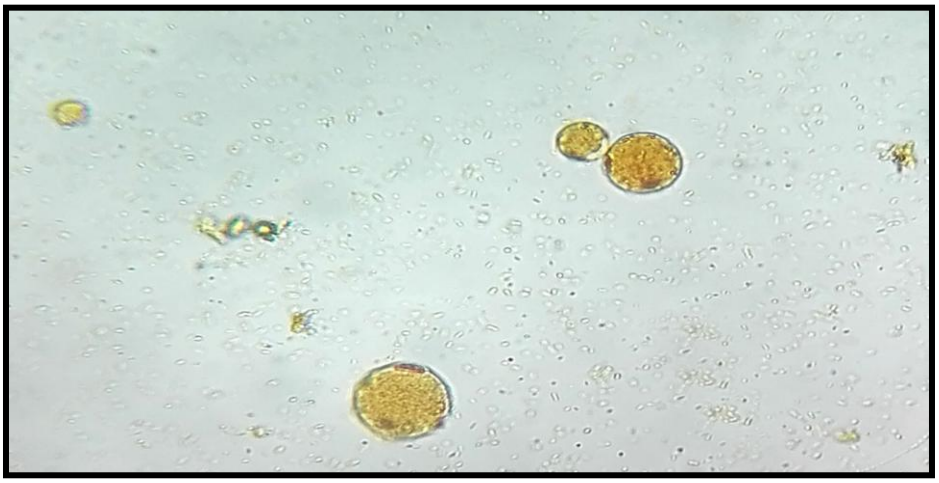

Fig (3): Culture smear stained with iodine stain, showing vacuolar form of *B. hominis* (x1000).

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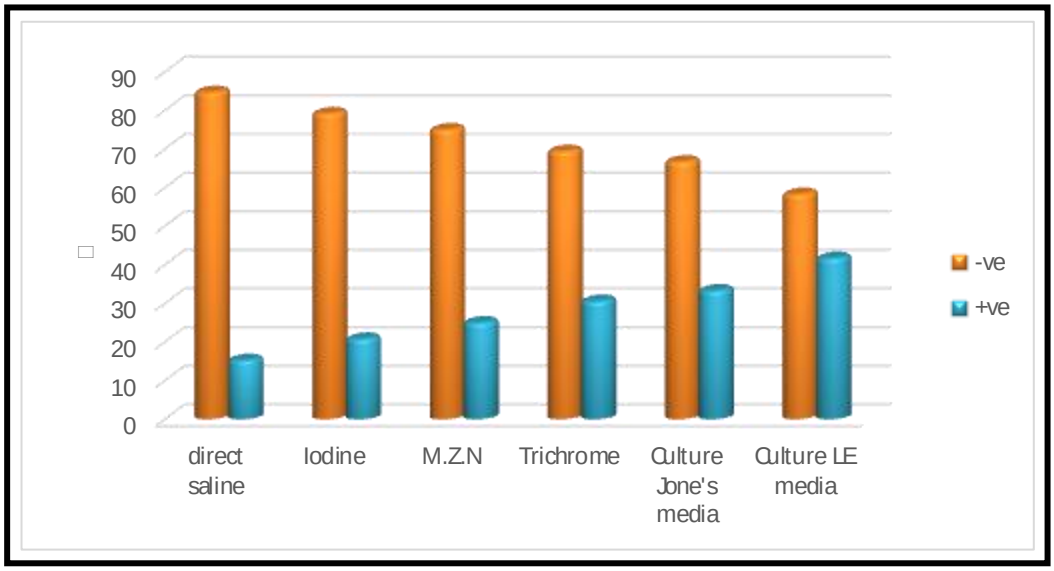

Fig. (4): Frequency of detection of Blastocystis hominis infection among the studied groups by different diagnostic methods.

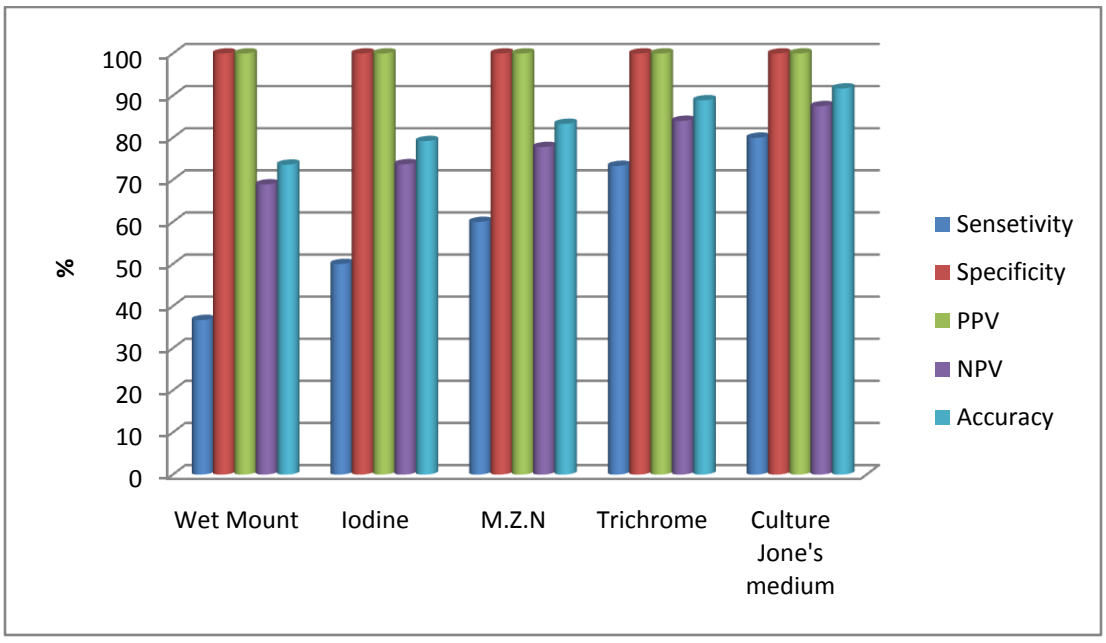

Fig. (5): Validity of different methods in diagnosis of *Blastocystis hominis* in comparison to LE medium as Gold standard

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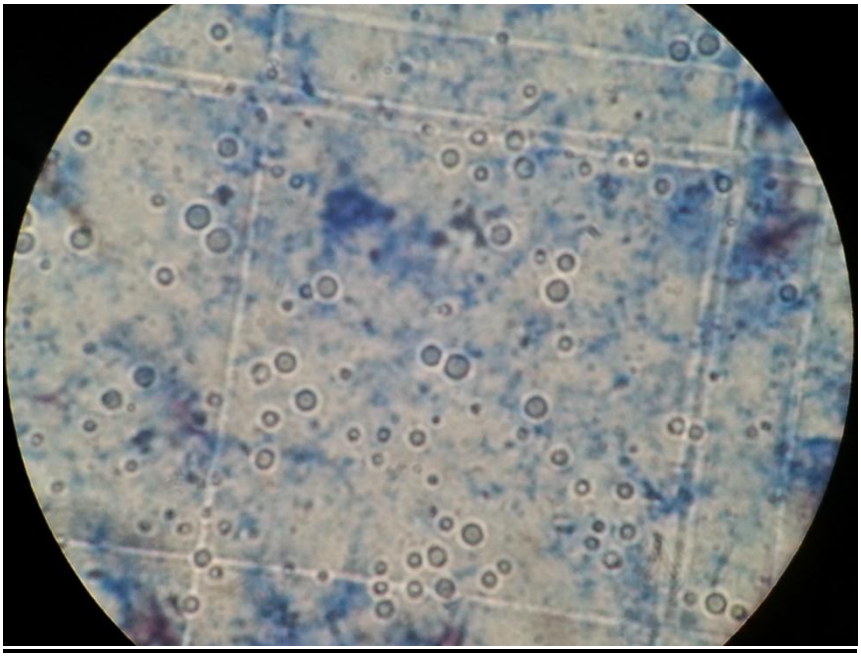

Fig. (6): Culture smear stained with trypane blue stain using hemocytometer showing live cell of B. hominis on LE medium without exposure to extracts or nitazoxanide (x400).

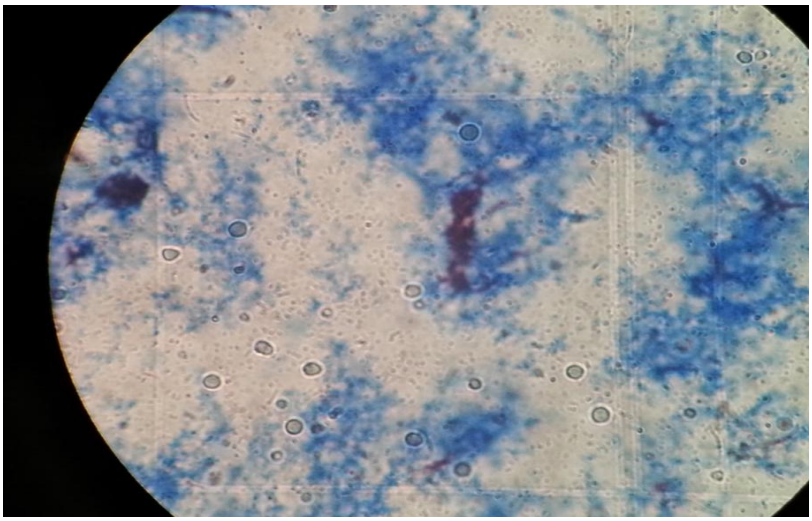

Fig (7): Culture smear stained with trypane blue stain using hemocytometer showing live cell and dead cell of *B. hominis* on jones medium without exposure to extracts or nitazoxanide. (x400).

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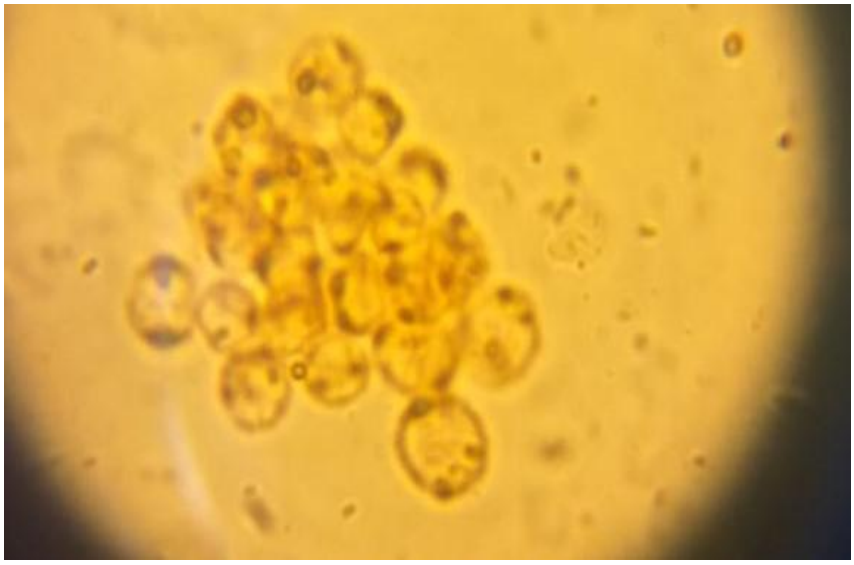

Fig (8): Culture smear stained with iodine stain showing *B. hominis* on LE medium without exposure to extracts or nitazoxanide

REFERENCES:

1. Yoshikawa, H, Koyama, Y, Tsuchiya, E, Takami, K. *Blastocystis* phylogeny among various isolates from humans to insects. Parasitol. Int.2016; 65, 750–759.

2. Wong, KH, Ng, GC, Lin, RT, Yoshikawa, H,Taylor, MB, et al. 2008: Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. Parasitol. Res. 2008;102:663-70.

3. El Safadi, D, Gaayeb, L, Meloni, D, Cian, A, Poirier, P, et al. Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infect. Dis.2014; 14, 1:1-9.

4. Nithyamathi, K, Chandramathi, S, Kumar, S. Predominance of *Blastocystis* sp. Infection among School Children in Peninsular Malaysia. Plos One,2016; 1-14.

5. Lepczy´nska, M, Białkowska, J, Dzika, E, Piskorz-Ogórek, K, Koryci´nska, J.Blastocystis: How do specific diets and human gut microbiota a_ect its development and pathogenicity? Eur. J. Clin. Microbiol. Infect. Dis. 2017;36, 1531–1540.

6. Gonzalez-Arenas, NR, Villalobos, G, Vargas-Sanchez, GB, Avalos-Galarza, CA, Marquez-Valdelamar, LM, Ramirez-Miranda,ME, Olivo-Diaz,A, Romero-Valdovinos,M, Martinez-

Hernandez, F, Maravilla, P. Is the genetic variability of Cathepsin B important in the pathogenesis of *Blastocystis* spp.? Parasitol. Res.2018; 117, 3935–3943.

7. Cifre, S, Gozalbo, M, Ortiz, V, Soriano, JM, Merino, JF, Trelis, M. *Blastocystis* subtypes and their association with irritable bowel syndrome. Med. Hypotheses, 2018 ;116:4–9.

8.***Krüger, K.; Kamilli, I. and Schattenkirchner, M.*** *Blastocystis hominis* as a rare arthritogenic pathogen. A case report. Z. Rheumatol.1994; 53 (2): 83-5

9. Tan, K, Singh, M, Yap, EH. Recent advances in *Blastocystis hominis* research: hot spots in terra incognita. Int. J. Parasitol., 2002; 32:789–804.

10. Suresh, K, Venilla,G, Tan ,T, Rohela, M. In vivo encystation of *Blastocystis hominis*. Parasitol Res .2009;104 (6): 13731380.

11. Tan, TC, Suresh, KG, Smith, HV. Phenotypic and genotypic characterization of *Blastocystis hominis* isolates implicates subtype 3 as a subtype with pathogenic potential. Parasitol. Res., 2008;104:85–93.

12. Smith, HV, Stensvold, CR, Nagel, R, Olsen, KE, Traub, RJ. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? J. Clin. Gastroenterol., 2010; 44: 85–90.

13. Stensvold, CR, Smith, HV, Nagel, R, Olsen, KE, Traub, RJ.Eradication of *Blastocystis* Carriage with antimicrobials: reality or delusion? J. Clin. Gastroenterol.,2010; 44 (2): 85-90.

14. Stensvold, CR, Arendrup, M, Jespersgaard, CC, Mølbak, K, Nielsen, HV, Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. Diagn. Microbiol. Infect. Dis.,2007; 59:303–307.

15. O ¨zcakir, O, Gu¨reser, S, Ergu¨ven, S, Yilmaz, YA, Topalo˘glu, R, Has¸celik, G.Characteristics of *Blastocystis hominis* infection in a Turkish university hospital. Turkiye Parazitol. Derg.,2007; 31:277–282.

16. Leelayoova, S, Taamasri, P, Rangsin, R, Naaglor, T, Thathaisong, U.In-vitro cultivation: a sensitive method for detecting *Blastocystis hominis*. Ann. Trop. Med. Parasitol.,2002; 96: 803–807.

17. Arendrup, MC, Jespersgaard, C, Molbak, K, Nielsen, HV.Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. Diagn. Microbiol. Infect. Dis.,2007; 59: 303–307.

18. Garcia, LS, Shimizu, RY, Bernard, CN.Detection of *Giardia lamblia, Entamoeba histolytica/Entamoeba dispar*, and *Cryptosporidium parvum* antigens in human fecal specimens using the triage parasite panel enzyme immunoassay. J. Clin. Microbiol .,2000; 38: 3337–3340.

19. Piekara-Stępińska, A, Gorczykowski, M, Piekarska, J.Suitability of selected culture media for *Blastocystis* spp. Polish Journal of Veterinary Sciences,2008; 21(4):815–817.

20. Saksirisampant, W, Nuchprayoon, S, Pradniwat P,et al.Boeck and Drbohlav Locke egg serum medium for detection of *Blastocystis hominis*. Chula Med. J.2010; 54, 6:527-36.

21. Cheesbrough, M.Medical Laboratory Manual for Tropical Countries. ELBS Tropical Health Technology Butterworth,1987; 1: 561-564.

22. Casemore, DP, Armstrong, M, Sands, RL, 1985: Laboratory diagnosis of cryptosporidiosis .J.Clin.pathol., 38:1337-1341.

23***. Taamasri, P, Leelayoova, S, Rangsin, R, Naaglor, T, Ketupanya, A.*** Prevalence of *Blastocytis hominis* carriage in Thai army personal based in Chonburi. ThailandMilit. Med.2002; 167:643-6.

24. Abd El-Wahab, MM, Selim, SM. Prevalance of *Blastocystis hominis* among food handlers from Shibin El Kom, Menoufiya Governorate, Egypt. Egypt J Med Sci; 2007; 28(1): 689-698.

25. Farghaly, A, Hamza ,RS, Abd El-Aal NF, Metwally S, Farag SM. Prevalence, Risk factors And Comparative Diagnostic Study Between Immunofluorescence Assay And Ordinary Staining Techniques In Detection Of *Blastocystis hominis* In Fecal Samples. J. Egypt. Soc. Parasitol. (JESP),2007; 47(3), 701 – 708.

2***6. Gabr, NS, Abdellatif, MZ, Rabie, MM, Belal, US, Sanadeki, MM, Abdel-ghany, WM.*** *Blastocystis* spp. Infection Among IBS Patients: Various Diagnostic Methods And Epidemiological Study**.** J. Egypt. Soc. Parasitol., (JESP), 2018; 48(1), 119 – 128**.**

27. Hammood, A, Ahmed, B, Salman, Y. Bl*astocystis hominis* Detection among Gastrointestinal Disorders' Patients in Kirkuk Province Using Three Different Laboratory Methods, Int. J Curr. Microbiol. Appl. Soc., 2016; 7:883-901.

28. El Safadi, D.; Cian, A.; Nourrisson, C.; Pereira, B.; Morelle, C.; Bastin, P.; Bellanger, A.; Botterel, F.; Candofli, E.; Desoubeaux, G.; Lachaud, L.; Morio, F.; Pomares, C.; Rabodonirina, M.; Wawrzyniak, I.; Delbac, F.; Gantois, N.; Certad, G.; Delhaes, L.; Poirier, P. and Viscogliosi, E. Prevalence, risk factors for infection and subtype distribution of the

intestinal parasite *Blastocystis* spp. from a large scale multi-center study in France. BMC Infect.Dis.,2016; 16: 1-11.

29. Rezan M.M. and Shahnaz A. A. A Study of *Blastocystis hominis* Infection in Sulaimani Pediatric Teaching Hospital, int, J, Curr.res, aca. rev., 2015;3(8):290-299.

30. Mehta, R, Koticha, A, Kuyare, S, Mehta, P.Are we neglecting *Blastocystis hominis* in patients having irritable bowel syndrome. J. Evol. Med. Dent Soc.,2015; 4(64):11164-71.

31. Darabian, A, Berenji, F, Ganji, A, Fata, A, Jarahi, L. Association between *Blastocystis hominis* and irritable bowel syndrome (IBS). Int. J. Med. Res. Hlth. Sci., 2016; 5 (9):102-105.

32. El ghareeb, AS, Younis, MS, El Fakahany, AF, Nagaty, IM. Laboratory diagnosis of *Blastocystis* spp. in diarrheic patients. Trop. parasitolo.,2015; 5(1): 36-41.

33. Nascimento, SA, Mda, LM. *Blastocystis hominis* and other intestinal parasites in a community of Pitanga City, Paraná State, Brazil. Rev. Inst. Med. Trop. Sao. Paulo.,2005; 47: 213-7.

34. El Marhoumy, S.M.; El-Nouby, K.; Shoheib, Z.S. and Salama, A.M. Prevalence and Diagnostic approach for a neglected protozoan Blastocystis hominis. Asian Pacific Journal of Tropical Disease,2015; 5(1):51-59.