# Detection of Blastocystis hominies in Diarrheic Patients with new Modified culture methodology

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# الكشف عن المتبرعمة الكيسية في مرضى الإسهال باستخدام طريقة زراعه جديدة معدلة

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**الملخص**

توفر المتبرعمة الكيسية تحديات كبيرة للتشخيص المختبري بسبب طبيعتها متعددة الأشكال في العينة الرطبة والتي يمكن أن تؤدي إلى الخلط مع غيرها من الكريات البروتوزوا أو الخميرة أو حتى الدهون. كشفت الدراسات أن المسحات البسيطة كانت أقل حساسية من زراعة المختبر باستخدام اوساط زرعية مختبفه مختلفة للكشف عن المتبرعمة الكيسية في عينات البراز. تم التعرف مجهريا وبعد ذلك تمت زراعتها وصيانتها بنجاح في اوساط زرعية في المختبر. لقد عدت دراسه مبسطه لصيانه وزراعه الطفيلي المتبرعمة الكيسية عزل من عينه البرازوتعرف عليه مجهريا وتمت عمليه زرعه والمحافظة على الطفيلي بنجاح في اوساط زرعيه مختبريا. جمعت مائه عينة من المرضى الذين كانوا يشكون من اضطرابات هضمية من مستشفيات بغداد المختلفة ومختبرات خاصة بشكل عشوائي و جمعت عينات البراز عن طريق اعداد عينه مباشره لأربع وسائط مختلفة باستخدام المياه، والمياه مع مادة البراز، والمياه مع مادة البراز والدم، والماء مع المصل والتي تم استخدام هذه الاوساط لزراعة الطفيلي والحفاظ عليه. حيث لوحظت الاوساط الزرعية المستنبتة بشكل (يوميا، أسبوعيًا، شهريًا) وفحصت مئه عينة من البراز التي كانت مصابه في عدوى الطفيلي المتبرعمة المتكيسة في المختبر. حيث وجدت 18 عينة بنسبة (18٪) للمتبرعمة الكيسية، كانت إيجابية لطفيلي لمتبرعمة المتكيسة في الفحص المباشر المجهري. ويعتبر الوسط الزرعي للدم WFB أعلى نمو للطفيلي، كما تم الكشف عن الصيانة لمدة تصل إلى 5 أشهر بنسبة 26٪ من العينات الإيجابية للمتبرعمة الكيسية على التوالي. وكان المصل مع الماء والبراز (WS) 17 عينه بنسبة (17٪) كانت إيجابية للمتبرعمة الكيسية التي هي الأفضل للحفاظ على الطفيلي لمدة تصل إلى 5 أشهر على التوالي. اما الماء مع البراز (WF) التي تحوي 4 عينه اي بنسبة (4 ٪) كانت عينات إيجابية لطفيلي المتبرعمة الكيسية وامتدت صيانه الطفيلي شهر واحد على التوالي بالإضافة الى ان الماء فقط لم يكن هناك ايه نمو لطفيليا المتبرعمة الكيسية.

**الكلمات المفتاحية:** المتبرعمة الكيسية، مرض الإسهال، اوساط زرعية في المختبر، المياه مع اوساط زرعيه البراز، المياه مع اوساط الدم والمصل.

**Abstract**

*Blastocystis hominis* provide significant challenges to laboratory diagnosis due to their nature of polymerization in wet sample that can lead to confusion with other protozoa, yeast. The current study was designed as new a simplified preparation for cultured parasites and preserved *blastocysts hominis* that isolated from a stool sample. *blastocysts hominis* was identified microscopically and then successfully cultivated and maintained in vitro-produced culture media. Hundred faces samples were

collected from patients complained with gastrointestinal disorders from different Baghdad hospitals and privet laboratories randomly. The samples were cultured in four various culture media using Water (W), water with fecal matter (WF), WF and blood (WFB), and water with serum (WS) were used to cultivate and maintain the parasite. Inoculated culture media were observed daily, weekly and then monthly. Cultures were then left for incubation and examined by direct microscopy to detect Blastocystis hominis. The results showed of 100 stool samples studied, 18 samples (26%) were positive results for *B. hominis* the culture media of WFB is highest growth of the parasite as well as maintenance up to 5 months were detected. However water fecal serum water fecal (WS) were 13 sample (17%). However, water with fecal was 4 (4%) sample were maintenance of the parasite up to 1 month respectively. Besides the water only was no growth for *Blastocystis hominis*.
**Keywords:** *B. hominis*, Diarrheal disease, In-vitro culture, water with fecal culture, water with serum and blood culture,

## 1- Introduction

In general, the prevalence of infectious parasites varies from region to region depending on the degree of personal and community hygiene, sanitation, and climate factors [1], [2] and various diagnostic techniques may influence their detection, Intestinal parasites are associated with diarrhea, dysentery, weight loss, malnutrition, anemia, abdominal pain, and other gastrointestinal diseases. [3] Chronic insomnia also weakens the physical development and cognitive functions of growing children. [4] Gastrointestinal parasites can cause infection in both humans and animals. Some of these organisms are potentially zoological. [5] [6] Furthermore, intestinal opportunistic parasitic infection is also a serious public health problem, which has increased in recent years in developing countries. [7] [8].Blastocystis hominis is a zoonotic disease and is acquired by humans via the fecal-oral route Blastocystosis is symptomatic infection caused by protozoa parasite called Blastocysts (Kevin, 2008), Humans may remain asymptomatic, or may develop dysentery similar to that caused by *Entamoeba histolytica* commonly known as traveler's diarrhea with the symptoms of characteristic diarrhea accompanied by abdominal pain, dizziness, nausea, anorexia, vomiting, weight loss and intestinal tympani (Moghaddam, et al.,2005 and Kuo, et al., 2008 ). *Blastocysts hominis* can become an opportunistic parasite in immunosuppressed hosts living in urban environments. As opportunistic organisms, *blastocystis hominids* tend to become invasive and penetrate the linings of the mucosa and submucosa of the damaged intestine and within the lymphoid tissue of affected hosts, from which they travel throughout the rest of the body Blastocystis is a unicellular, an obligate anaerobic, eukaryotic protest which found in the intestinal tract of different host including humans (Tan et al., 1997). Establishment of all the organisim is very difficult and expensive in addition to labor-intensive to maintain in the diagnostis .The parasite has three distinct morphological forms includes vacuolar, granular and amoeboid, were distinguished in stools and culture, but recent studies found other forms such as cystic, vacuolar and multi vacuolar(Singh, et al.,1995 and Vdovenko,2000). *Blastocysts hominis* is an often-neglected pathogen in the present study.

Currently, the detection of infection with *B.hominisis* usually based on microscopic examination on fecal samples either by wet mount or by trichome-stain smears(Surcsh and Smith,2004) , also the parasite maybe difficult to distinguish from leukocytes or from trophozoites or cysts of other protozoa(Stenzel&Boreham,1996). Thus resulting of diagnosis probably in misdiagnosis of numerous cases. Culturing has been shown to be more reliable of identification infection (Dogruman (*et al.*2010)

The new medium was designed because of the need of an economic medium without the inter lot variability.The main objective of this study in vitro techniques was to demonstrate that the new medium facilitates proper blastocystis *hominis* growth without affecting virulence factors.

## 2- Materials and Methods

### 2.1. Isolation of *blastocystis hominis*

About (100) feces samples were collected from patients complained about gastrointestinal disorders from different Baghdad hospitals and privet laboratories randomly. Microscopic examination was done by light microscopic, the samples were taken into the slide and examined whether it is positive or not for Blastocystis A freshly collected diarrheic stool sample from the patient was subjected to routine examination. Fresh wet mounts were carried out to detect *blastocystis hominis* using a light microscope viewed at low power (×100) and high power (×400) magnifications. *blastocysts hominis* can readily be recognized in wet mount slide preparations, even at a low magnification...

### 2.2- Culture preparation

Four different liquid media were used as follows: 1-The first medium Water (W): Water was collected in a sterile bottle from a water cooler to that a water purifier was already installed. Culture and microscopic examination were done to rule out any bacterial and parasitic contamination. 10 mL of water was used for culture of *blastocystis hominis*. Water + Fecal mater (WF): 2–3 g of fecal mater, that is, negative for any parasite is mixed in 10 mL of water in another sterile bottle. Water + Fecal + Blood (WFB): In another sterile bottle, 0.5 mL of fresh blood was mixed with water and fecal matter. Water + Serum (WS): 0.5 mL of serum was mixed with 10 mL of the water.

### 2.3- Inoculum

A measured inoculum (0.05 mL) of that diarrheic specimen, which was positive for *blastocystis hominis*, is passed to all four culture bottles containing a fresh medium. Duplicate cultures were also set up. All bottles were incubated at room temperature (25°C) and observed after 24 h, 48 h, 72 h, and then weekly regularly.However, various subcultures were done using 0.05 ml of inoculum from previously positive culture and inoculated in fresh medium to observe the unceasing growth of *blastocysts hominis*

## 3- Results

In the present study100 samples of faces were presented by direct smear microscopy from (Water (W), water with fecal matter (WF), WF and blood (WFB), and water with serum (WS) were used to cultivate and maintain the parasite *B.hominis*. This method is cost-effective and easily because it does not require a horse or human serum addition during its preparation is not necessary. High detection rate of parasites was observed by water fecal blood (WFB) culture media at percentage 26% **a**lso in this method culture become positive quickly and the parasite identification after 24hr&48h in number was (4%), and the number became decrease after 4 month still the contractile vacuoles are clearly seen. Respectively However, Water serum (WS) was 17 at a percentage of (17%) positive samples for *B. homini* were better maintenance of the parasite up to 5 months respectively. However, Water fecal (WF) was 4 at a percentage of (4%) positive samples for *B. homini* were maintenance of the parasite up to 1 month respectively. And the water only was no growth for *Blastocystis hominis*. with make subculture every 3days results are presented in (Table 1). Only two forms of B.hominis obtained from positive cultures, vacuolar forms are shown in Fig1 and granular forms are shown in Fig2 (G). Zerpa et al. (2000) also made a new culture media and considered an easy and fast method because it does not need to add horse serum and the parasite identification after 24hr. After 1-month slightly, decrease in the count was observed as shown in (Table 1). *blastocystis hominis* survived for 4 months, 7 months, but a decrease in the count and no growth in culture W, WF, WFB, and WS, respectively. Growth was observed in subcultures except for WS. The cultures grow and maintained well at room temperature up to several months. The different forms of *B.hominis*.

**Table 1: Detection of protozoan parasites in culture media with different time.**

| culture media | water + fecal | water +fecal+ blood | water + fecal+ serum | water |
|---|---|---|---|---|
| After 24 h | +1 | +4 | +3 | Scan |
| After 48 h | +1 | +4 | +3 | Nil |
| After 72 h | +1 | +4 | +3 | Nil |
| After 1 week | +1 | +4 | +2 | Nil |
| After 2 month | scanty | +4 | +2 | Nil |
| After 3 month | scanty | +2 | +1 | Nil |
| After 4month | scanty | +2 | +1 | Nil |
| After 5month | scanty | scanty | Nil | Nil |
| After 7month | Nil | scanty | Nil | Nil |

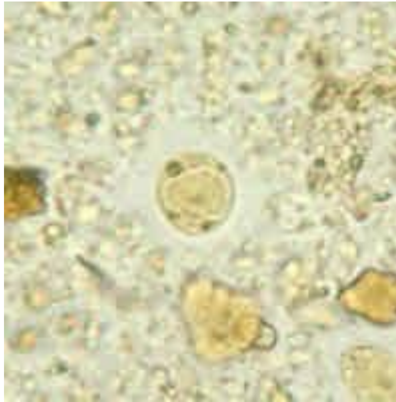

**Figure 1. Light microscopy of *B.hominis* showing vacuolar forms (100x)**

### 3.1. Discussion

In Iraq hospital, diagnosis of intestinal parasitic infections in clinical sampleis made by routine direct smear microscopy of stools in normal saline or iodine preparations. Evaluation of different new culture procedures has not been done for clinical specimens. Therefore, data are available for comparison. The present study demonstrate comparison of 4 culture media techniques for the detection of parasites in stools.

The results of this study describes the value of using all culture media procedures. Water (W), water with fecal matter (WF), WF and blood (WFB), and water with serum (WS) confirmed an increase of detection efficiency of blastocystis hominis parasites in stools compared to other culture media techniques used in the study. This observation agrees favorably with other similar studies, (Stenzel, D.J. and Boreham 1996 ) which reported prevalence.

The results of this study revealed the distinct superiority of the culture media techniques on the microscopy of direct formation ($P < 0.05$). This result is consistent with other studies Stenzel, D.J. and Boreham 1996 ) Diamond's trypticase (casein digest), yeast extract, serum, and porcine gastric mucin (TYSGM) medium for Entamoeba and other enteric parasites also supports the xenic growth of blastocystis hominis The medium contains TYSGM. The starch powder is added to tubes at the time of inoculation of the medium. A defined' medium with cysteine HCl and i-inositol was used for short-term physiologic experiments with the organisms, but little in the way of results appeared in the literature.

Which clearly indicated that Water (W), water with fecal matter (WF), WF and blood (WFB), and water with serum (WS) are advantageous over direct smear preparations.

cultures of all parasites handled in the similar way. Xenic cultures of *Entamoeba. histolytica* and Dientamoeba fragilis are surely passaged at 48–72 hours intervals. Xenic isolates of B. hominis and B. coli can usually be also kept on this schedule. The preparation and cultivation of these xenic cultures are complex, costly, and difficult to handle. Studies showed the results of these media were also compromising. Cultures have been established in (LE Lock-egg slant medium) modification of Boeck and Drbohlav's medium), and Robinson's media, but before as mentioned ,it is difficult to keeping many isolates for a long. Whether this is a deficiency of the media or is dependent on the bacterial flora composition or on some other factor is not known Positive cultures containing blastocystis *hominis* survived for 14 days with subcultures have done every 24 h. Cultures of the isolate observed at 48 h without subcultures resulted to an increased bacterial density and the protozoa gradually decreased in number

To overcome all these difficulties, in this study, some new culture media are devised. These media are not only cost-effective but also easy to handle and yielding good results. There is no need to passage again and again for maintenance of this parasite. The culture mediums "WFB" and "WFS" showed better cultivation and maintenance of the parasite. They can maintain the parasite up to 5 and 7 months, respectively. The culture medium "WF" also showed growth respectively the culture medium "W" showed no any cultivation. One of the frustrations faced by some authors was the a lot to variable in the medium components. The media was used in this study do not require any specification and dependability on a manufacturer. We can prepare it in our laboratory easily, whenever required.

Staining such as hematoxylin-eosin could not stain the internal structure of parasite, but the stain concentrates progressively in the cytoplasm, obscuring all internal detail (Figure1)

Trophic ciliates did not survive longer than 24–48 h in cultures maintained at temperatures over 40°C. We kept the culture positive for *blastocystis hominis* at 42°C in water bath for 24 h to observe the survival of this parasite but could not be discovered it. The cultures grew well at room temperature

**Conclusions**

This work showed the prevalence of *blastocystis hominis* protozoan parasites is relatively high in the population studied. This study also indicated that culture media development of these new techniques will encourage propagation and longer survival of *blastocysts hominis* in culture and good method for the routine examination of the intestinal parasites in clinical stool samples. With those mediums "WFB" and "WFS" support the growth of the organisms and routine maintenance of parasite that was difficult or time-consuming. We belived the difficulties associated with cultivating and maintenance the organism , and the disability to prevent people to chooses to study them. Also Culture proved to be a more sensitive and specific tool for dignosis of *blastocystis hominis* than microscopy.

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