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Preparation Simplified Culture for Culturing Blastocystis Hominis Parasite

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Abstract
Blastocystis hominis is an obligate and aerobic protozoa, a world waded distribution between human and animals. Anew simplified of culturing parasite is prepared. One hundred fresh positive stool samples were inoculated in tube containing a new media. This media contain simple compound like ringer solution, water rice cooked and don't need serum add. Tubes were incubated at 37c°, culture's was examined after 24, 48 and 72hours, culture became positive after 24hr, and the number of parasite was (1830) while after 48hr was (1525) but after 72hr the numbers of parasite began decreasing and became (381.25). The multiform of parasite described but vacuolar and granular form more observed. This culture is a new, quick and easy method for culturing *Blastocystis homini* also not require to add serum.
Keywords: *Blastocystis hominis,* vacuolar, granular, culture media.

1. INTRODUCTION

Blastocystosis is symptomatic infection caused by protozoal parasite called *Blastocystis* (Kevin, 2008), which 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 tic(Moghaddam, *et al.*,*2005* and Kuo, *et al*., *2008*).

Blastocystis is a unicellular, an obligate anaerobic, eukaryotic protest which found in the intestinal tract of different host including humans (Tan *et al*., *1997*), it is found in stool specimens of infected human and animals (stenzel *et al*., *1996*). 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). *Blastocystis hominis* early in last century was classified as protozoa (Zierdt,*1988*). Although several studies considered *B.hominis* a commensal parasite (Chen,*et al.,2003* and Leder *et al.,2005*) but recent studies characterized as a pathogenic and causative agent of diarrhea (Rossingnol *et al.,2005*; Tan &Suresh,*2006*;Kaya *al.,2007*).

Currently, the detection of infection with *B.hominis* is usually based on microscopic examination on fecal samples either by wet mount or by trichome-stain smears(Markell& Udkow,*1986*; Zierdt,*1991* ;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*).

There are many types of cultures which used for this parasite such as Jone's solution medium (Jones, *1946*), Loffler medium, ringer solution medium(Stenzel and Boreham,1994), modified whole-egg Slant medium Locke solution(Zierdt,*1991*), Xenic culture(Dogruman,*et al.,2010*) and methods made by Rttozepra(zepra *etal*.,2000), but most of these culture media require addition serum for animals or humans and contain different type of salts and other materials,

So, the aim of the present study is to make simplified culturing methods to detect the presence of *B.hominis* in stool by using simplified component and without need additional serum for human or animals.

2. MATERIALS AND METHODS

2.1 Collection of samples

About (100) feces samples were collected from patients complained with gastrointestinal disorders from different Baghdad hospitals and privet laboratories randomly. Microscopic examination was done by light microscopic, the samples were taken into slide and examined whether it is positive or not for *Blastocystis hominis*, (100) specimen containing *B.hominis* were later used for in vitro culture.

2.2Preperation of culture

The new medium contains:

500 ml	Ringer solution
0.5 gm	Yeast extract
5 gm	Pepton

Mixed well and adjust the pH to 7.2-7.4 after sterilization, then 20ml of water cooked rice (Cook rice well with water and boil after that taking a part of the cooking water) was added ,streptomycin 50-100mg was add after sterilization with a Seitz filter. The medium was distributed in sterile glass tubes 10ml each.

2.3 Establishment of culture

Small amount of inoculums from stool fraction similar to those used to microscopical examination. The tubes were tightened and incubated at 36c°. Examination was performed after 24, 48 and 72 hours.

2.4 Examination of culture media

One drop of sample was examined on direct microscopic examination and counted the number of parasite in 1ml of sample by use this equation (Al-Idrrise ***et al., 2008***)

No. of (C) or (T) ×15.25= No. of (C) or (T) in one ml sample.

3. RESULT AND DISCUSSION

In the present study prepared a new culture media for *B.hominis*. This method is cost-effective and easily because it does not require horse or human serum addition during its preparation is not necessary. Also in this method culture become positive quickly and the parasite identification after 24hr in number was (1830), while after 48hr reach to(1525), but the number became decrease after 72hr reach to(381.25) so stock culture need to be make subculture every 3days (Table 1). Only two forms of *B.hominis* obtained from positive cultures, vacuolar forms are shown in Fig1 (A&B) and granular forms are shown in (C&D).

Zerpa ***et al.*** **(2000)** also made a new culture media and considered as easy and fast method because it does not need add horse serum and the parasite identification after 24hr.

The different forms of *B.hominis* obtained from positive culture also described by Zierdt, *(1991),* Moe *et al.* (2015) reported that when the parasite cultured in Jone's medium containing 10% horse serum the cyst developed into large number of vacuolar forms within 24hr, and binary fission was the only mode of reproduction observed. Barua *et al.* (2015) reported that Xenic culture is a good media but it contains about twelve substances.

Finally, this method is easy, fast and inexpensive way, it consists of materials available plus they don't need to add serum.

Table 1: The number of parasite in culture media with different time.

Time (hour)	48	24	72
Number of parasites(cell/ml)	1525	1830	381.25

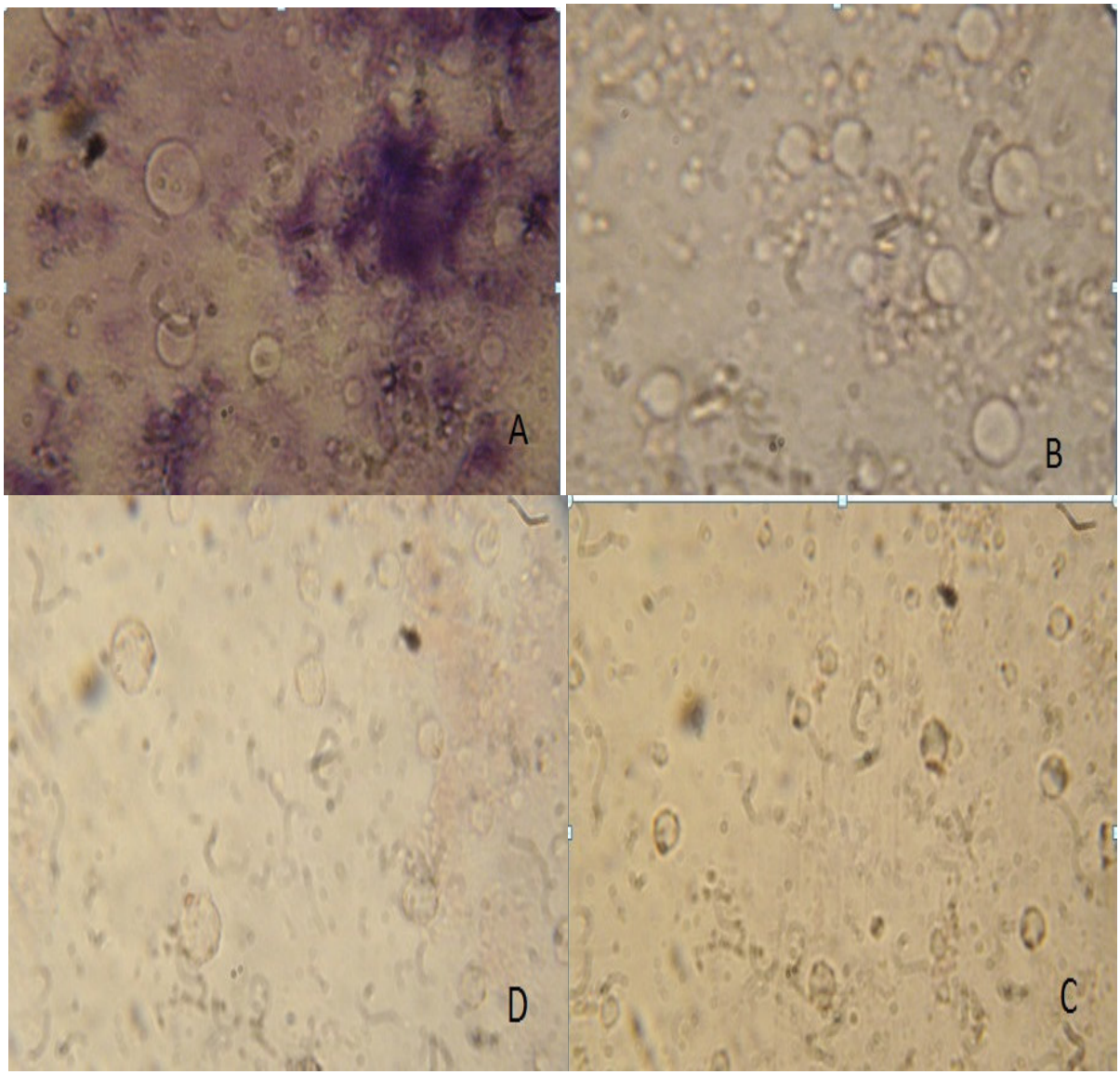

Figure1 :(A&B) Light microscopy of *B.hominis* showing vacuolar forms (100x)
(C&D) Light microscopy of *B.hominis* showing granular forms (40x)

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