In vitro culture technique: a better diagnostic tool for *Blastocystis hominis*

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Abstract

A total of 189 stool specimens obtained from HIV-infected inmates from a drug rehabilitation center in Selangor were examined for *Blastocystis hominis* using the direct faecal examination, formalin-ether concentration and the *in vitro* culture techniques. The *in vitro* culture technique using Jones medium was 4 times (29.6 % (56/189)) more sensitive in detecting the parasite than the direct-faecal examination (7.4 % (14/189)) and almost 2 times more sensitive than formalin-ether concentration technique (15.3% (29/189). The detection of *Blastocystis* using the *in vitro* culture method is very much easier, less tiresome and more time-saving. Furthermore this method can easily be performed in the field. The *in vitro* culture method has the added advantage of maintaining isolates obtained from the field for further biological, biochemical and molecular characterization.

Key words: *Blastocystis hominis*, *in vitro* culture; Jones medium

Blastocystis hominis, a protozoan parasite, has been incriminated in recent years to be responsible for causing diarrhoea in humans (Garcia *et al.*, 1984; Ricci *et al.*, 1984; Vannatta *et al.*, 1985; Sheehan *et al.*, 1986; El Masty *et al.*, 1988; Zierdt, 1988). The usual method of detecting the parasite is by light microscopy of wet mounts of either fresh stools or concentrates.

Many diagnostic laboratories have taken the vacuolar forms of 5-8 µm as the standard morphology, but in the light of recent observations of the form *in vivo* (Stenzel *et al.* 1991), it is essential also to look for smaller and multivacuolar forms in fresh stools to avoid missing positive cases. It is also known that the parasite, often, exists in very low numbers in the stools of infected persons.

Staining with permanent trichrome mounts may aid in diagnosis but such staining procedures require fixing which can distort these fragile parasites. Recently, acridine orange has been used to identify the various stages of the parasite (Suresh *et al.*, 1994). Although this staining procedure does not require fixing the parasite, the need to use a fluorescent microcope to examine the parasite can be cumbersome and not practical when the diagnosis has to be done in the field.

Hence there is a need for cheaper and easier alternative methods to detect this parasite in stools. Jones medium (Jones, 1946) has been used to culture *Entamoeba histolytica* successfully and very recently *Blastocystis* (Zaman & Khan, 1994). The present study was undertaken to compare the sensitivity of three techniques namely the direct fecal examinantion, formalin-ether concentration technique and the *in vitro* culture technique using Jones medium for the detection of *Blastocystis* in stools from HIV-infected patients.

Materials and Methods

A total of 189 stool specimens was obtained from HIV-infected inmates from a drug rehabilitation center in Selangor. Stool specimens were collected in labelled stool cups which were distributed one day prior to the actual collection of stool samples. They were examined in batches of 50 each.

Fresh faecal samples were mixed with a drop of saline and examined directly under a microscope. The samples were also concentrated using the formalin-ether concentration technique and examined for *Blastocystis* in 100 fields for both the techniques under 400 x magnification. The readings were done blindly so as to avoid bias. Faecal materials were cultured in bijou bottles containing two ml of Jones medium. The cultures were kept for 24 hours at 37°C and the contents examined using a light microscope under 400 x magnification.

Results and Discussion

Direct faecal examination, formalin-ether concentration and *in vitro* culture techniques revealed 7.4 % (14/189), 15.3% (29/189) and 29.6 % (56/189) *B. hominis* in stools respectively. Most of the faecal samples received were soft and mushy.

Formalin-ether concentration technique was almost 2 times more sensitive in detecting *Blastocystis* than direct faecal examination. Cysts of *Giardia* were the only other parasite seen and that too in three faecal samples. Both the direct faecal examination and the formalin-ether concentration techniques were time-consuming as the parasites must be searched for and differentiated from fat globules and other particles resembling *Blastocystis* in the faeces. The parasites that were detected by the direct faecal examination technique were more of the amoeboid forms while the vacuolar forms were

mostly seen in the other two techniques. Miller & Minshew (1988) reported that *B. hominis* could be detected in stained smears of faecal sample but not in concentrated specimens from the same fecal sample. However findings in other studies (Aldeen & Hale, 1992; Garcia *et al.*, 1984; Guimaraes & Sogayar, 1993; Hussain *et al.*, 1989) concur with our observation that the concentration methods are more effiective.

Kukoschke *et al.* (1990) compared the effectiveness of microscopy and culture in the idenrification of infected stools, and found that culture had no benefit over microscopy. Zierdt (1988; 1991) reporred that culture was successful only if large numbers of *B. hominis* were present in the stool. In our present study the *in vitro* culture technique using Jones' medium was 4 times more sensitive than the direct-faecal examination and almost 2 times more sensitive than formalin-ether concentration technique. Only very small amounts of fecal material were needed for the culture of the parasite. Although Kukoschke & Muller (1992) reported that the detection of the parasite in culture requires increased time, costs and personnel, the reverse was true in our study. It is likely that the patients in Kukoschke *et al* (1990)'s study were chronic carriers of *Blastocystis* and hence they could easily detecr the parasites in stools using the direct method. Our results in rhe present study are similar to that of Zaman & Khan (1994) where they detected *Blastocystis* in 32% and 18% using the cuture and direct stool examination methods respectively. However, when the cultures in their study were further incubated up to 72 hours the percentage increased from 32 to 45. In our study we did not detect any similar increase. Guimaraes & Sogayer (1993) suggested that spontaneous sedimentation was a suitable technique to separate *B. hominis* from faecal material but this is too time-consuming.

In another study 44% of a cohort of 49 homosexual men, confirmed to be infected with HIV, were positive for *Blastocystis* (Church *et al.*, 1992). However no correlation was found between the occurrence of any enteric parasitic infection and gastrointestinal symptoms. In a prospective cross-sectional study of children in Tanzania, *B. hominis* was detected only in HIV-infected individuals and not in children with chronic diarrhoea (Cegielski *et al.*, 1993). In our present study 29.6 % of the 189 HIV-patients were positive for *Blastocystis*. Some of the inmates had loose watery stools and since no other parasites were found in these stools other than *Blastocystis* it is extremly tempting to incrimnate the cause of diarrhoea to this parasite. However we are currently collecting more samples and correlating clinical signs and symptoms with the presence of the parasite so as to obtain a better undetstanding of the pathogenic aspects of *Blastocystis*.

So far permanent smears appear to be the procedure of choice for light microscopic diagnosis of *B. hominis* as the organism is difficult to identify in wet mounts (Zaki *et al.*, 1991). Trichrome staining of permanent smears has been recommended for routine use in the diagnosis of *B. hominis* (Garcia & Bruckner, 1988; 1993, Markell & Udkow, 1990) although many other stains have been used succesfully. These include iron hematoxylin (MacPherson & MacQueen, 1994), Giemsa (Dawes *et al.*, 1990), Gram (Zierdt, 1991) and Wright's (Vannatta *et al.*, 1985). However variability in staining particularly of the central vacuole has been reported (Garcia & Bruckner, 1993; MacPherson & MacQueen, 1994). Stenzel (1995) has provided evidence that the variability in size and shape of the organism is a result of osmotic changes and the time of storage of unfixed faecal material prior to microscopy. It should be noted that the predominant forms seen in the faecal samples could sometimes be the cystic stages which are not only small (3-5 μm) but also store large lipid inclusions and glycogen deposits, thus affecting staining patterns.

Serological testing has not been successful so far and although invasive techniques have been occasionally used to detect *B. hominis* in the intestine, these are not recommended as routine methods.

Jones medium is cheap and simple to prepare, dispense into bijou bottles and store at 4°C until use. The quantity of faecal material needed is very small and after 24 hours of incubation at 37°C, a drop of the culture medium in cultures where the stool is positive for *Blastocystis* should show more than 10 parasites per field under 400 x magnification.

The detection of *Blastocystis* using the *in vitro* culture method is very much easier, less tiresome and more time-saving. Furthermore this method can easily be performed in the field. The *in vitro* culture method has also the added advantage of maintaining isolates obtained from the field for further biological, biochemical and molecular characterization.

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