SMALL FORMS OF *BLASTOCYSTIS HOMINIS*

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ABSTRACT: **Unusually small forms of *Blastocystis hominis* were found in the stools of Indonesian immigrants. The size of the parasites seen in faecal samples of the Indonesian isolates were 3 - 5 mm whereas larger vacuolar forms measuring 10 - 15 mm were seen in Malaysian and Bangladeshi isolates. These small forms showed a distinct growth profile with an optimum parasite yield as high as 11.7 x 10^5 when cultured in Jone's medium compared to yield of 3.9 x 10^5 and 0.5 x 10^5 for Malaysian and Bangladeshi isolates respectively. The unusually small forms of *Blastocystis*, unlike the other isolates, was found to grow in cultures at 37°C even after being kept at room temperature for as long as 9 days.** *(JUMMEC 2002; 1:77-79)*

KEYWORDS: *Blastocystis hominis*, **small forms; immigrant; morphology, growth profile.**

Introduction

Blastocystis hominis (1), a human intestinal protozoan parasite, has been reported in a wide range of hosts e.g. humans, pigs, monkeys, fowls, ostriches, turkeys, guinea pigs (2, 3), reptiles including snakes, crocodiles and iguana lizards (4, 5). However, very little is understood about the parasite's mode of reproduction and growth.

Previous studies on morphology, growth profile and karyotypic patterns of *Blastocystis* were on isolates obtained from one geographical region or unspecified (6, 7, 8). To the best of our knowledge, no study has been undertaken to compare the morphology, growth profile and karyotypic pattern of *Blastocystis* isolates obtained from different geographical regions. In the present study we report unusually small forms of *Blastocystis* isolated from *Blastocystis*-infected Indonesian migrants. These forms show distinct growth patterns and different biological characteristics compared to the normal vacuolar form seen in Malaysian and Bangladeshi isolates.

Material and Methods

Malaysian *Blastocystis* isolates were obtained randomly from faecal samples of patients admitted at the University Malaya Medical Centre (UMMC), Kuala Lumpur. Only two out of ten patients had diarrhoea and all the patients were confirmed to have acquired the infection locally. Faecal samples were also collected from immigrant workers who had recently arrived from Bangladesh and Indonesia, at the outpatient clinic, University Malaya Medical Centre (UMMC), Kuala Lumpur. These *Blastocystis*-infected individuals were apparently healthy and asymptomatic.

Direct faecal smears were made from ten *Blastocystis*-infected persons from each of the three nationalities. The morphology and size of *Blastocystis* were observed under both light and phase contrast microscopy as well as fluorescent microscopy after acridine orange staining. The faecal samples were subsequently maintained in bijou bottles containing 3 ml of Jone's medium at 37°C (9, 10). After several sub-cultures three isolates originating from stool specimen of each nationalities were randomly selected for the growth profile study. The parasites of each of these isolates were pooled together to make a concentration of 5 x 10^5 parasites/ml of Jone's media and added into 3 screw capped tubes of 10ml volume respectively containing 4 ml of fresh Jone's medium. The experiment was therefore done in triplicates and an average parasite count was made from each of these isolates. The screw-capped tubes containing the cultures were then maintained at 37°C and the parasite count was determined every day until the parasites were absent. The parasites from culture samples were assessed for its viability by sub-culturing into fresh Jone's medium daily after the 5th day culture.

In a separate experiment, parasites of each of the isolates were pooled together to make up a concentra-

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tion of 1×10^5 parasites/ml. They were then maintained at room temperature in bijou bottles containing Jone's medium. Every day 50 ml from each of the culture sample was transferred into bijou bottles containing Jone's medium and maintained at 37°C for 24 hours. A drop from the culture sample was then observed the next day under light microscopy and an average parasite count was made from 10 fields. This observation was continued until parasites were absent on sub-culture.

Results

Most parasites seen in the faecal samples of all three nationalities were vacuolar and granular forms. Under phase-contrast microscopy, parasites of Malaysian and Bangladeshi isolates were 10-13 mm in sizes whereas those of Indonesian isolates were less than 5 mm in size. For day 1 cultures, both Bangladeshi and Malaysian parasites showed the same size and the peripheral nuclei were as distinct as those seen directly in the faecal samples. The size of the parasites of the Indonesian isolates did not increase when observed in day 1 cultures and continued to remain at 3 – 5 mm in size. Other than the size, the forms of all three isolates were morphologically similar. Cyst-like forms showing viscous cytoplasm with thicker walls were seen only in cultures from Indonesian isolates.

Parasites in all nine tubes decreased in numbers until day 2 and most of the parasites were of the granular form. The parasites from Malaysian and Bangladeshi isolates peaked on day 5 and 6 with a parasite count of 3.9×10^5/ml and 0.5×10^5/ml respectively. The parasite count for the Indonesian isolate increased until day 8 to 11.7×10^5. The average size of the vacuolar form at peak parasite count for the Malaysian, Indonesian and Bangladesh isolates was 16.1 mm, 10.5 mm and 12.3 mm respectively. The percentage of vacuolar form at peak parasite count for the Indonesian isolate on day 8 was 93.2% whereas for Malaysian and Bangladeshi isolates this was 82.5% and 52.7% respectively.

In cultures both vacuolar and granular forms were present but the percentage of the granular form increased in older cultures. The size of vacuolar and granular forms of the Malaysian isolates ranged from 3.8 mm to 100 mm and 3.8 mm to 65mm respectively. The size of the vacuolar form in the Indonesian isolates ranged from 2.5 mm to 50 mm and the granular form from 2.5 mm to 35mm. the size of the vacuolar form from Bangladeshi isolates ranged from 5mm to 37.5 mm while that of the granular form was from 5 mm to 80 mm. Intact whole parasites were observed until day 11 and 12 in cultures from Malaysian and Bangladeshi isolates but these parasites were not viable when subcultured in Jone's medium. Intact whole parasites from Indonesian isolates were seen in cultures until day 25 but the parasites were not viable after day 12. The observation supports the report that the *in vitro* culture technique (10) is still the best method to detect viable *Blastocystis*.

Discussion

Size variation of *Blastocystis* in faecal samples were noted as early as 1917 (11, 12). Several earlier reports also noted a spore-like smaller *Blastocystis* surrounded by a thick wall (13). However, in the present study the smaller forms did not have a thick wall surrounding the parasites when seen under light microscopy. The sizes of the cystic stages of *Blastocystis* range from 3.7 mm to 5mm (14). The small forms of *Blastocystis* seen in the present study lysed in distilled water confirming that they were not cysts. Ultrastructural studies should be carried out to elucidate the detailed morphology of the small forms of the parasite. Generally the vacuolar form has been reported to be approximately 10 to 15 mm in diameter with a large central vacuole (2). However, there have been reports that smaller forms of the parasite approximately 5 mm in size do exist in faecal samples (3, 14, 15)

Intact whole parasites from the Indonesian isolates were observed as long as 25 days in Jone's medium but the parasites were not viable after day 12. The observation supports the report that the in vitro culture technique (10) is still the best method to detect the parasites.

Blastocystis hominis has been shown to grow successfully at 37°C (2,17) and not at other temperatures. Parasites from the Indonesian isolate continued to remain viable even up to day 9 at room temperature while Malaysian and Bangladeshi isolates remained viable only until day 5 and 6 respectively. A drop from the original culture sample maintained at room temperature of all three isolates after the above periods, showed intact whole granular form of *Blastocystis* but they were not viable as they could not multiply when sub-cultured. When parasites from Indonesian isolates, maintained at room temperature for 6 days was subcultured and viewed under 40x magnification the following day, an average of 35 parasites was seen within a field compared to 1 per field in the other isolates, thus showing the high reproductive potential of the Indonesian isolates. This would also explain why Indonesian isolates showed the highest parasite count during the growth profile study.

The variation in morphology among isolates of *B. hominis* has important implications for diagnosis. The central body of the vacuolar form of (as small as 3 mm) in the Indonesian faecal samples was clearly seen when stained with acridine orange. Acridine orange staining was shown previously to be useful for the identification of

the parasite stages (18) and in the present study was shown to be extremely useful in the identification of these smaller forms.

Whether these small forms of *Blastocystis hominis* which show distinct growth characteristics when compared to other isolates, represent another species or strain, can only be answered by karyotypic studies. If these is not so, the effect of diet, host immune response, environmental factors, and possibly host life styles influencing the size of these parasites must be considered.

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