Microbiology

The isolation of *Blastocystis* by using small capped tube cultivation in medical laboratory

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Blastocystis hominis is an intestinal protozoa that can cause diarrhea and irritable bowel syndrome among healthy. This study was aimed to detect the intestinal protozoa *Blastocystis hominis* by using small capped tube cultivation method. This is a simple step of preparation and minimum waste management. The stool specimen was collected from a study of intestinal parasitic survey among healthy. The small capped tubes cultivation methods were provided the optimal carbon dioxide condition and growing protozoa cultivation. This application method was used in our medical laboratory during this study. Total of stool samples were 475 specimens and positive results in amount of 58 specimens (12.21%). *Blastocystis* isolates were capable to subculture more than weeks prior to confirm with conventional PCR. It was identified the morphological related to subtyping. This isolated was *Blastocystis* spp. subtype 3 and highly similarity (89%) that refer to most strains found in humans. This conclusion shown an usefulness and simply step of *Blastocystis* cultivation in medical laboratory. Small capped tube cultivation methods can distribute the innovative idea of application test in medical field.

Introduction

Blastocystis hominis is an anaerobic intestinal parasite and it is often caused the health problem among developing countries and local community area. Mostly infections are caused by low hygiene with contaminated food and water. Prevalence of *Blastocystis* spp. infection and the zoonotic are potential raised and impact on public health consideration[1,2]. The symptoms were increased an irritable bowel syndrome and gastrointestinal diseases as watery diarrhea[3,4].

Developing the new technique on *Blastocystis* infection testing method among the community based sites is useful[5,6]. This study would like to apply the new cultivation tool for use in detection of *Blastocystis hominis* and collection in survey field for further advanced diagnostics test and enumerated intestinal infection among villagers by using simple smear method and Jones medium cultivation.

Methods

Procedure for collecting specimens

Participants were sent a stool in a clean closure container for intestinal parasitic examination. Specimens were kept in cool box and sent to the laboratory unit at Faculty of Medical Technology, Rangsit University. Fecal cultivation tubes were used a small capped plastic tubes and incubated at 37 ºC and remains were kept at -20 ºC for molecular diagnostic test.

Diagnosis with simple direct smear method

Specimens were examined under microscopy and mixed with NSS and Iodine solution on glass slides. Specimens were labeled on each slide prior to the cultivation tubes method. Confirmatory with microscopy under 40x magnification were examined for intestinal ova and parasites. The results were recorded the characteristic of *Blastocystis hominis* and continued in the fecal cultivation in figure 1-2.

Jones medium cultivation

The culture medium was prepared and sterilized in a 2.5ml capped cultivation tubes. The culture medium was kept at 4 °C and working medium was thawed and added 10% inactivated bovine serum as completed medium ready to use. Stools were placed in a cultivation tube as 0.2 milligrams and incubated at 37 °C for 3-7 days. The small capped tubes cultivation methods were provided the optimal carbon dioxide condition and growing protozoa cultivation. Fecal sediment was transferred by a sterilized dropper and added 500ul sediment to the new cultivation tube. All cultivation sediments were examined for 14 days daily under microscopy for *Blastocystis hominis* different forms in figure 3a-3c.

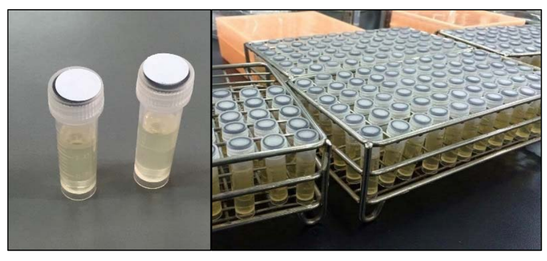

Figure 1-2: Small capped plastic cultivation tubes **(Left)** and cultivation rack for fecal cultivation method**(Right)**.

Molecular diagnosis of *Blastocystis hominis*

Blastocystis spp. isolate was enumerated in a capped tube with Jones' medium. After incubation, the sediment were extracted by innuPREP® DNA spin kit (Germany). Genomic solution was ready for conventional PCR method. Taq polymerase and dNTPs from Solis biodyne® (Estonia) was transferred to mixture tube with specific primer set. Then DNA amplification was performed by TC-300 thermal cycler for 30 cycles as following the previous protocol recommended[7]. The PCR product was approximately 195 bp. Sequenced PCR product was performed by Macrogen® (Korea). *Blastocystis hominis* was showed the highly similarity (89%) as *Blastocystis* subtype 3.

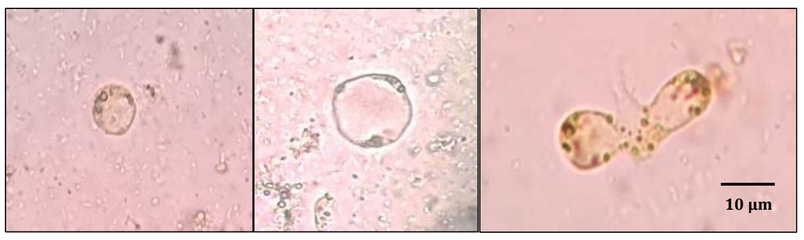

Figure 3a-b: *Blastocystis* spp. vacuolar forms (early and large vacuole), **3c:** Amoeboid form and binary fission.

Results

Positive laboratory result was efficiently determined a growth of *Blastocystis* spp. in cultivation method. Nevertheless, simple direct smear is a simple procedure and showed difficulty with low sensitivity. In other hand, the cultivation method with Jones medium can increase the probability of detection of *Blastocystis* in case of low infection numbers[8]. Also, participants were not found intestinal symptoms but stool cultivation can give a positive results. Small stools can showed the higher yield of diagnosis and detect in just a week with high sensitive diagnostic result in table 1.

Detections	Participants (N=475)	Positive results
Simple direct smear	23	5.05%
Jones medium	58	12.21%

Table 1. Laboratory diagnosis between simple direct smear and Jones' medium cultivation.

Conclusion

We are providing a preliminary study for diagnosis associated with healthy participants among community area. The prevention of parasitic infection can be determined by a sensitive diagnostic test to stop a spread and/or outbreak of intestinal parasites and protozoa to human. Awareness of pet contact and consumed the low hygiene of food and drinking water with contaminants. Also, we are recommended an usefulness and simply step of *Blastocystis* cultivation in medical laboratory with small capped tube cultivation methods distributed the minimum waste management and innovative idea of application diagnostic test in medical field.

References

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