Original Article

Detection of Blastocystis hominis using Simple direct smear compared with Jones' medium cultivation in Thatu Subdistrict, Chiang Khan District, Loei Province

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Abstract

Introduction: This study aimed to detect *Blastocystis hominis* for intestinal infection from participants in Thatu subdistrict, Chiang Khan district, Loei province.

Methods: The cross-sectional study was conducted using questionnaires and laboratory examinations. This study was approved by the Ethical Committee of the Research Institute of Rangsit University (RSPE01/2560).

Results: Questionnaires were collected from 209 males (44%) and 263 females (55%). Participants with no intestinal symptoms (38.9%) and who consumed bottled drinking water (36.1%) accounted for the majority of responses. Total 475 of stool specimens were examined by simple direct smear and cultured in Jones'medium. Intestinal parasites infections were found in specimens which showed Blastocystis hominis infection rate were 5.05% in simple smear and 12.21% in cultivation. Jones'medium cultivation method was significantly tool for detection test ($P<0.05$).

Conclusion: This is the first time study to minimize stools with micro-cultivation tubes. Most participants showed no intestinal symptoms but a positive cultivation found. Minimum stools with micro-cultivation tubes will be a useful tool to help investigation of intestinal parasite infection and confirmation with this applicable new diagnostic test.

Key words: Blastocystis hominis, Simple direct smear, Intestinal protozoa, Jones'medium Cultivation

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Introduction

Blastocystis hominis is an anaerobic intestinal parasite, and it is often caused the health problem among developing countries and local community area. This protozoan is found at a higher rate in immunocompromised hosts.[1, 2] *Blastocystis hominis* infections are caused by poor hygiene causing contaminated food and water. Prevalence of *Blastocystis spp.* infection and the zoonotic are potentially raised and have an impact on public health.[3, 4] The symptoms were increased an irritable bowel syndrome and gastrointestinal diseases as watery diarrhea.[5, 6] *Blastocystis spp.* infection was associated with a variety of gastrointestinal disorders and might play a significant role of inflammation.[6-8] Among community-based, a new technique was needed identify clinical features of *Blastocystis* infection for treatment.[9,10] A new tool was focused on the growth of this pathogenic parasite in anaerobic condition. Significant advances of this method could explain in the genetic diversity and identify the pathogenic strains. Using the genetic identification of extracellular proteases as virulence factors and subtyping techniques were established.[11-13] This study aims to apply new applicable cultivation tool for *Blastocystis hominis* detection and general health information in community for further advanced diagnostic tests.

Methods

Samples collection and questionnaires

Stool samples and questionnaires were collected along with general health information from healthy participants in the local community center. Participants signed a consent form before answering questionnaires and stool collection. The minimum samples were at least 250 samples which were calculated by using W.G.Cochran formulation. Stool samples were tested for simple direct smear and Jones'medium cultivation at Rangsit University. This study was approved by Ethical Committee of the Research Institute of Rangsit University (RSPE 01/2560).

Procedure for collecting specimens

Participants collected stool sample in a small clean container. The samples were kept in a cool box and sent to the laboratory unit at Faculty of Medical Technology, Rangsit University. Fecal cultivation tubes were incubated at 37°C. The remaining samples were kept at -20°C for molecular diagnostic test.

Simple direct smear method

Specimens were examined for stool formation and mixed with 0.85% NSS and 1% Iodine solution on glass slides. Specimen numbers were labeled on each slide prior to collect the cultivation tubes and health information. Stool sample (0.2mg) was taken with small plastic spatula and dissolved in saline and iodine solution and placed on a slide. Under microscopic examination were used 100 magnification and 400 magnification to identify for intestinal ova and parasites. The results were recorded *Blastocystis hominis* morphology and continued compare with the cultivation part.

Jones'medium cultivation for *Blastocystis hominis*

Jones'medium was prepared and sterilized in a 2.5 ml capped micro-cultivation tubes. The culture medium was kept at 4°C and working medium was thawed at room temperature and 10 % inactivated bovine serum was added as completed. The media was aliquoted into 1.5 ml cultivation tubes shown in Figure 1. Amount of 0.2 milligrams stools were placed in a cultivation tube and incubated at 37°C for 3-7 days for positive result. Fecal sediment was transferred by a plastic dropper and added in a new cultivation tube. All negative cultivation sediments were examined for 14 days under microscopy. Positive results were found *Blastocystis hominis* cysts and trophozoites in cultivation shown in Figure 2.

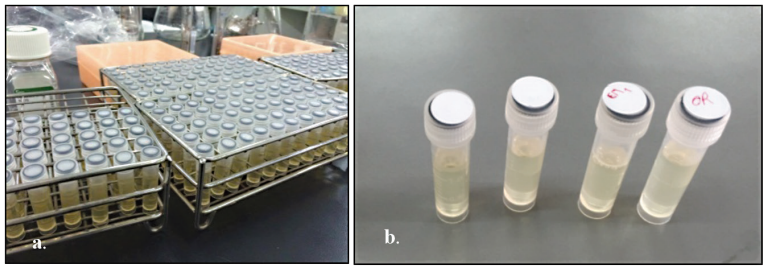

Figure 1 (a,b) Jones'medium in capped cultivation tubes and preparation (a.) Tube culture racks, (b.) Capped tubes.

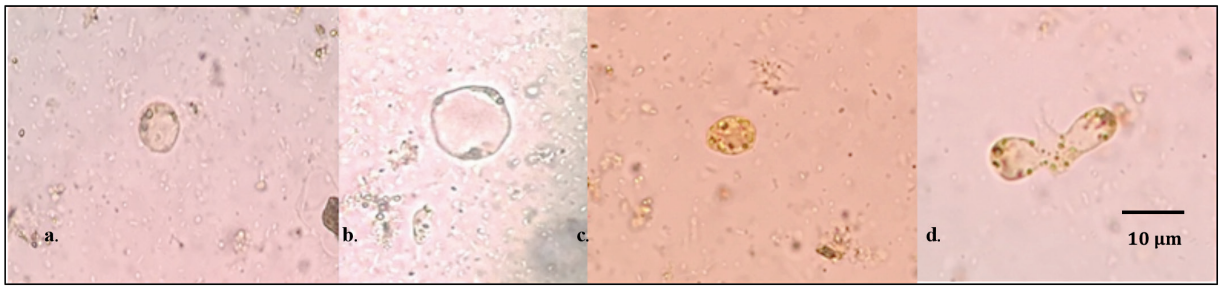

Figure 2 (a-d) Blastocystis spp. vacuolar form, granular form and amoeboid form during binary fission in Jones'medium (a) Vacuolar form, (b) Large vacuolar form, (c) Granular form and (d) Amoeboid form, respectively.

Data collection and analysis

The questionnaires were summarized and presented as the percentages of participants in general information and testing results. Detection of positive results of *Blastocystis hominis* between simple direct smear method and Jones'medium cultivation method were compared the positive result. General information was given the percentages of participants who have a healthy status. Statistical analysis was performed by using the chi-square correlation test for detection methods. SPSS statistic software version 17.0 was used in this study.

Results

General health information

The questionnaires responses showed the health status, history of infection and water sources among the participants. Most of the participants were female and no history of parasite infection in the community at Thatu subdistrict, Chiang Khan district, Loei province, Thailand. Participants were no intestinal symptoms and contact with the pet. Drinking water was most water source, then home reservoir and mixed more sources were shown in Table 1. Details of general health information were showed only returned questionnaires.

Table 1 The general health information of participants in this study

General Information	Participants (n=475)	% of participants (n)
Sex	Male	44.3% (209)
	Female	55.7% (263)
Water sources	Home reservior	32.1% (136)
	Well water	4.4% (19)
	Drinking water	36.1% (153)
	Public water plant	5.2% (22)
	Boiled water	0.7% (3)
	More than 2 mixed	20.0% (85)
History of infection	Parasite	25.5% (47)
	No parasite	74.5% (137)
Symptoms	No symptom	38.9% (155)
	Diarrhea	4.5% (18)
	Constipation	7.5% (30)
	Flatulance	8.2% (39)
	Stomach pain	2.8% (11)
	Uticaria i.e. hives	4.8% (19)
	more than 2 mixed	30% (119)
Contact with pet	Less than 3 times	24.2% (77)
	More than 3 times	28.6% (91)
	No pet	47.2% (150)

Simple direct smear

Total of 475 stools were found intestinal parasites and protozoa (23.26%). The simple direct smear method found intestinal protozoa such as *Blastocystis hominis* (12.04%), *Endolimax nana* (6.24%), *Giardia lamblia* (0.43%) and *Iodamoeba butschlii* (0.43%); nematodes such as, *Strongyloides stercoralis* (1.51%); trematodes such *Opisthorchis viverrini* (0.22%); and cestodes such *Taenia spp*. (0.84%). Mixed infection with more than one intestinal protozoa infections (1.05%) such as *Endolimax nana, Trichomonas spp., Blastocystis hominis, Entamoeba histolytica*-like, *Iodamoeba butschlii*.

Jones'medium cultivation for Blastocystis hominis

Total of 475 stools were tested for *Blastocystis hominis* using the Jones'medium cultivation. *Blastocystis hominis* cyst forms were detected by the microscopy of sediment under 400 magnification. Negative results of *Blastocystis hominis* were followed for up to 14 days. 58 specimens were found to be positive for *Blastocystis hominis* vacuolar, granular and amoeboid forms (12.21%) by Jones'medium cultivation (Table 2). Simple direct smear showed positive results for *Blastocystis hominis* in 5.05% of the samples. The data was analyzed by statistical program and showed significantly of chi-squares correlation test ($P<0.05$). *Blastocystis* cultivation sediments were kept in preservative solution for further morphology study.

Table 2 Comparison positive results between simple direct smear and cultivation

Detection of *Blastocystis hominis*	Percentages of Results (n=475)
Simple direct smear	5.05% (23)
Jones'medium cultivation	12.21% (58)

Discussion

Blastocystis spp. caused the intestinal infection. Health prevention concerns were increasingly concerned.[1-2] This surveillance was collecting data that have provided health status and showed risk of infection among the community at Chiang Khan district, Loei province. The most participants providing a preliminary data for association between clinical features with health status such as consumed drinking water in local water sources and no intestinal symptoms. Participants without diarrhea were presented *Blastocystis hominis* in both testing. It could provide a major risk of poor hygiene guidance and healthcare services for general consideration.[14-16] The prevention of parasitic infection showed awareness of pet contact and consumed the low hygiene of food and drinking water with caused of contamination.[17-20] The communities who serve as healthcare volunteers can help to support and provide the relationships of the causes and risks of infection and report to the community. It could distribute those participants on the treatment with physician in the prevention of drug resistant protozoan.

Laboratory result showed the detection of *Blastocystis* cultivation method is efficiently. However, simple direct smear is a simple procedure and minimum cost. The detection of low infection rate under a simple smear method was less specific and sensitivity as previous reported.[21-23] The cultivation method with Jones'medium revealed that the probability of detection of *Blastocystis* was increased in case of low infection numbers.[23] Moreover, most participants had no intestinal symptoms but stool cultivation detected the parasite with small amount of stool for higher yield of diagnosis and detection. By increasing the number of *Blastocystis* can grow as pathogens for detection. In conclusion, this method was helpful to improve detection compared to low sensitivity of direct smear. Jones'medium cultivation provides an extended laboratory leading to better diagnostic test.

Declaration of Interests

The authors declare that there are no conflicts of interest.

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บทคัดย่อ

การตรวจหาเชื้อบลาสโตซิสติส โฮมินิส ด้วยวิธีการตรวจสเมียร์อุจจาระ เปรียบเทียบกับการเพาะเลี้ยงในอาหารเหลวโจนส์ ในชุมชน ต.ธาตุ อ.เชียงคาน จ.เลย

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บทนำ: ในการศึกษาวิจัยครั้งนี้ วัตถุประสงค์เพื่อทำการศึกษาตรวจหาการติดเชื้อโปรโตซัว บลาสโตซีสติส โฮมินิส ของประชากรในตำบลธาตุ อำเภอเชียงคาน จังหวัดเลย

วิธีศึกษา: สำรวจแบบภาคคัดขวางจากผู้เข้าร่วมโครงการ ใช้โดยใช้แบบสอบถามสุขลักษณะเบื้องต้นและ เก็บตัวอย่างส่งตรวจทางห้องปฏิบัติการ ซึ่งได้ผ่านการพิจารณาของคณะกรรมการจริยธรรมมหาวิทยาลัยรังสิต เลขที่ 01/2560

ผลการศึกษา: ตัวอย่างเก็บได้จาก เพศชาย 209 รายและเพศหญิง 263 ราย คิดเป็นร้อยละ 44 และ 55 ตามลำดับโดยประชากรส่วนใหญ่มักไม่มีอาการร้อยละ 38.9 นิยมน้ำดื่มขวดร้อยละ 36.1 สิ่งส่งตรวจทั้งหมดจำนวน 475 ราย ถูกส่งตรวจและเพาะเชื้อลงในหลอดทดลอง พบว่าอัตราการติดเชื้อเชื้อโปรโตซัว บลาสโตซีสติส โฮมินิส ระยะแวคิวโอล ร่วมด้วย เมื่อทำการตรวจอุจจาระด้วยวิธีสเมียร์อุจจาระและการเพาะเลี้ยงเชื้อในอาหารเหลวโจนส์ พบว่าอัตราการติดเชื้อโปรโต ซัว บลาสโตซีสติส โฮมินิส ร้อยละ 5.05 และ 12.21 ตามลำดับ พบว่าวิธีการเพาะเลี้ยงเชื้อในอาหารเหลวโจนส์ มีประสิทธิภาพในการตรวจมีความจำเพาะของวิธีการตรวจมากกว่าอย่างมีนัยสำคัญที่ความน่าจะเป็นน้อยกว่า 0.05 ของการเปรียบเทียบความสัมพันธ์ด้วยสถิติไคสแคว

สรุปผลการศึกษา: การวิจัยนี้เป็นครั้งแรกที่ใช้อุจจาระปริมาณน้อยและนำหลอดทดลองจุกเกลียวมาปรับใช้ อีกทั้งผลส่วนใหญ่ของผู้ที่ไม่มีอาการทางระบบทางเดินอาหารแต่เมื่อนำมาเพาะเชื้อพบว่ามีการติดเชื้อ ดังนั้น การใช้วิธีทดสอบทางห้องปฏิบัติการนี้สามารถช่วยในการหาแหล่งโรคและยืนยัน เพื่อเป็นปัจจัยเพิ่มที่ช่วยในการวินิจฉัยเชื้อในหลอดทดลองได้

คำสำคัญ: บลาสโตซีสติส โฮมินิส, สเมียร์อุจจาระ, โปรโตซัวในลำไส้, การเพาะลี้ยงในอาหารเหลวโจนส์