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Boeck and Drbohlav Locke egg serum medium for detection of *Blastocystis hominis*

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- Objective** : *To study Blastocystis hominis infection, positivity by using Boeck and Drbohlav Locke egg serum medium (LE) cultivation and Jones' medium.*
- Design** : *Cross-sectional study.*
- Setting** : *Bann Kew Savag Mai, Mae Chame District, Chiang Mai Province and Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*
- Materials** : *One hundred and fifty-eight stool specimens from hill-tribe children, aged 1-18 years old.*
- Methods** : *All 158 stool specimens were cultivated in Jones' and LE media, transported to the laboratory and examined for B.hominis under the microscope. The parasites were detected after a 72 hr incubation at 37°C. Each stool sample was also identified for B.hominis by simple smear and concentration techniques.*
- Results** : *The prevalence rate of B.hominis was 0.91% and 1.09%, examined by simple smear and formalin ether concentration technique, respectively. The Jones' medium demonstrated 22.15% parasite positivity. The LE medium showed a high efficiency of organism detection with 43.67% parasite positivity.*

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Conclusion : *The classical LE medium which is routinely used to detect the presence of intestinal amoeba, primarily Entamoeba histolytica in many hospital-based laboratories has no available report for B.hominis detection. In this study, the standard LE medium performed the most efficiently for B.hominis production when compared to Jones' medium. The LE medium may be used as a reliable medium which has low-cost. This medium can eliminate the problem of the expiration date of the yeast extract used in the Jones' medium.*

Keywords : *Blastocystis hominis, Jones' medium, Boeck and Drbohlav Locke egg serum medium.*

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วิไล ศักดิ์ศิริสัมพันธ์, สุรางค์ นุชประยูร, ปวีณา ปราชญ์นิวัต, ดาวเรือง ลำจวน. การใช้อาหารเลี้ยงเชื้อ Boeck and Drbohlav Locke egg serum medium เพื่อตรวจหาเชื้อ *Blastocystis hominis*.
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- วัตถุประสงค์** : เพื่อตรวจหาเชื้อ *Blastocystis hominis* โดยใช้อาหารเลี้ยงเชื้อชนิด Boeck and Drbohlav Locke egg serum medium (LE) เปรียบเทียบกับอาหารเลี้ยงเชื้อชนิด Jones' medium
- รูปแบบการศึกษา** : การศึกษา ณ. จุดเวลาใดเวลาหนึ่ง
- สถานที่ที่ทำการศึกษา** : บ้านกิวสะแวกใหม่ อำเภอแม่แจ่ม จังหวัดเชียงใหม่ และภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร ประเทศไทย
- ตัวอย่างที่ศึกษา** : อุจจาระจำนวน 158 ตัวอย่าง จากเด็กไทยภูเขา อายุ 1 - 18 ปี
- วิธีการศึกษา** : อุจจาระแต่ละตัวอย่างจาก 158 ราย ถูกเพาะในอาหารเลี้ยงเชื้อ ชนิด Jones' medium และ LE medium ส่งมาที่ห้องปฏิบัติการ เพื่อการวินิจฉัยด้วยกล้องจุลทรรศน์ โดยผ่านการเพาะที่ 37°C เป็นเวลา 72 ชั่วโมง อุจจาระตัวอย่างแต่ละรายที่นำมาถึงห้องปฏิบัติการ ยังได้ตรวจหาเชื้อ *B. hominis* ด้วยวิธี simple smear และ concentration
- ผลการศึกษา** : อัตราความชุกของเชื้อ *B. hominis* คือ 0.91% และ 1.09% จากการตรวจวินิจฉัยโดยวิธี simple smear และ concentration การเพาะเลี้ยงในอาหารเลี้ยงเชื้อชนิด Jones' medium ให้ผลบวก 22.15% ส่วน LE medium มีประสิทธิภาพสูงให้ผลบวกมากถึง 43.67%
- บทสรุป** : LE medium ที่ใช้กันแพร่หลายเป็นประจำ เพื่อใช้เพาะเชื้อ amoeba มานานกว่าศตวรรษ โดยเฉพาะเชื้อ *Entamoeba histolytica* ยังไม่มีรายงานทดลองใช้เพื่อตรวจหาเชื้อ *B. hominis* ในการศึกษาครั้งนี้ LE medium มีประสิทธิภาพเพิ่มจำนวนเชื้อ *B. hominis* ได้มากกว่าเมื่อเทียบกับ Jones' medium อาหารเลี้ยงเชื้อชนิด LE medium น่าจะสะดวก ซึ่งมีราคาถูก อาหารเลี้ยงเชื้อชนิดนี้ ไม่มีปัญหาของการหมดอายุของสารสกัดยีสต์ที่ใช้เป็นส่วนประกอบใน Jones' medium
- คำสำคัญ** : *Blastocystis hominis* , อาหารเลี้ยงเชื้อ Jones' medium และ อาหารเลี้ยงเชื้อ Boeck and Drbohlav Locke egg medium

Blastocystis hominis, an intestinal organism, was first described in 1912. Over the years, it has been reclassified as a protozoan, not yeast. ⁽¹⁾ Although most patients infected with *Blastocystis hominis* are asymptomatic, they may have diarrhea and other gastro – intestinal symptoms both in immunocompetent and immunocompromised individuals. ⁽²⁾ The infection is more common in tropical countries, and is certainly more prevalent in developing countries which have the prevalence of 30 - 50%, compared to 1 - 10% in industrial parts of the world. ⁽³⁾

B.hominis is a polymorphic protozoan, consisting of vacuolar, avacuolar, multivacuolar, amoeboid, granular, and cystic forms. Its transmissions is via the fecal-oral route, through contaminated food or water, much like that other protozoa of the alimentary system. ⁽⁴⁾ Although there is no experimental confirmation, the cyst form is probably the infective stage. Most laboratories recognize only the vacuolar form as the diagnostic stage, since it can be easily distinguished from other protozoa. This classical form varies tremendously in size from 6 - 40 μ m characterized by a large central body, which is usually like a large vacuole. ⁽³⁾

There are several techniques routinely used to identify this protozoan including simple smear, concentration, and staining techniques. They basically demonstrate the parasite in faecal specimens. Permanent stained smear is the method of choice because the stool is directly smeared on wet preparation, but it also yields false negative reports. In the technique, the organism is easily destroyed if fresh stool is rinsed in water before fixation. ⁽⁵⁾ In order to gain more sensitive diagnostic

test, *in vitro* cultivation in Jones' medium of fresh specimens for the protozoa prior to light microscopy has been recently described and now widely used. ^(2-4, 6, 7) However, the classical Boeck and Drbohlav Locke egg serum (LE) medium consisting of whole eggs, serum and rice powder which is routinely used to detect the presence of intestinal amoeba, primarily *Entamoeba histolytica* in many hospital-based laboratories has not been studied for *B.hominis* detection. ⁽⁶⁾ In this study, however we introduce LE medium for *in vitro* cultivation of *B.hominis* from faecal specimens from hill-tribe children of Bann Kew Savag Mai, Mae Chame District, Chiang Mai Province, in Northern Thailand.

Materials and Methods

Area of study

The study area was Bann Kew Savag Mai, Mae Chame District, Chiang Mai. (Fig. 1). It was performed from March to November 2008. The total population of this study area included adults and children; all are 300 hill-tribe Karens, with 160 individuals aged ≤ 18 years old.

One hundred and fifty-eight children ranging in age from 1 to 18 years old (male: 85, female: 73) were recruited for participation in the trial. Her Royal Highness Princess Maha Chakri Sirindhorn provided her private fund for the establishment of the school in this village. All Karens in this study were informed about the purpose of the present study under the help of local translators and volunteer teachers, education and documents of the parasitic infections were given and distributed. The study protocol has been approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University.

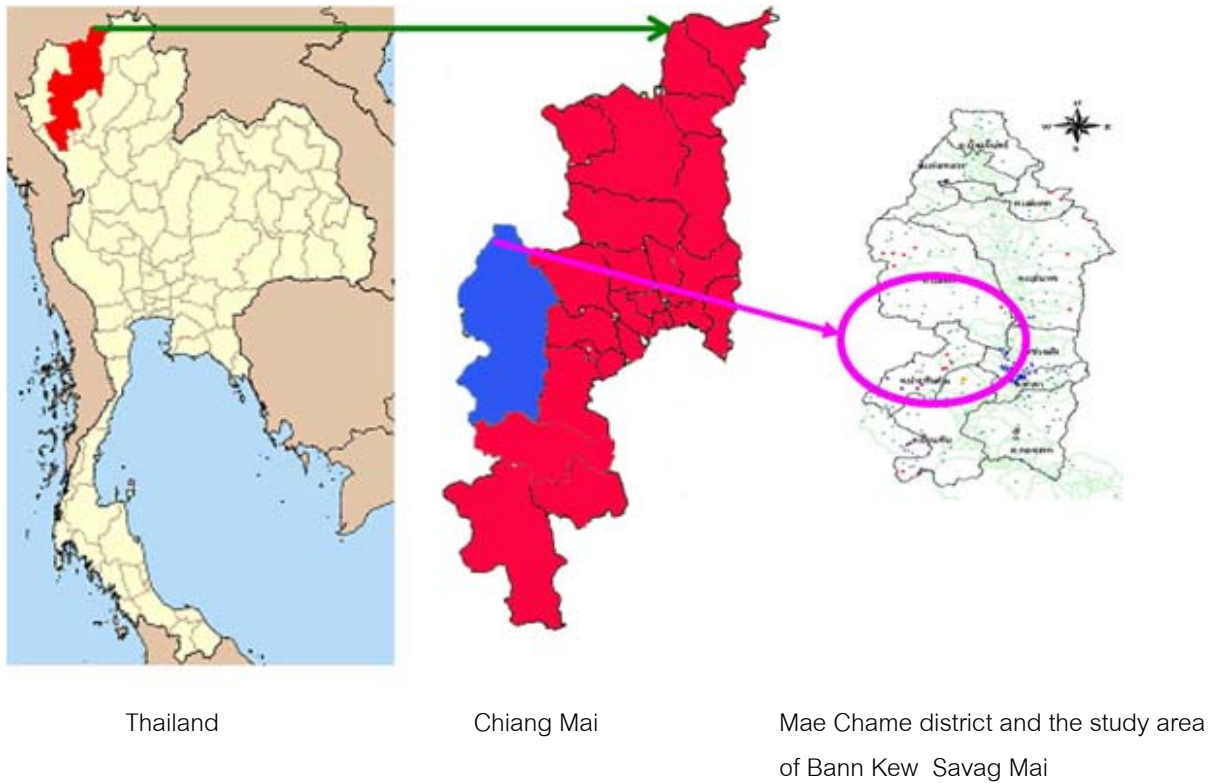


Figure 1. A map of Thailand; Chiang Mai Province and the survey point (Bann Kew Savage Mai in Mae Chame District).

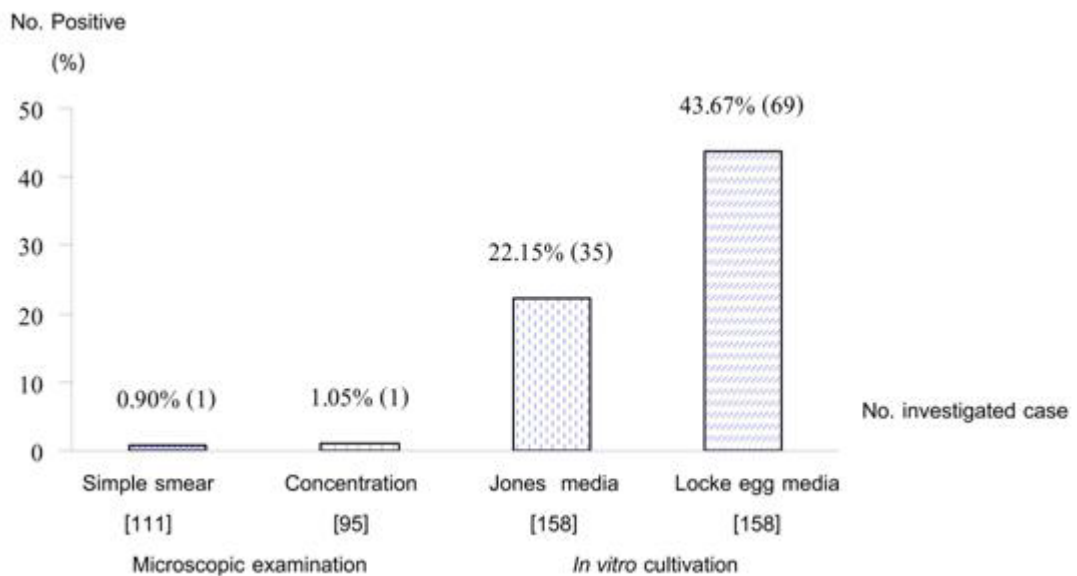


Figure 2. The comparison of *B. hominis* infection rates determined by microscopic examination (simple smear and concentration techniques) and *in vitro* cultivations (Jones' media and LE media techniques). The number of positive cases and the number of samples investigated are indicated in () and [], respectively.

Stool examination and cultivation

The techniques used for stool examination were simple smear and formalin-ether concentration techniques. ⁽⁷⁾ Approximately, 50 mg of each stool sample were cultivated immediately in 5 ml screw-capped tube with medium of Jones' and LE media after collection. Each of the cultured media and the collected stool samples were then carried to the laboratory of the Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. We found some stool samples were inadequate for simple smear or (and) concentration, therefore, only culture technique was performed.

The cultured tubes were then incubated at 37 °C for 72 hrs, before examined by two individuals independently. Sub-cultivation was performed every 72 hrs. The organism demonstrated in Jones gradually decreased after two weeks of cultivation. In contrast to LE medium, the organism could be maintained for more than three months.

Data analysis

The statistically significant differences were analyzed by unpaired student's t-test.

Results

We introduced the reliable medium of LE medium to detect *B. hominis*. A total of 158 stool samples obtained from 85 male and 73 female children from the mountainous areas of in Bann Kew Savag Mai, Mae Chame District of Chiang Mai. The prevalence of *B.hominis* using stool simple smear was 0.90% which was not significantly different when compared to the stool concentration technique (1.05%). For this protozoa detection, we recognized the vacuolar form which normally presented in majority when compared with other forms. This classical form varies from 6 - 40 µm with a large vacuole at central body which was easily distinguished from other protozoa. When cultivation of Jones' medium was used as the standard cultivation of this protozoan,

Table 1. Composition of Jones and LE medium per 1 liter of purified water.

Jones medium			Boeck and Drbohlav Locke egg serum medium		
Na ₂ HPO ₄	1.244	gm	NaCl	8.0	gm
KH ₂ PO ₄	0.397	gm	KCl	0.2	gm
NaCl	7.087	gm	CaCl ₂		0.2
gm					
Yeast extract f..dfs(((10	gm	MgCl ₂		0.1
gm					
(BBL, MD21030 USA)					
Horse serum	100	ml	NaH ₂ PO ₄ 2H ₂ O	0.1	gm
			NaHCO ₃	0.1	gm
			Hen egg	4	eggs
			Horse serum	100	ml
pH	7.0		pH	7.0	

the higher positive could demonstrate (22.15%). Interestingly, this high infection rate was more obvious by using LE medium (43.67%), and showed statistically significant difference from the Jones' medium (P value = 0.001). However the high production of organism in both media of LE and Jones' was mostly the vacuolar form. Interestingly, the size of this organism in media cultivation tended to bigger than that detected in fresh stool specimen.

There was no statistically significant difference between male and female. The age 11-15 children had the highest infection rate (73.33%), when compared with other age groups (Table 2).

Discussion

The infection rate of intestinal parasitic infections varied from one area to another depending on the degree of personal and community hygiene, sanitation and climatic factors. By using Kato Thick smear technique the intestinal parasitic infection rate in the hill-tribes children of Chiang Mai reported in 1982 was 76.76%.⁽⁹⁾ By using formalin ether sedimentation technique, another survey in 1989

revealed the rate of intestinal parasite at 48 - 70%.⁽¹⁰⁾

Since the last decade, the rate of parasitic infection in the northern part has been ranged from 45.7% to 60%.⁽⁹⁻¹¹⁾ However, *B.hominis* infection has not been reported. In addition, our recent report in different areas (Sedosa, Rajaprajanugroh 31 school and Baan Mai Pattana Santi of Mae Chame district) of Chiang Mai province has also shown negative result for *B.hominis*.⁽¹²⁾ It is possible that conventional simple smear and concentration techniques provided low sensitivity for this protozoan detection.

The data of protozoan infections have usually been a byproduct of survey for helminth infections, which is normally performed by stool simple smear or stool concentration of the formalin ether or Kato's thick smear techniques.^(6, 7) However, to provide reliable data of *B.hominis* prevalence, a more appropriate method is required. The stool concentration technique is basically more sensitive than simple smear in diagnosis, but due to the low infection rate, and duration to carry stool samples from remote areas to the laboratory, the protozoan might have decreased or degenerated.

Table 2. Prevalence of *B.hominis* infection among 158 children detected by LE medium cultivation from Bann Kew Savage Mai Chiang Mai, Northern Thailand, in 2008 classified by age and sex.

Age group (year)	No. of Male		No. of Female		Total	
	Investigated	Positive (%)	Investigated	Positive (%)	Investigated	Positive (%)
1-5	13	8 (61.53%)	14	6 (42.86%)	27	14 (51.85%)
6-10	44	25 (56.82%)	37	21 (56.76%)	81	46 (56.79%)
11-15	6	5 (83.33%)	9	6 (66.67%)	15	11 (73.33%)*
15-19	22	3 (13.64%)	13	1 (7.69%)	35	4 (11.43%)
Total	85	41 (48.24%)	73	34 (46.58%)	158	75 (47.47%)

* p – value < 0.001

In this study, other protozoa could also be found in the stool samples (data not shown). *B.hominis* could be found together with other intestinal pathogenic protozoa: *G.lambli*a, *E.histolytica*, *E.coli* and *E.nana*.⁽²⁾ The children aged 11 - 15 had the highest infection rate when compared to the other studies.^(12 - 17) This protozoan infection can be an indicator of poor personal hygiene. Communities with high prevalence of *B.hominis* infection have to improve their sanitation to prevent, not only *B.hominis* but also other intestinal protozoa.

Recent reports have showed that infections are more common in residents of developing countries (infection rate 18.9 - 77.9%)^(17 - 20) than those of developed countries (infection rate 1.0 - 2.6%).^(21, 22) Immigrants, refugees, and adopted children from developing countries also have high infection rates (26 - 59.0 %).⁽²³⁻²⁶⁾

However, in this study we have demonstrated that LE medium performed most efficiently well for *B.hominis* reproduction. This medium, consists of whole eggs, serum and rice powder; it is routinely used to detect the presence of intestinal amoeba, primarily *Entamoeba histolytica* in our and many other hospital-based laboratories.⁽⁶⁾

LE medium has the advantage of lower cost than that of the Jones' medium. Since the hen egg consisting in LE medium is cheaper than yeast extract, which must be imported (BBL, MD21030 USA). Moreover, hen egg could eliminate concerns about expiration dates of yeast extracts.

This consideration is particularly relevant for laboratories in developing countries. In addition, it was found to be more efficient than the standard medium of Jones' for cultivation of parasites from stool

specimens. These two media have in common several ingredients. The LE medium might be a suitable alternative to the Jones' medium. The *B.hominis* infection rate determined by simple smear and concentration techniques may be under- reported. The combination of simple smear and cultivation may provide the standard approach for detecting *B.hominis* in patient specimens. However, the culture technique is more cost-effective and need less expertise to perform than those of molecular biological and immunological techniques for laboratory diagnosis.^(15, 27) Moreover, the efficient reproduction of *B.hominis* in LE medium may also be suitable for antigen preparation, *in vitro* drug sensitivity studies, and organism harvesting prior to PCR.

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