Parasites &Vectors

Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand

Duangnate Pipatsatitpong[1], Ram Rangsin[2], Saovanee Leelayoova[3], Tawee Naaglor[3] and Mathirut Mungthin[3*]

Abstract

Background: *Blastocystis* sp. is one of the most common intestinal protozoa in humans. Unlike other intestinal parasitic infections such as giardiasis and cryptosporidiosis, the epidemiology of blastocystosis in children who live in crowded settings such as day-care centers and orphanages has been rarely explored.

Methods: A retrospective cohort study was conducted to evaluate incidence and risk factors of *Blastocystis* infection in an orphanage every two consecutive months during April 2003 to April 2004, in Bangkok, Thailand. *Blastocystis* sp. was identified using direct simple smear, and *in vitro* cultivation in Jones' medium.

Results: The incidence rate was 1.8/100 person-months and the independent risk factors associated with *Blastocystis* infection were age, nutritional status and orphans living in the room where their childcare workers were infected.

Conclusions: Person-to-person transmission was most likely to occur either from orphans to childcare workers or from childcare workers to orphans living in the same room. Universal precautions such as regular hand washing and careful handling of fecally contaminated materials are indicated.

Background

Blastocystis sp., one of the most common intestinal protozoa in humans, has a worldwide distribution and is frequently seen in stool samples identified in parasitological surveys. *Blastocystis* sp. had been thought to be a commensal organism since most cases of blastocystosis have been reported as asymptomatic carriages [1-3]. However, a few studies have identified *Blastocystis* sp. as a causative agent of gastrointestinal diseases [4-6]. The high prevalence of 10-40% of *Blastocystis* sp. has been reported in developing countries [7,8] while in Thailand, the prevalence of *Blastocystis* carriage in various groups is as high as 11-37% [9-11]. Risk factors for acquiring blastocystosis have been identified, and include contact with animals and consuming contaminated food or water [12-14]. Most studies of *Blastocystis* infection in young Thai children were performed using cross-sectional design [15-18] so the epidemiological information including incidence, true risk factors and natural history of this infection is still lacking. In addition, compared to other intestinal

parasitic infections such as giardiasis and cryptosporidiosis, the epidemiology of blastocystosis in children who live in crowded settings such as day-care centers and orphanages has been rarely explored. Thus, the objectives of this study were to determine the incidence of and risk factors for *Blastocystis* infection in an orphanage, Bangkok, Thailand.

Methods
Study population

A retrospective cohort study was conducted in Thai orphans who lived in an orphanage located in Bangkok, Thailand from April 2003 to April 2004. This orphanage is for orphans aged less than five years and consisted of 10 rooms for orphans, accommodating 30 to 40 orphans with 3 childcare workers and a food and milk preparation room. The orphans were raised in the institute until they were adopted. Otherwise, they were transferred to other orphanages for older children at the age of five. These children were rarely brought out of the area. The childcare workers in each room were asked to collect stool samples and complete standardized questionnaires for these orphans for whom they were responsible every two consecutive months. The study was reviewed and approved by

* Correspondence: mathirut@hotmail.com
[3]Department of Parasitology, Phramongkutklao College of Medicine, Ratchathewi, Bangkok 10400, Thailand
Full list of author information is available at the end of the article

the Ethical Committee of the Medical Department, the Royal Thai Army.

Stool collection and examination

A total of 697 fecal samples were collected and transported to the Department of Parasitology, Phramongkutklao College of Medicine, Bangkok, Thailand. To identify *Blastocystis* sp., each stool sample was screened by direct simple smear. Stool samples were then cultured in Jones medium supplemented with 10% horse serum. After incubation for 48 to 72 h at 37°C, each sample was examined by light microscopy. A *Blastocystis*-infected case was defined as the presence of vacuolar, cyst, trophozoite or other forms in the culture of stool specimens.

Questionnaire

To determine the risk factors and outcomes of *Blastocystis* infection, standardized questionnaires were collected regarding age, sex, weight, height, underlying disease, HIV infection status, and present illnesses including diarrhea, abdominal pain and other gastrointestinal symptoms. Diarrhea was defined as a change in their normal pattern of bowel movements and at least three loose stools during a 24-hour period. Dysentery was defined as at least one passage of mucous bloody stool in one day. The weight and the height of individuals were recorded at the nursing room. To determine their nutritional status, criteria from the Institute of Nutrition, Mahidol University (INMU) Thai Growth Study was used [19].

Statistical analysis

Incidence was defined as the number of new cases occurring during the observation period. The estimated date of infection for the incident cases was taken as the midpoint between the last negative result and first positive result. Survival analysis was used to calculate median time to clearance of *Blastocystis* infection. The estimated date of the time to clearance was taken from the period between the first positive results to the first negative results of *Blastocystis* identification. Difference between the median times to clearance was analyzed by Log-rank test. Possible risk factors were analyzed using incidence rate ratios and their 95% confidence intervals. The chi-square or Fisher's exact test was used to compare proportions. Poisson regression using STATA/SE for Window version 9.2 (StataCorp LP, College Station, TX) was performed for multivariate analysis to assess the independent association of the risk factors and *Blastocystis* infection.

Results

Study population

A total of 697 participants, composed of 606 orphans and 91 childcare workers, were enrolled in the study. The median ages of the orphans and childcare workers were 10.5 months (0.3 months to 9.0 years) and 37.0 years (20.0 to 52.0 years), respectively. The number of participants who enrolled every two consecutive months was 343, 336, 336, 319, 287, 339 and 305, respectively (Table 1). Ninety-eight orphans (14.1%) were HIV positive (57 males and 41 females). Information on $CD4^+$ T-lymphocyte count was not available. All HIV-positive orphans were prescribed antiretroviral therapy (i.e., zidovudine and didanosine).

Characterization of persons with *Blastocystis* infection

The characteristics of the enrolled participants are shown in Table 2. *Blastocystis* infection was significantly different when analyzed by age group ($p < 0.001$), rooms ($p < 0.001$) and reception of care from an infected childcare worker. ($p = 0.002$). No significant difference was found between sex, HIV infection, nutritional status and diarrhea. In addition, no other symptoms including abdominal pain, flatulence, anorexia and nausea/vomiting were associated with *Blastocystis* infection ($p = 1$, 0.150, 0.233 and 0.610, respectively).

Prevalence and incidence of *Blastocystis* infection

From a total of 697 participants, the prevalences, incidences and the incidence rates of *Blastocystis* carriage during each round are shown in Table 1. A total of 414

Table 1 Prevalence, incidence and incidence rate of *Blastocystis* infection in the orphanage, Bangkok, Thailand from April 2003 to April 2004.

	Prevalence N (%)	Incidence N (%)	Time (person-months)	Incidence rate (100 person-months)	Total
April 2003	94 (27.4)	-	-	-	343
June 2003	35 (10.4)	9 (2.7)	450.2	1.9	336
August 2003	29 (8.6)	9 (2.7)	470.9	1.9	336
October 2003	23 (7.2)	5 (1.6)	259.1	1.9	319
December 2003	19 (6.6)	9 (3.1)	283.3	3.2	287
February 2004	26 (7.7)	6 (1.8)	462.6	1.3	339
April 2004	26 (8.5)	8 (2.6)	433.1	1.8	305

Table 2 Characteristics of the enrolled subjects and the incidence of *Blastocystis* infection from April 2003 to April 2004.

Characteristics	No. of enrolled subjects	No. (%) infected	*p value*
Sex			
Male	232	22 (9.5)	
Female	182	15 (8.2)	0.660
Age (months)			
0-12	202	1 (0.5)	
13-24	93	3 (3.2)	
25-36	24	7 (29.2)	
37-48	26	6 (23.1)	
49-60	12	7 (58.3)	
> 60	56	13 (23.2)	< 0.001
Room no. (specific group)			
No. 1 (36-60 months)	32	15 (46.9)	
No. 2 (new born to 8 months)	47	1 (2.1)	
No. 3 (new enrolled)	67	0 (0.0)	
No. 4 (HIV- infected children)	69	9 (13.0)	
No. 5 (32-36 months)	21	5 (23.8)	
No. 6 (24-32 months)	22	3 (13.6)	
No. 7 (new born to 8 months)	37	1 (2.7)	
No. 8 (8-12 months)	42	1 (2.4)	
No. 9 (12-18 months)	50	1 (2.0)	
No. 10 (18-24 months)	23	0 (0.0)	
Food and milk preparation room	4	1 (25.0)	< 0.001
Nutritional status			
Normal nutrition	281	29 (10.3)	
Undernutrition	86	4 (4.7)	
Overnutrition	33	3 (9.1)	0.275
HIV infection			
No	330	27 (8.2)	
Yes	67	9 (13.4)	0.172
Infected childcare worker(s) in the room			
No	257	14 (5.4)	
Yes	119	18 (15.1)	0.002
Diarrhea			
No	371	31 (8.4)	
Yes	7	1 (14.3)	0.464

participants who were negative for *Blastocystis* infection at the baseline survey in April 2003 were enrolled and followed up every two consecutive months until April 2004. Of these *Blastocystis*-negative participants, 232 (56.0%) were male and 182 (44.0%) were female. The incidence rate of blastocystosis was 1.8/100 persons-months.

Time to clearance of *Blastocystis* infection
Analysis of time to clearance was performed in a total of 115 infected participants whose first positive and first negative results were identified. Of these, 89 and 26 were children and childcare workers, respectively. The median time to clearance of *Blastocystis* infection in this

population was 2.62 months (95%CI, 2.57-2.67). Subgroup analysis showed that the median time to clearance was 2.59 months (95%CI, 2.55-2.63) in children and 10.03 months (95%CI, 5.56-14.51) in childcare workers. Significant difference was found between the median times to clearance of these 2 groups with the *p* value of < 0.001.

Risk factors of *Blastocystis* infection
Univariate analysis showed an increasing risk of acquisition of *Blastocystis* infection with age (IRR = 1.04, 95% CI, 1.03-1.05). Additionally, children who lived in rooms where childcare workers were infected with *Blastocystis*

sp. had a higher risk of acquiring blastocystosis (IRR = 3.3, 95%CI, 1.7-6.7). Multivariate Poisson regression analysis showed that age was independently associated with *Blastocystis* infection. The risk of acquiring *Blastocystis* infection increased at a rate of 1.04 for each additional year of the subject's age. However, classification of age into 3 groups (< 2 yr, 2-5 yr, > 5 yr) showed that those who were 2 to 5 and greater than 5 years had higher risk of *Blastocystis* infection than those who were less than 2 years (*p* < 0.001, data not shown). Since each room was assigned for orphans within a specific age group, rooms were not included into the multivariate analysis model. Nutritional status showed that orphans who had undernutrition status had lower risk of infection than those with normal or overnutrition status. Additionally, children who lived in a room where childcare workers were also infected with *Blastocystis* sp. had a 3.1 times higher risk of acquiring *Blastocystis* infection (95%CI, 1.3-7.6) than those who lived in the rooms without infected childcare workers after adjusting for sex and HIV infection status. In contrast, sex and HIV status were not associated with blastocystosis (Table 3).

Discussion

In this study, *Blastocystis* sp. was identified using both direct wet smears and stool culture. Short term *in vitro* cultivation is considered to be a diagnostic method with a high sensitivity for the detection of *Blastocystis* sp. [20]. The incidence rate of *Blastocystis* infection was 1.8/100 person-months in this setting, which was low compared with a previous study in children at an older age group [21]. The prevalences of *Blastocystis* carriage in orphans at each time point varied from 7.2% to 27.4%. The highest prevalence was shown at the beginning of the study, and then gradually decreased after the time of follow up. This might be due to the notification of the first finding. Additional interventions that occurred during early 2004 to prevent transmission of parasitological infections included health education and the cleaning of clothes and bedding accessories using autoclave heat treatment. Since *Blastocystis* is not a host-specific organism, transmission from human-to-human and animal-to-human could have occurred [22,23]. It is indicated that the orphans acquired *Blastocystis* infection in this orphanage since no newly-enrolled orphans were infected by *Blastocystis* at the enrollment. In this orphanage, zoonotic transmission was unlikely to have occurred since no pets were allowed to be raised in this community setting. Water contamination has been identified as a method of transmission [16,18,24]. In this orphanage, filtered water was supplied from the same source to be used as drinking water in every room. Because significant differences of *Blastocystis* infection were found among different

rooms, it was less likely that water was the main route of transmission. In this study, the prevalence and incidence of blastocystosis was significantly different among rooms. Thus waterborne transmission of *Blastocystis* infection was less likely since drinking water for orphans was from the same source.

Univariate and multivariate analysis showed that older orphans had a higher risk of acquiring the infection than those at younger ages. This independent risk factor was probably related to the children's behaviors such as playing with each other on unclean floor areas or playground, poor toilet training and poor hygienic practices, e.g., food-handling. In contrast, younger children had been taken care of by their childcare workers. These results were similar to those reported in several studies, where *Blastocystis* sp. was often identified in older children and adults [9,15,25]. Most infected children in this orphanage had normal nutritional status. Analysis of risk associations showed that children who had undernutritional status had a significantly lower risk for acquiring the infection. From personal communication, these undernourished children were usually among the new enrollments. After living in the orphanage, they had received better care, and, thus, were less likely to be undernourished. This may indicate that acquiring blastocystosis was related to the period of time that they stayed in this orphanage.

The possibility of person-to-person transmission was shown when the association of infected orphans and their childcare workers was analyzed. A significant association was found between the infected childcare workers and the orphans under their responsibility. Orphans who lived in the room where childcare workers were infected with *Blastocystis* sp. had a higher risk of acquiring blastocystosis than those who lived in the rooms without infected child care workers. This evidence suggests person-to-person transmission of *Blastocystis* sp. in this community although no subtyping or phylogenetic analysis was performed to corroborate this finding. Survival analysis showed that the median time to clearance of *Blastocystis* infection in the childcare workers was 10.03 months. Suresh et al. (2009) studied *in vivo* encystation of *Blastocystis* sp. and found that *Blastocystis* survived in infected individuals over a 6-month period [26]. Zhou and colleagues (2010) inoculated *Blastocystis* sp. into the abdominal cavity of mice, and found that only the cyst form existed for more than 6 months [27]. Both studies showed that encystations enable *Blastocystis* sp. to survive and avoid an immune attack from the host. The time to clearance of *Blastocystis* infection in the childcare workers was similar to those found in these studies. Therefore, it is likely that the infected childcare workers could consistently transmit *Blastocystis* sp. for a period of time. However, the median time to clearance of *Blastocystis* infection in the orphans

Table 3 Univariate and multivariate analysis for risk factors of *Blastocystis* infection.

Characteristics	No. positive for *Blastocystis*	Person-months of follow up	IR (100 person-months)	IRR (95% CI)			
				Crude	p value	Adjusted	p value
Sex							
Male	29	1491.1	1.9	1		1	
Female	17	1128.9	1.5	0.8 (0.4-1.5)	0.408	0.7 (0.3-1.5)	0.366
Age (months)	46	2620.0	1.8	1.04 (1.03-1.05)	< 0.001	1.04 (1.03-1.05)	< 0.001
Nutritional status							
Normal nutrition	37	1653.9	2.2	1		1	
Undernutrition	4	655.8	0.6	0.3 (0.1-0.8)	0.006	0.3 (0.1-0.9)	0.044
Overnutrition	4	215.9	1.9	0.8 (0.2-2.3)	0.851	0.5 (0.1-2.5)	0.417
HIV infection							
No	34	2116.9	1.6	1		1	
Yes	10	444.4	2.3	1.4 (0.6-2.9)	0.352	1.7 (0.6-4.9)	0.313
Infected childcare worker(s) in the room							
No	16	1613.8	0.9	1		1	
Yes	24	723.4	3.3	3.3 (1.7-6.7)	0.002	3.1 (1.3-7.6)	0.014

Data were adjusted for sex and HIV infection status. IR = incidence rate; IRR = incidence rate ratio

was significantly shorter. This finding may reflect the recurrent exposure to *Blastocystis* in the childcare workers since they lived outside the orphanage. Further studies should be performed to identify the factors contributing to the difference in the time to clearance such as host responses and *Blastocystis* serotypes.

Blastocystis infection in these orphans and childcare workers caused no symptoms. This is similar to several studies reporting asymptomatic *Blastocystis* infection in healthy individuals [3,9,28]. It is still unclear why blastocystosis can cause symptoms in some patients. Some specific subtypes of *Blastocystis* sp. such as subtype 3 have been speculated to cause gastrointestinal symptoms; however, no compelling evidence exists for this association [29-32]. It is possible that these orphans and childcare workers were infected by nonpathogenic subtypes. Unfortunately, we have no information of *Blastocystis* subtypes in this study. In addition, it has been shown that immunological status of the infected individual influenced the prevalence of *Blastocystis* infection and its clinical outcomes [33-36]. A few studies identified a significantly higher prevalence of *Blastocystis* infection in an immunocompromised host than in healthy individuals. However, in this orphanage the incidence rate of *Blastocystis* infection in HIV-positive children (IR = 2.3) was not significantly higher than in the HIV-negative group (IR = 1.6). Asymptomatic infection in the HIV-infected group with no higher incidence might be due to their intact immune status. No higher incidence of *Blastocystis* infection may be also the result of strict adherence to universal precautions of the childcare workers in the room for HIV-infected children.

Conclusions

Our study was the first retrospective cohort study of *Blastocystis* infection conducted in an orphanage with a low incidence of *Blastocystis* infection. Risks factors for *Blastocystis* infection were age, nutritional status and orphans living in the room where their childcare workers were infected. Person-to-person transmission was the most likely to occur either from orphans to childcare workers or from childcare workers to orphans living in the same room. In an institution such as an orphanage, where *Blastocystis* sp. could spread easily, universal precautions such as regular hand washing and careful handling of feces contaminated materials should be taken.

Author details
[1]Biomedical Sciences Program, Faculty of Allied Health Sciences, Thammasat University, Khlong Luang, Pathum Thani 12121, Thailand. [2]Department of Military and Community Medicine, Phramongkutklao College of Medicine, Ratchathewi, Bangkok 10400, Thailand. [3]Department of Parasitology, Phramongkutklao College of Medicine, Ratchathewi, Bangkok 10400, Thailand.

References
1. Eroglu F, Genc A, Elgun G, Koltas IS: **Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR.** *Parasitol Res* 2009, **105**:1589-1592.
2. Leder K, Hellard ME, Sinclair MI, Fairley CK, Wolfe R: **No correlation between clinical symptoms and *Blastocystis hominis* in immunocompetent individuals.** *J Gastroenterol Hepatol* 2005, **20**:1390-1394.
3. Udkow MP, Markell EK: ***Blastocystis hominis*: prevalence in asymptomatic versus symptomatic hosts.** *J Infect Dis* 1993, **168**:242-244.
4. Dogruman-AI F, Yoshikawa H, Kustimur S, Balaban N: **PCR-based subtyping of *Blastocystis* isolates from symptomatic and asymptomatic individuals in a major hospital in Ankara, Turkey.** *Parasitol Res* 2009, **106**:263-268.
5. Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P: **Is *Blastocystis hominis* a cause of diarrhea in travelers? A prospective controlled study in Nepal.** *Clin Infect Dis* 1995, **21**:97-101.
6. Hussein EM, Hussein AM, Eida MM, Atwa MM: **Pathophysiological variability of different genotypes of human *Blastocystis hominis* Egyptian isolates in experimentally infected rats.** *Parasitol Res* 2008, **102**:853-860.
7. Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IK, Hossain MB, Zaman V, Haque R, Takahashi Y: **Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries.** *Parasitol Res* 2004, **92**:22-29.
8. Nimri LF: **Evidence of an epidemic of *Blastocystis hominis* infections in preschool children in northern Jordan.** *J Clin Microbiol* 1993, **31**:2706-2708.
9. Yaicharoen R, Sripochang S, Sermsart B, Pidetcha P: **Prevalence of *Blastocystis hominis* infection in asymptomatic individuals from Bangkok, Thailand.** *Southeast Asian J Trop Med Public Health* 2005, **36**:17--20.
10. Thathaisong U, Worapong J, Mungthin M, Tan-Ariya P, Viputtigul K, Sudatis A, Noonai A, Leelayoova S: ***Blastocystis* isolates from a pig and a horse are closely related to *Blastocystis hominis*.** *J Clin Microbiol* 2003, **41**:967-975.
11. Taamasri P, Leelayoova S, Rangsin R, Naaglor T, Ketupanya A, Mungthin M: **Prevalence of *Blastocystis hominis* carriage in Thai army personnel based in Chonburi, Thailand.** *Mil Med* 2002, **167**:643-646.
12. Parkar U, Traub RJ, Kumar S, Mungthin M, Vitali S, Leelayoova S, Morris K, Thompson RC: **Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential.** *Parasitology* 2007, **134**:359-367.
13. Stensvold CR, Alfellani MA, Norskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG: **Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype.** *Int J Parasitol* 2009, **39**:473-479.
14. Cheng HS, Haung ZF, Lan WH, Kuo TC, Shin JW: **Epidemiology of *Blastocystis hominis* and other intestinal parasites in a Vietnamese female immigrant population in Southern Taiwan.** *The Kaohsiung Journal of Medical Sciences* 2006, **22**:166-170.
15. Yaicharoen R, Ngrenngarmlert W, Wongjindanon N, Sripochang S, Kiatfuengfoo R: **Infection of *Blastocystis hominis* in primary schoolchildren from Nakhon Pathom province, Thailand.** *Trop Biomed* 2006, **23**:117-122.
16. Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M: **Drinking water: A possible source of *Blastocystis* sp. subtype 1 infection in schoolchildren of a Rural Community in Central Thailand.** *Am J Trop Med Hyg* 2008, **79**:401-406.
17. Taamasri P, Mungthin M, Rangsin R, Tongupprakarn B, Areekul W, Leelayoova S: **Transmission of intestinal blastocystosis related to the**

quality of drinking water. *Southeast Asian J Trop Med Public Health* 2000, **31**:112-117.

18. Leelayoova S, Rangsin R, Taamasri P, Naaglor T, Thathaisong U, Mungthin M: **Evidence of waterborne transmission of** *Blastocystis hominis.* *Am J Trop Med Hyg* 2004, **70**:658-662.

19. Institute of Nutrition, Mahidol University: **INMU Thai-growth.,** Available at: http://www.inmu.mahidol.ac.th/thaigrowth/. Accessed September 1, 2009.

20. Leelayoova S, Taamasri P, Rangsin R, Naaglor T, Thathaisong U, Mungthin M: **In-vitro cultivation: a sensitive method for detecting** *Blastocystis hominis.* *Ann Trop Med Parasitol* 2002, **96**:803-807.

21. Herwaldt BL, de Arroyave KR, Wahlquist SP, de Merida AM, Lopez AS, Juranek DD: **Multiyear prospective study of intestinal parasitism in a cohort of Peace Corps volunteers in Guatemala.** *J Clin Microbiol* 2001, **39**:34-42.

22. Yoshikawa H, Abe N, Iwasawa M, Kitano S, Nagano I, Wu Z, Takahashi Y: **Genomic analysis of** *Blastocystis hominis* **strains isolated from two long-term health care facilities.** *J Clin Microbiol* 2000, **38**:1324-1330.

23. Parkar U, Traub RJ, Vitali S, Elliot A, Levecke B, Robertson I, Geurden T, Steele J, Drake B, Thompson RC: **Molecular characterization of** *Blastocystis* **isolates from zoo animals and their animal-keepers.** *Vet Parasitol* 2010, **169**:8-17.

24. Eroglu F, Koltas IS: **Evaluation of the transmission mode of** *B. hominis* **by using PCR method.** *Parasitol Res* 2010, **107**:841-845.

25. Wongstitwilairoong B, Srijan A, Serichantalergs O, Fukuda CD, McDaniel P, Bodhidatta L, MASON CJ: **Intestinal parasitic infections among pre-school children in Sangkhlaburi, Thailand.** *Am J Trop Med Hyg* 2007, **76**:345-350.

26. Suresh K, Venilla G, Tan T, Rohela M: **In vivo encystation of** *Blastocystis hominis.* *Parasitol Res* 2009, **104**:1373-1380.

27. Zhou XB, Zhang X, Qiao JY, Cai J, Cheng S, Yuan Y, Li J: **Encystation–survival of** *Blastocystis hominis* **in immunocompetent mice abdomen cavity.** *Parasitol Res* 2010, **106**:1315-1320.

28. Hellard ME, Sinclair MI, Hogg GG, Fairley CK: **Prevalence of enteric pathogens among community based asymptomatic individuals.** *J Gastroenterol Hepatol* 2000, **15**:290-293.

29. Tan TC, Suresh KG, Smith HV: **Phenotypic and genotypic characterisation of** *Blastocystis hominis* **isolates implicates subtype 3 as a subtype with pathogenic potential.** *Parasitol Res* 2008, **104**:85-93.

30. Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, Olsen KE, Arendrup MC, Nielsen HV, Mølbak K: *Blastocystis*: **unravelling potential risk factors and clinical significance of a common but neglected parasite.** *Epidemiol Infect* 2009, **137**:1655-1663.

31. Dominguez-Marquez MV, Guna R, Munoz C, Gomez-Munoz MT, Borras R: **High prevalence of subtype 4 among isolates of** *Blastocystis hominis* **from symptomatic patients of a health district of Valencia (Spain).** *Parasitol Res* 2009, **105**:949-55.

32. Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R: **Irritable bowel syndrome: is it associated with genotypes of** *Blastocystis hominis.* *Parasitol Res* 2010, **106**:1033-1038.

33. Albrecht H, Stellbrink HJ, Koperski K, Greten H: *Blastocystis hominis* **in human immunodeficiency virus-related diarrhea.** *Scand J Gastroenterol* 1995, **30**:909-914.

34. Cirioni O, Giacometti A, Drenaggi D, Ancarani F, Scalise G: **Prevalence and clinical relevance of** *Blastocystis hominis* **in diverse patient cohorts.** *Eur J Epidemiol* 1999, **15**:389-393.

35. Hailemariam G, Kassu A, Abebe G, Abate E, Damte D, Mekonnen E, Ota F: **Intestinal parasitic infections in HIV/AIDS and HIV seronegative individuals in a teaching hospital, Ethiopia.** *Jpn J Infect Dis* 2004, **57**:41-43.

36. Idris NS, Dwipoerwantoro PG, Kurniawan A, Said M: **Intestinal parasitic infection of immunocompromised children with diarrhoea: clinical profile and therapeutic response.** *J Infect Dev Ctries* 2010, **4**:309-317.